Proceedings of the 54th Annual Coccidioidomycosis Study Group Meeting

2010

March 27, 2010 • Surprise City Council Chambers • Surprise, Arizona

Proceedings of the 54th Annual Coccidioidomycosis Study Group

Meeting Number 54
March 25, 2010
Surprise City Council Chambers
Surprise, Arizona

Antonino Catanzaro, M.D.
Chairperson, Coccidioidomycosis Study Group

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Meeting Program

8 – 8:30 **Registration and Welcome**

8:30 am. **Epidemiology and Ecology**
Moderator: John Galgiani

8:30 am. **Investigation of High Incidence of Coccidioidomycosis in the Northwest Valley of Arizona: Results of a Serosurvey.** Chang LS, Sunenshine R, Lindsley M, Danielson C, Gomez B, Saubolle M, Imolte S, Bolden C, Tsang C, Ahlquist A, Harris J, Anderson S, Erhart L, Schumacher M, Santana S, Nesset A, Komotsu K, Chen S, Chiller T, Park B.

8:45 am. **Prolonged Drought, Pink Skies, Brown Snow: Can Arthroconidia be Far Behind? Noteworthy Events within and on the Margins of the *Coccidioides* Endemic Zones.** Fisher FS, Johnson SM, Pappagianis D, Bultman MW.

9:05 am. **Temporal Variation of Coccidioidomycosis Incidence and Associations with Precipitation in Arizona.** Comrie A, Tamerius J.

9:20 am. **Timeseries Exposure Analysis of Coccidioidomycosis in the Southern Arizona Endemic Corridor.** Yool SR, Pianalto FS, Daly E.

9:45 am. **Basic Science and Immunology**
Moderator: Karl Clemons

9:45 am. **Cloning and Characterizing *Coccidioides* Cupin Protein.** Johnson SM, Carlson EL, Ampel MN, Lunetta JM Pappagianis D.

10:00 am. **Immunological Analysis of Bronchoalveolar Lavage Cells in the Assessment of Coccidioidomycosis.** Ampel NM, Nesbit L, Knox K.

Meeting Program

10:20 am. **Phylogeography of the fungal pathogen *Coccidioides posadasii* and C. immitis in Mexico.** Luna-Isaac JA, Muñiz-Salazar R, Baptista-Rosas RC, Castañón-Olivares LR, González GM, González-Martínez MR, Bazán E, Contreras-Perez C.

10:35 – 11 am. **Break**

11:00 am. **Identification of Peptides from *Coccidioides* in Plasma by Mass Spectrometry.** Antwi K, Stolper R, Ruiz Y, Lake D.

11:15 am. **Targete*d Large Scale SNP Genotyping of* Coccidioides immitis and *C. posadasii*.** Schupp J, Driebe E, Gillece J, Sheff K, Pearson T, Colvin J, Beckstrom-Sternberg S, Barker B, Rounsley S, Keim P, Engelthaler DM.

11:35 am. **Diagnostics**
Moderator Neil Ampel

11:35 am. **Delayed Hypersensitivity Skin Testing for Coccidioidomycosis: The Reevaluation of Spherulin—4 studies.** Johnson R, Nielsen S, Ampel N.

11:50 am. **Anti-CSA Antibodies Detected by ELISA Compared to Conventional Diagnostic Coccidioidal Antibodies Detected by Immunodiffusion.** Peng T, Lewis ML, Galgiani JN.

12:10 pm. **Utility of Positive Enzyme Immunoassay Results for Detection of *Coccidioides* specific IgM.** Oubsuntia, V, Bhogal, N, and Lancaster, M

12:25-12:55 pm. **Lunch**

12:55 – 1:25 pm **Business Meeting**

Meeting Program

1:30 pm.	**Clinical Issues and Treatment** Moderator: Janis Blair
1:30 pm.	**The Role of Surgery in Diagnosis and Treatment of Pulmonary Coccidioidomycosis.** Meyerson SL, Galgiani JN
1:45 pm.	**Update on Human and Animal PK/PD studies involving Nikkomycin Z.** Nix DE, Hoover S, Shubitz L, Galgiani JN.
2:05 pm.	**Pulmonary Coccidioidomycosis and Tuberculosis Co-infection in a Teaching County Hospital.** Gulle A., Heidari A., Munoz A.
2:20 pm.	**Refractory Coccidioidomycosis: an Experiment in Nature.** Kuberski T.
2:45 – 3:45 pm.	**Symposium: Coccidioidomycosis in Pregnancy** Moderator: Tony Catanzaro 1. Case discussion: Rob Bercovitch 2. Coccidioidomycosis in Pregnancy review: Demo Pappagianis 3. Discussion: How to handle coccidioidomycosis in pregnancy: Neil Ampel a. Old coccidioidomycosis in newly pregnant woman b. New coccidioidal acquisition in pregnancy
3:45 – 4:15 pm.	**Break**
4:15 pm.	**Unusual Cases of Coccidioidomycosis** Moderator: Rafael Laniado-Laborin
5:15 pm.	**Adjourn**

Poster Session

Treatment of Prosthetic Joint Infections Associated with Coccidioidomycosis. Ianas V, Kuberski T.

Evaluation of Cell Lysis and DNA Extraction Methods for Real-time PCR Detection of Coccidioides in Clinical Specimens. Bowers JR, Driebe EM, Nibecker JL, Ampel NM, Hoover S, Wojack B, Saubolle M, Keim PS, Engelthaler DM.

10 year Review of Surgical Management for Pulmonary Coccidioidomycosis, Mayo Clinic Arizona. Jaroszewski D, et al.

Mutants of the *Coccidioides STE12* Transcription Factor are Hypervirulent in Mice, Suggesting a Role in Host Recognition. Narra HP, Shubitz LF, Kellner EM, Li L Orbach MJ.

Prolonged Drought, Pink Skies, Brown Snow:
Can Arthroconidia be Far Behind?
Noteworthy Events within and on the
Margins of Endemic Zones (EZ)
Fisher FS, Johnson SM, Pappagianis D, Bultman MW

Dust from Arizona and southern Utah has clouded skies and coated mountain snowpacks over large areas of Colorado. Major dust generating storms have increased in frequency and intensity from four storms in 2003 to twelve in 2009. Winds in these storms have been measured from 60 to 90 miles/hr while the dust has degraded air quality, often reducing visibility to less than a mile. Dust composition and satellite photos suggest that some of the dust may have been eroded from desert areas near St. George, Utah and also southern Nevada. Regions considered endemic for *Coccidioides* based on infection rates and the identification of coccidioidomycosis in wild rodents. No evidence exists at this time that the dust storms are transporting arthroconidia, but coccidioidomycosis is not reportable in Colorado so infections may go unnoticed or not be associated with the storms. However, increased soil erosion and changing wind patterns due to drought and climate changes within and adjacent to EZs bear monitoring, because of the potential of large unexposed populations outside of known EZs being infected by wind borne arthroconidia. The dust storms may be due to a decades long, northward migration of the western storm tract resulting in less moisture in Arizona and Utah, which in turn reduces, dust anchoring, ground covering, vegetation. Prolonged drought in the southwest is also contributing to soil erosion. Anthropogenic activities including increased areas of livestock grazing, recreation, and construction, all have added to the natural dust loads. USGS maps (2003) predicted that parts of northern Arizona and southern Utah would be areas of dust generation. These predictions have been validated by 2009 satellite images depicting wind events in Arizona and Utah. Similar predictions have also been validated for areas in Texas.

Temporal Variation of Coccidioidomycosis Incidence and Associations with Precipitation in Arizona

Andrew Comrie, James Tamerius

University of Arizona
School of Geography & Development

Incremental progress has been made in identifying the environmental factors that can account for inter-annual and seasonal variability in incidence of coccidioidomycosis, but the precise mechanisms remain unclear. In this study, we use Arizona coccidioidomycosis case data for 1995-2006 to investigate temporal trends and variability in case rates, and identify relationships with precipitation using correlation and regression techniques. We find a seasonal autocorrelation structure in case rates for Arizona that may be related to the ecology of the fungus, or an artifact of differential diagnosis and reporting lags. Regression analysis indicates that October-December precipitation is positively associated with case-rates the following fall and winter in both Maricopa County ($R^2 = 0.52$, $p = 0.013$) and Pima County ($R^2 = 0.48$, $p = 0.019$). In addition, fall and winter case rates are negatively associated with concurrent precipitation in Maricopa ($R^2 = 0.69$, $p = 0.002$) and Pima ($R^2 = 0.46$, $p = 0.02$), possibly due to dust inhibition. However, a strong negative correlation between precipitation in successive falls and winters during the study period make it difficult to assess the independence of these associations. Our results are consistent with partially-comparable independent data from the landmark early study by Hugenholtz in 1957.

Time Series Exposure Analysis to Coccidioidomycosis in the Southern Arizona Endemic Corridor

Stephen R. Yool, F. Scott Pianalto, Erin Daly

School of Geography and Development
The University of Arizona

Abstract: Results of lagged regression modeling suggest moist soil resulting from winter precipitation may be associated with increased incidence up to a year later in Pima, Pinal and Maricopa counties (Fig. 1 $R^2 = 0.6$). Similar time lags were
found to exist between precipitation vs. populations of the rodent *D. merriami* (Fig. 2 $R^2 = 0.80$) and remote-sensing analysis of soil disturbance vs. incidence (Fig.3 Rho = 0.65). Such findings appear to favor further ecological modeling of rodent habitat and disturbed soils as potential exposure sites.

Questions or PDF: yools@email.arizona.edu

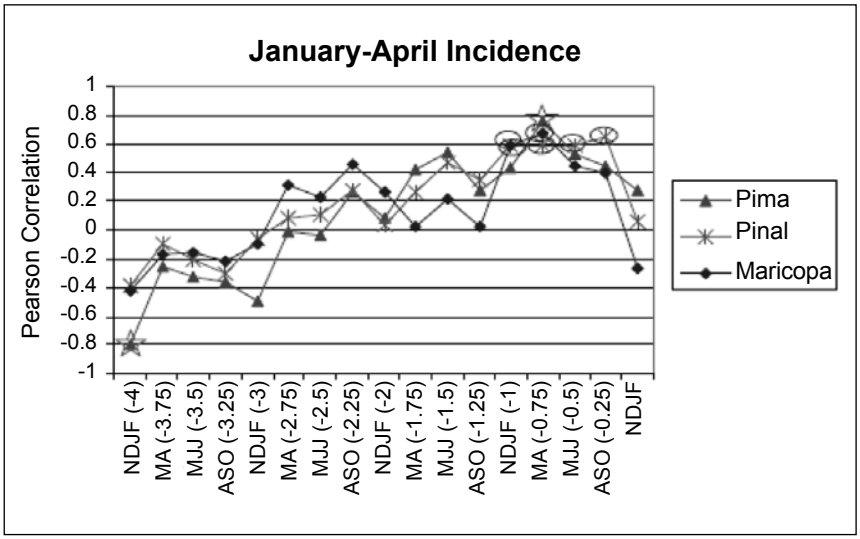

Figure 1.

(Continued)

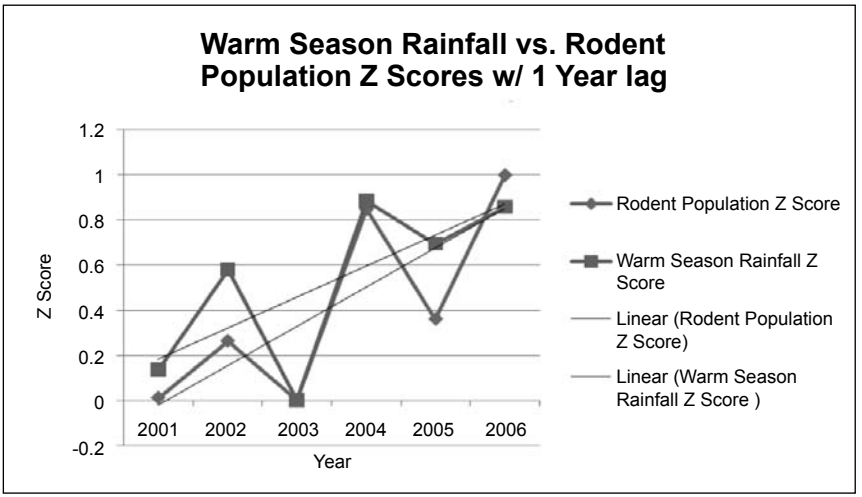

Figure 2.

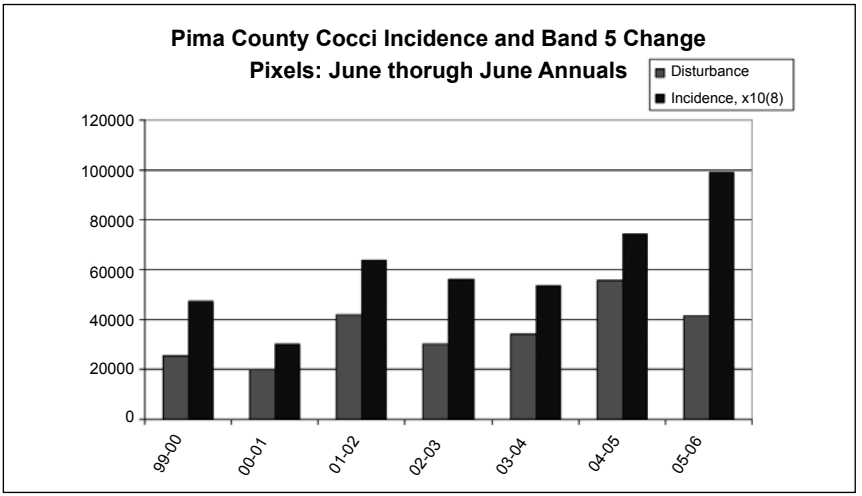

Figure 3.

Cloning and Characterizing *Coccidioides'* Cupin Protein

Suzanne M. Johnson[1], Erin L. Carlson[1], Neil M. Ampel[2], Jennine M. Lunetta[1], Demosthenes Pappagianis[1]

[1]Department of Medical Microbiology and Immunology, University of California, Davis, Davis, CA; [2]Valley Fever Center for Excellence and the Southern Arizona Veterans Affairs Health Care System, Tucson, AZ

Coccidioidomycosis is a fungal disease of humans and other animals caused by the inhalation of the infectious spore of *Coccidioides immitis* or *C. posadasii*. Previous studies have shown that the T27K vaccine, which is prepared from inactivated *C. posadasii* strain Silveira mature spherules, can protect mice against a lethal respiratory challenge. However, the component(s) responsible for inducing the immunoprotection have not been fully identified and/or characterized. We used continuous elution electrophoresis to separate the T27K proteins into pools and tested each for immunostimulation. Mass spectrometry identified the proteins present in the respective pools by comparing trypsin digests with peptides predicted from genomic sequencing projects. Peptides from one pool matched those predicted from contig CIMG_06609 which was annotated as a hypothetical protein.

The coding sequence of the 21 kDa hypothetical protein (21HP) was amplified from *C. posadasii* strain Silveira cDNA. The PCR product was cloned into an E. coli expression vector that encoded a 6X His tag and the recombinant fusion protein was isolated by affinity chromatography to nickel. Amino acid sequence analysis indicated that the protein contained a cupin, or barrel domain. Proteins with cupin domains typically bind a metal and are highly diverse. Because of this diversity, the protein function cannot be predicted. While motifs for N- and O-linked glycosylation were detected, a signal peptide was not. Therefore it is not clear if the native protein is glycosylated.

Antibody was prepared by hyperimmunizing rabbits with the recombinant 21HP purified protein. This antibody bound to a single protein in the T27K migrating at approximately 20 kDa when analyzed by Western blot. This antibody was then used to recover the native protein from the T27K. When coccidioidal immune human cells were incubated with the recombinant protein, no stimulation as measured by release of IL-2 was observed. However, when the same cells were incubated with T27K or an enriched native 21HP preparation, stimulation was apparent. Efforts are underway to isolate the native protein from the T27K and determine if glycosylation is present which may be responsible for the immunostimulory activity.

Immunological Analysis of Bronchoalveolar Lavage Cells in the Assessment of Coccidioidomycosis

Neil M. Ampel, MD; Lance Nesbit, BS; Kenneth S. Knox, MD

University of Arizona Department of Immunobiology and the Southern Arizona Veterans Affairs Medical Center, Tucson, AZ

Background: Polyfunctional T lymphocytes (PTL), which produce the T-helper type 1 cytokines interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) simultaneously, are considered to be markers for a robust and protective cellular immune response. We have recently detected such cells in peripheral blood mononuclear cells (PBMC) obtained from coccidioidal immune donors when incubated with the antigen preparation T27K. We now examine if these cells exist in the bronchoalveolar lavage (BAL) fluid of patients with pulmonary disease of unclear etiology living in the coccidioidal endemic region.

Methods: Patients undergoing diagnostic BAL for pulmonary disease of unknown etiology at a single medical center gave their consent to use the excess BAL fluid (BALF) for research purposes. The excess fluid was collected on ice, transported in 50 ml tubes to the laboratory, and centrifuged at 300 x g for 7 min in 15 ml conical tubes within 1 hour of collection. The cell pellets were suspended at 1×10^6 viable cells/ml of AIM-V, plated onto 12 mm flat-bottom wells and incubated for 48 hr. Plates received nothing (control) or 20 μg of T27K (a generous gift from Suzanne Johnson, Ph.D. and Demosthenes Pappagianis, M.D., Ph.D., University of California at Davis). Intracellular flow cytometry was performed as previously described (Nesbit et al, Infect Immun 2010; 78:309).

Results: Data were generated on 12 subjects. Based on intracellular cytokine expression in BALF, five subjects demonstrated pulmonary coccidioidal cellular immunity and three demonstrated CD4+ PTL in response to T27K. All five had negative BALF fungal cultures and three had negative coccidioidal serologic tests. In one donor, simultaneous studies were done with PBMC and these also contained both CD4+ and CD8+ PTL in response to T27K.

Conclusions: Immunological analysis of BALF using intracellular flow cytometry can indicate pulmonary coccidioidal infection even when other clinical assays are negative.

ABSTRACT 7: Comparison of Coccidioidomycosis State Surveillance and Self-Report Survey Cases in Tucson, Arizona

Joseph A. Tabor[1,2] and Mary Kay O'Rourke[2]

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State-reported coccidioidomycosis cases in Arizona have dramatically increased since 1997 and indicate an epidemic of unknown causes. Changes in disease reporting-compliance, misdiagnosis, and changing demographics of susceptible populations can mask the true disease frequency. Address-level state-reported disease cases were compared with self-reported cases from a telephone survey collected in 2002 and 2003 in greater Tucson, Arizona. Disease frequencies from 1992 to 2003 surveillance data and self-reported cases from 1994 to 2001 were analyzed at census block group resolution and by strata based on three landscape types and two demographic classes. Disease frequency is highly variable in space and time at the census block-group and coarser geographies. Disease outbreaks could be detected at census block group resolution that corresponded to soil disturbance events. Differences in disease frequencies by strata indicate *Coccidioides* exposures and host susceptibility are important predictors of coccidioidomycosis. There was no dramatic increase in state-reported cases between 1992 and 2003 that met criteria for an epidemic after adjusting for reporting compliance.

Identification of Peptides in Plasma from *Coccidioides* by Mass Spectrometry

Douglas F. Lake[1], Kwasi Antwi[1], Richard Stolper[2]

[1]School of Life Sciences, Arizona State University
[2]Scottsdale Ranch Animal Hospital

Objective: The objective of this study was to determine if Coccidioidal peptides and/or proteins could be detected in plasma from sero-positive dogs. The rationale for this work is that serological tests are not always able to identify infected dogs due to immunosuppression or a delayed immune response to the fungus. As such, the presence of Coccidioidal proteins or peptides in blood would provide a definitive diagnosis.

Methods: Blood was drawn from dogs that were clinically suspected to have Coccidioidomycosis. One tube of blood was sent to a veterinary laboratory for serological testing by ELISA to obtain CF titers. Plasma (1-2ml) from another tube of blood was ultrafiltered and subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a Thermo LTQ mass spectrometer. Mass spectra were searched against protein databases of *Coccidioides posadasii* str. Silveira and *Canis lupus familiaris*.

Results: From 19 dogs clinically suspected of having coccidioidomycosis, 9 were seropositive, and 10 were seronegative. In the seropositive dogs, peptides were identified corresponding to 59 different coccidioidal proteins and 136 canine proteins. Two coccidioidal peptides were common among all 9 seropositive dogs. In the 10 seronegative dogs, neither peptide was found in 6 of the dogs. However, one coccidioidal peptide was found in low levels in 2 dogs and both coccidioidal peptides were found in 2 dogs. Interestingly, neither coccidioidal peptide was found in a seronegative dog that was on maintenance fluconazole therapy and previously seropositive.

Conclusions: Coccidioidal peptides can be identified in plasma from dogs seropositive for coccidioidal CF antigen using LC-MS/MS techniques. Remarkably, 2 peptides were identified that were common in all 9 seropositive dogs. Although the 2 coccidioidal peptides were detected in 4 of 10 seronegative samples, these dogs were all suspected of having canine coccidioidomycosis. Follow-up studies are planned with the seronegative dogs whose plasma contained one or both peptides. These preliminary studies suggest that LC-MS/MS can detect coccidioidal infection in dogs that are symptomatic and seropositive. We also postulate that LC-MS/MS can detect coccidioidal peptides in symptomatic, but seronegative dogs due to delayed immune seroconversion.

Targeted Large Scale SNP Genotyping of *Coccidioides immitis* and *C. posadasii*

Schupp J[1], Driebe E[1], Gillece J[1], Sheff K[1], Pearson T[2], Colvin J[1], Beckstrom-Sternberg S[1,2], Barker B[3], Rounsley S[3], Keim P[1,2], Engelthaler DM[1]

[1]The Translational Genomics Research Institute, Flagstaff, AZ
[2]Northern Arizona University, Flagstaff, AZ
[3]The University of Arizona, Department of Plant Sciences, Tucson, AZ

Phylogeographic analysis can facilitate epidemiological understanding of microbial organisms important to public health, such as *Coccidioides.* However, previous genetic analyses of these organisms using microsatellite marker loci, while resolving the two species and providing some resolution within them, has not resulted in robust meaningful fine scale phylogeographic characterization. *C. posadasii* and *C. immitis* are known to be highly recombinogenic and contain significant genetic sequence repeat structure throughout their genomes, the latter suggestive of a Repeat Induced Point mutation (RIP) mechanism, found in other fungal organisms. There is also suggestion of interspecies hybridization, resulting in genetic introgression of genomic regions from one species to the other. These genomic characteristics can confound phylogenetic analyses through the generation and propagation of homoplastic (*i.e.,* conflicting) genetic polymorphisms, including both microsatellites (genetically unstable) and SNPs (genetically stable). However, the use of large numbers of SNPs located outside of known genomic repeat regions can overcome the effects of homoplasy, resulting in meaningful phylogenetic characterization within these two species. We analyzed over 100 *C. posadasii* and *C. immitis* samples with 1000 SNPs using a custom microarray genotyping chip. The SNPs were identified from a whole genome sequence analysis of 14 available *Coccidiodies* sequences, targeting locations outside of known repeat regions. Subsequent phylogenetic analysis revealed significant phylogeographic correlation within both species, with multiple major clades identified. The large number of SNPs used also provided for fine scale resolution among isolates as well as accurately identifying closely related and replicate isolates. New methodologies can provide interrogation of even greater numbers of SNPs, such as whole genome sequence typing (WGST). Using next generation sequencing technology, we recently sequenced 18 genomes of *Coccidioides* spp. WGST can provide for even more accurate and, therefore, meaningful phylogeographic analyses of these important fungal pathogens.

Delayed Hypersensitivity Skin Testing for Coccidioidomycosis: The Re-evaluation of Four Studies on the road to a Marketable Test Antigen

R Johnson, NM Ampel, S Nielsen, S Kernerman, B Sawtelle

Skin testing for coccidioidomycosis was first attempted by Cooke in 1915 as an emulsion of mycelia and spherules1. Coccidioidin was developed and made a standard test by C.E. Smith. This was a mycelial derived antigen2. Spherulin or the original spherule derived skin test antigen was developed by H. Levine and produced and marketed by Berkley Biologicals. Subsequently the product was sold twice and marketing was discontinued. The product was purchased by Allermed and a reformulation and testing project was undertaken.

Concerns arose over thimerosal as a preservative additionally thimerosal controls demonstrated delayed hypersensitivity reactions. This data prompted a new formulation of the thimerosal preserved antigen base stock in a phenolic preservative.

The following studies have been undertaken with the newly formatted phenolic preserved spherule derived coccidioidin (SDC) over the last ten years. All studies were IRB approved. A dose response study was undertaken at Kern Medical Center with doses of 0.4 – 2.4 µg/ml in individuals with a demonstrable history of coccidioidal disease. Linear regression analysis predicted a 1.27 µg dose would yield a mean induration of 22mm. € (induration/concentration) = 5.592 ±13.106 concentration.

A study of individuals with no history of or likely exposure to coccidioidal infections was undertaken in Spokane Washington on 59 evaluable patients only. One individual had a 5 mm response. A study was undertaken in Blair, Nebraska an area endemic for histoplasmosis. Twelve individuals were tested. All twelve were negative. A phase III sensitivity study was undertaken in Bakersfield, California and Tucson, Arizona. 1.27 µg of SDC was evaluated. Negative controls saline, thimerosal and positive controls candida and trichophyton were simultaneously placed according to a randomized blinded plan. 52/53 reacted positively. One thimerosal control developed an 8 mm reaction. The adverse event rate was low. SDC was demonstrated to have sensitivity, specificity and safety such that it should be available for use in evaluating individuals for coccidioidomycosis.

[1]Cooke, J.V. Immunity Tests in Coccidioidal Granuloma. Arch Intern Med. 1915;XV(3):479-486.
[2]Smith, CE, Whiting, EG, Baker, EE, et al. The Use of Coccidioidin. Am Rev Tuberc. 1948 Apr;57(4):330-60.

Anti-CSA Antibodies Detected by ELISA Compared to Conventional Diagnostic Coccidioidal Antibodies Detected by Immunodiffusion

Peng T, Lewis ML, Galgiani JN

Valley Fever Center for Excellence and Bio5 Institute, University of Arizona

Background: Early infection due to *Coccidioides* spp. (Valley Fever) requires specific laboratory tests, usually standard assays for anti-coccidioidal antibodies, to distinguish this fungal infection from other causes of community acquired pneumonia. Unfortunately, perhaps half of first sera tested in this way are falsely negative.

Methods: We have expressed "Coccidioides-specific antigen" (CSA) in *S. cerevisiae* and measured antibodies by ELISA in deidentified sera from 20 Tucson patients without known coccidioidal infection (Controls) and 50 sera from 25 patients with either tube-precipitin only (TP+, n=8) or complement-fixing (CF+, n=17) antibodies and a previous sera obtained at some time prior to the TP+ or CF+ specimen (Antecedents).

	IgG ELISA anti-CSA antibody titer (x100)							
Group	4	8	16	32	64	128	256	>512
Control	18	1	1					
TP+	7			1				
CP+	6		2	5			1	3
Antecedents	17		2	3	1	1		1

Results: Only 13% of the TP+ sera and 65% of CF+ sera showed anti-CSA ≥1:1,600. None of the antecedent sera in TP+ patients showed significant anti-CSA titers, but, 47% of antecedent sera in CF+ patients with intervals ranging from 7 to 227 days (median=34 days) had anti-CSA titers ≥1:1,600. Analysis with recombinant CSA truncations is underway.

Conclusions: Further development of an anti-CSA ELISA may improve the detection for early coccidioidal infections.

Utility of Positive Enzyme Immunoassay Results for Detection of Coccidioides Specific IgM

Oubsuntia V, Bogul N, Lancaster M

Kern County Public Health Laboratory, Bakersfield, CA, USA

Background: Meridian Premier® *Coccidioides* enzyme immunoassay for IgM (EIA IgM) assay may produce false positive results when compared to conventional immunodiffusion. This work evaluated frequency, and distribution of EIA IgM positive results as defined by the assay's package insert. Our objective was to characterize EIA IgM positive and indeterminate results by comparison to conventional immunodiffusion method (RIDIgM), and to a high sensitivity modification of immunodiffusion (KIDIgM), to determine utility of EIA IgM in detecting *Coccidioides* infection.

Methods: Serum samples presented for routine serologic analysis for Coccidioides infection were evaluated for evidence of IgM with Meridian EIA kit, (EIA IgM), and with conventional immunodiffusion (RIDIgM) and a high sensitivity modified immunodiffusion method (KIDIgM).

Results: 3,364 consecutive serum specimens were evaluated for EIA IgM reactivity and distribution of their respective EIA reading evaluated. Sample distribution was: 74% negative; 26% indeterminate or positive (8% indeterminate, 18% positive). 1,682 specimens evaluated by all three methods demonstrated, 15% positive or indeterminate by EIA IgM, with 44% of confirmed by RIDIgM or KIDIgM. 523 EIA IgM positive/KIDIgM negative samples demonstrated overlapping range of EIA IgM A_{450} values with 101 EIA IgM positive/KIDIgM positive samples.

Conclusions: Comparison of EIA IgM to RIDIgM does suggest that a high level of false positives are observed. However, when compared to a high sensitivity immunodiffusion method, the rate of false positives drops significantly. This suggests that the "false positives" may be more a reflection of the increased sensitivity of EIA IgM rather than true false positive results. However, considerable overlap of EIA IgM reading values for both confirmed and not confirmed samples suggests that EIA IgM may have no added utility over high sensitivity immunodiffusion for detecting positive samples.

Pulmonary Coccidioidomycosis and Tuberculosis Coinfection

Apollo Gulle, MD[1]; Arash Heidari, MD[2]; Augustine Munoz, MD[3]

[1]Department of Medicine
[2]Division of Infectious Diseases
[3]Division of Pulmonary and Critical Care
Kern Medical Center/UCLA, Bakersfield, CA

Background: Coccidioidomycosis is endemic in the Southwest US, South and Central America. San Joaquin Valley of California accounts for 2/3 of the reported cases in this State and majority of these patients were from Kern County. Mycobacterium tuberculosis (MTB) infection is virtually found all over the world. In the US, California remains in the top 5 states with highest incidence of MTB. Coccidioidomycosis and MTB share many similarities in clinical presentation, radiologic characteristics, demographics, and risk factors. Therefore, coinfection of MTB and coccidioidomycosis can be overlooked which can lead to under diagnosis and underreporting. The objective of this study is to describe the clinical, epidemiologic, laboratory, and radiologic features of pulmonary MTB and Coccidioidomycosis coinfection.

Methodology: This is a retrospective review of medical records of patients in Kern Medical Center. All patients with diagnosis of MTB or Coccidioidomycosis were identified and then matched for the coinfection. Patients' demographics, radiologic, serologic, microbiologic and laboratory results were reviewed.

Results: Thirteen patients with pulmonary MTB and Coccidioidomycosis coinfection were identified. All patients were immigrants from Mexico or Philippines. Majority of the patients were male (85%) and Hispanics (85%). Coccidioidomycosis and pulmonary MTB were diagnosed at the same time in 62% of the cases. On 15% of the cases, Coccidioidomycosis was diagnosed first before MTB, and the order of diagnosis was opposite in the rest of the cases. Risk factors and comorbidities associated with the coinfection were Diabetes, Positive HIV serology, alcohol abuse, tobacco abuse, being a field worker, and homelessness. Majority of the patients had apical or bi-apical lesions (50%) on their pulmonary imaging and others had miliary, mediastinal, or unilateral lower lung infiltrates.

Conclusion: Pulmonary tuberculosis and Coccidioidomycosis can coexist in the same host. The similarities between both diseases in clinical presentations, demographics, risk factors and imaging, urges clinicians to look for the coinfection.

Refractory Coccidioidomycosis: An Experiment in Nature

T. Kuberski, MD

2006: A nineteen year old black male was diagnosed with disseminated coccidioidomycosis in 2006. He presented with fevers, shortness of breath and military infiltrate on chest x-ray. Work up revealed lytic lesions ninth right rib, cervical and thoracic spine. He had been in Phoenix ten months coming from Chicago. He was a student, previously healthy and no drug use. Laboratory studies notable for a negative HIV test, 10% eosinophilia and Coccidioides CF titer of > 1:256. He underwent two neurosurgical procedures, debridement with fusion of C2 and C3 and thoracic spine fusion and stabilization with Harrington rods T4 thru T8. He was treated with liposomal amphotericin B, total dose >7,000 mg, followed by oral fluconazole 400 mg daily. He was lost to follow-up.

2007: Insurance did not cover his fluconazole and it was stopped because he could not afford it. The coccidioidomycosis relapsed and he was hospitalized at another hospital. He was transferred back because of renal failure (creatinine > 5.0) related to liposomal amphotericin B. No fevers, but weight loss of twenty pounds. The lytic bony lesions were worse. His renal failure improved and he was treated with oral intraconazole 200 mg bid. Itraconazole levels were therapeutic.

2008: Hospitalized at another facility again for a right chest mass. An attempt was made to debride the mass, but created a non-healing fistula. He was transferred back because of the fistula. He had clear progression with more bony lesions and bilateral psoas abscesses despite oral itraconazole. He was treated with amphotericin B lipid complex (ABLC) and developed renal failure again. His coccidioides isolate was sent for sensitivity testing and reported sensitive to both itraconazole and fluconazole. He was switched to oral fluconazole 800 mg daily.

2009: Admitted because of enlarging multiple soft tissue masses and failure to thrive despite fluconazole. Large soft tissue masses were present right chest, bilateral psoas muscles, left flank and upper chest. The largest mass (8x5 cm) had formed at the site of the prior surgery on his right chest. He was also experiencing back pain associated with a thoracic spine abscess at the site of prior fusion. He had drainage of the spine abscess and a debulking procedure of the right chest mass. He was treated with ABLC and discharged on intravenous outpatient therapy.

Refractory Coccidioidomycosis: An Experiment in Nature *(Continued)*

T. Kuberski, MD

2009: Readmitted because of enlarging soft tissue masses despite outpatient ABLC. He had weight loss and failure to thrive. The ABLC was increased to tolerance and he was placed on oral posaconazole 400 mg bid and gamma interferon subcutaneous qod. The patient gradually stabilized as an inpatient over six weeks. He could not be discharged to an outpatient facility because of the expensive treatment medications. The patient was sent to the National Institutes of Health for further evaluation and treatment. What can be learned from this patient?

Teratogenicity of Azole Antifungals

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Azole antifungals are an important tool in the management of coccidioidomycosis. However, there have been reports of teratogenicity that make their use during pregnancy problematic. While first developed in the 1960's, azole use has expanded greatly since the 1990's with the development of the triazoles, including fluconaconazole, itraconazole, posaconazole and voriconazole. Animal studies of fluconazole have demonstrated dose-dependent effects on both the mother and the fetus which led to a pregnancy class C warning from the U.S. Food and Drug Administration. The first case of possible human teratogenicity related to fluconazole was reported in 1992 and described a fetus with several cranial dysmorphic abnormalities similar to Antley-Bixler Syndrome (ABS) in a woman on 400 mg daily of fluconazole for coccidioidal meningitis. Since then, four other cases of cranial-facial abnormalities have been reported, one of which involved the same mother as the initial case. That these abnormalities are associated with fluconazole is biologically plausible because of the dose-dependent effects observed in animals and because ABS is linked to mutations in steroid biosynthesis, the site of inhibitory activity of azoles. An epidemiologic study of low-dose fluconazole during the first trimester did not demonstrate an increased risk of congenital malformations. Moreover, animal studies suggest that the effect should be limited to the first trimester. In addition, two reports of relatively high-dose fluconazole therapy of women during the second trimester found no evidence of teratogenicity. It is likely that the effects seen with fluconazole would also occur with other triazoles but data are limited. In summary, fluconazole and possibly other triazoles are teratogenic at high doses during the first trimester of pregnancy. Their safety is unclear during the second and third trimester but limited human data suggests possible safety.

Immunological Basis of Coccidioidomycosis during Pregnancy

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The immunological paradox of pregnancy was first addressed by Sir Peter Medawar in 1953. Despite containing foreign antigen, no clear mechanism has been delineated for the lack of rejection of the fetus by a mother. While there are data to suggest that there is a general suppression of immunological function during gestation, healthy women are not considered to be at an increased risk for infection. However, three infections have been associated with worse outcomes among pregnant women than among the general population. These are mycobacterial diseases, influenza A, and coccidioidomycosis. Catanzaro reviewed the problem of coccidioidomycosis during pregnancy (Chest 1985; 84 [3 Suppl]:14S-18S) and noted that there is moderate generalized immunosuppression throughout pregnancy, but could identify no risk of increased infection among other systemic mycoses. He promoted the idea of Drutz (Infect Immun 1981; 32:897) that high levels 17 β-estradiol might increase the growth of Coccidioides during gestation and so lead to more severe disease. A review of PubMed in 2010 again demonstrated that no mycoses other than coccidioidomycosis are associated with a poor outcome during pregnancy. These include histoplasmosis, blastomycosis, sporotrichosis, paracoccidioidomycosis, and cryptococcosis. Except for an increase in vulvovaginal disease, there appears to be no propensity for an increase in the clinical severity of candidiasis. Barbee et al (Chest 1991; 100:709) studied coccidioidal specific in vitro cellular immune responses among 3 pregnant women who developed coccidioidomycosis during the first trimester and compared them to 4 pregnant women with previous coccidioidomycosis. While those with infection acquired during pregnancy had low in vitro cellular immune responses throughout gestation and into the post partum, those with previous infection had only modest declines during gestation and significant increases in their response post partum. While there are no more recent studies, these data suggest that there is a depression in specific cellular immune response in coccidioidomycosis when infection is acquired during pregnancy. These results are in accordance with clinical observations that women who acquire new coccidioidal infection during the second and third trimester have increased risks for severe and disseminated disease, while those with coccidioidomycosis acquired prior to pregnancy generally do well during gestation. Further research in this area is clearly warranted.

Most Unusual Cases of Coccidioidomycosis - Bilateral Osseus Olecranon Coccidioidomycosis

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This is a case of an 18-year-old Filipino male who lives in Delano located in central valley of California (San Joaquin Valley). His story started four month earlier when he developed influenza like illness with fever, body ache, fatigue and dry cough.

Three weeks later while still suffering from fatigue and dry cough he developed generalized “rash” on his hands, forearms and shins. He was seen by his primary care physician and diagnosed with viral infection and received anti histamines. His skin rashes resolved over 2 weeks but cough and fatigue persisted. He started practicing Karate in order to gain more energy about six weeks later and during one of his practices he traumatized both of his elbows without any skin breaks.

He noticed that his painful elbows became swollen, tender and red over 2 days. Warm compressions and other home remedies for 2 weeks were not helpful. Finally, he went to the emergency department (ED) and was diagnosed with bilateral bursitis and received a course of oral antibiotics. Two weeks later without any improvement he noticed breaking of the skin over both elbows and drainage of purulent discharges bilaterally. This time he came back to ED and he was admitted to the hospital.

The initial imaging work up revealed osteomyelitis of both olecranons bilaterally. This was then confirmed with whole body bone scan afterward. Also he was found to have a large left lower lobe infiltration. He underwent incision and drainage of both elbows. Deep tissue cultures were positive for fungal elements resembling *Coccidioidoes immitis* bilaterally. Subsequently, microscopic pictures from pathology confirmed existence of spherules with endosporulation on both sides.

Most Unusual Cases of Coccidioidomycosis - Bilateral Osseus Olecranon Coccidioidomycosis *(Continued)*

Arash Heidari, MD[1]; Rushabh Shah, MD[2]

His serologies for *Coccidioidal* EIA(IgM) and immunodiffusion, IgM and IgG came back reactive with complement fixation titers of 1:256.

He was initially started on oral fluconazole. When it was decided by plastic surgery team to perform skin grafting after more debridement his antifungal treatment switched to Amphoterin B Liposomal Complex (ABLC). Parenteral ABLC continued for 4 more weeks and changed to oral Fluconazole. Since then he has been followed up in the clinic closely and his wounds and his pulmonary infiltration have been improved.

This is a case of bilateral osseus *Coccidioidomycosis* due to Locus Minoris Resistencia due to trauma to the affected area.

Treatment of Prosthetic Joint Infections Associated with Coccidioidomycosis

V. Ianas, MD; T. Kuberski, MD

Prosthetic joint infection due to coccidioidomycosis is an uncommon problem. A small series of prosthetic knee infections due to *Coccidioides* was previously reported at the 2000 Coccidioidomycosis Study Group meeting. The latter report generated interest from clinicians about how to treat their patient with a prosthetic joint infection associated with disseminated coccidioidomycosis.

We reviewed available clinical cases and the literature to assess treatment and outcomes in order to propose a course of treatment for patients with this type of infection. It is a general clinical observation that fungal infections of prosthetic joints are unusual. The rarity of coccidioidomycosis causing this infection suggests that it is the result of dissemination to the joint irrespective of the presence of a prosthesis.

We propose the following treatment options depending on the clinical circumstances:

1. **Fusion of the joint:** preoperative and postoperative amphotericin , debridement of joint with fusion, followed by life-long fluconazole;
2. **Removal of prosthesis**: debridement of the joint with synovectomy, preoperative and post operative amphotericin, followed by eventual replacement of prosthesis and life-long fluconazole;
3. **Maintain the prosthesis in place:** a course of amphotericin, followed by life-long fluconazole.

The rational for these approaches are discussed.

Mutants of the *Coccidioides STE12* Transcription Factor are Hypervirulent in Mice Suggesting a Role in Host Recognition

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A number of genes conserved among fungi have been shown to play a role in pathogenicity in both animal and plant pathogens. One such gene is *STE12,* an ortholog of a yeast homeodomain transcription factor that is known to regulate invasive growth in other filamentous fungi. To determine whether the *Coccidioides* ortholog of *STE12* (designated *CST12 for Coccidioides STE12*) was important for virulence, we generated *cst12* mutants of *Coccidioides posadasii* strain *Silveira* using an *Agrobacterium*-mediated gene knock-out strategy. A hygromycin resistance gene cassette was cloned between the *CST12* 5' and 3' flanking sequences and transformed into Silveira to generate gene replacement mutants. When compared with the wild type strain, in vitro growth seemed normal, but spherulation was significantly reduced in Δ*cst12* mutants, not growing beyond the size of 48 hr WT spherules. Surprisingly, the Δ*cst12* mutant was found to be more virulent in mice than Silveira. This was indicated by virulence and fungal burden present in C57BL/6 and Swiss Webster mice. Based on these findings, we propose that *cst12* in *Coccidioides* spp. is essential for host signal recognition during invasion and that this recognition leads to the pathogen detecting the host as a hostile environment and entering a quiescent state. In the mutant, recognition of host signals does not occur and the pathogen proliferates leading to increased virulence.

Abstract presented at meeting, not available for publication.

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Annual Meetings of the Coccidioidomycosis Study Group

Number	Date(s)	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	California Thoracic Society
10.	December 7, 1965	Phoenix, AZ	2nd Coccidioidomycosis Conference
11.	April 19, 1967	Palm Springs, CA	California Thoracic Society
12.	May 1, 1968	Fresno, CA	California Thoracic Society
13.	April 15, 1969	San Diego, CA	California Thoracic Society
14.	April 1, 1970	San Francisco, CA	California Thoracic Society
15.	April 6, 1973	Newport Beach, CA	California Thoracic Society
16.	April 5, 1974	Sacramento, CA	California Thoracic Society
17.	September 30, 1974	San Francisco, CA	Coccidioidomycosis Cooperative Treatment Group
18.	April 2, 1975	San Diego, CA	California Thoracic Society
19.	July 31, 1975	San Diego, CA	Coccidioidomycosis Cooperative Treatment Group
20.	January 14-15, 1976	San Diego, CA	Coccidioidomycosis Cooperative Treatment Group
21.	April 7, 1976	Palo Alto, CA	California Thoracic Society
22.	May 18, 1977	San Francisco, CA	American Lung Association
23.	April 5, 1978	Beverly Hills, CA	California Thoracic Society
24.	May 15, 1979	Las Vegas, NV	American Lung Association
25.	April 11, 1980	Sacramento, CA	California Thoracic Society
26.	March 28, 1981	San Francisco, CA	California Thoracic Society

Annual Meetings of the Coccidioidomycosis Study Group

Number	Date(s)	Location	Held In Conjunction With
27.	May 15, 1982	Los Angeles, CA	American Lung Association
28.	March 20, 1983	La Jolla, CA	California Thoracic Society
29.	March 14-17, 1984	San Diego, CA	4th Coccidioidomycosis 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 Coccidioidomycosis “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.	August 23-26, 2006	Stanford, CA	6th International Symposium on Coccidioidomycosis
51.	March 29, 2007	Tempe, AZ	
52.	April 5, 2008	San Diego, CA	
53,	April 4, 2009	Bakersfield, CA	
54.	March 27, 2009	Surprise, Arizona	

• Publishing courtesy of Mayo Clinic in Arizona •