



Coccidioidomycosis
S T U D Y G R O U P

2012

Proceedings of the 56th Annual
Coccidioidomycosis Study Group Meeting

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University of Arizona

Tucson, Arizona



Antonino Catanzaro, M.D.

Chairperson, Coccidioidomycosis Study Group

Janis E. Blair, M.D.

Program Chairperson



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Address editorial correspondence to:

Janis E. Blair, M.D.

Mayo Clinic Hospital

5777 East Mayo Boulevard

Phoenix, Arizona 85054

Meeting Program

- 7:00 a.m.** **Breakfast and Registration**
- 7:45 a.m.** **Welcome and Open Business Meeting Part 1**
- 8:00 a.m.** **Epidemiology**
Moderator: Peter Kelly
- 8:00 a.m.** **Coccidioidomycosis Surveillance in Arizona:
Comparison of 2007 and 2011 Data**
C. Benedem, C. Tsang
- 8:15 a.m.** **Skin Test with Coccidioidin in 13 Mexican Sites**
L.R. Castañón-Olivares, A. Higuera-Iglesias,
M. Arce-Ramírez, et al.
- 8:30 a.m.** **Epidemiology of Coccidioidomycosis in
Maricopa County from 2006-2011**
R. Sunenshine, J. Galgiani, A. Ayala, M. Kretschmer
- 8:45 a.m.** **Natural Distribution and Basic Characteristics
of Coccidioidomycosis**
Moderator: John Galgiani
- 8:45 a.m.** **PCR Survey for *Coccidioides spp.* from Presumed
Highly Endemic soils**
S.M. Johnson, E.L. Carlson, D. Pappagianis, R.F. Hector
- 9:00 a.m.** **A Strategy for Optimization of Detection of
Coccidioides in Soil Samples**
J.L. Beebe, C. Khoushabian, M. Applebaum, R. Hendrickson
- 9:15 a.m.** **Molecular and Serological Detection of *Coccidioides spp.* in
Soil and Rodent's Serum from Baja California, Mexico**
J. Catalan-Dibene, R. Baptista-Rosas,
A Romero-Olivarias, M. Riquelme
- 9:30-9:50 a.m.** **Break**
- 9:50 a.m.** **Laboratory and Experimental Coccidioidomycosis**
Moderator: Karl Clemons
- 9:50 a.m.** **Population Genetics and Genomics of *Coccidioides***
B.M. Barker, J. Tabor, L.F. Shubitz, M.J. Orbach
- 10:05 a.m.** **Expression, Purification, and Immunoreactivity of
Coccidioides posadasii Heat Shock Protein 10**
E.L. Carlson, S.M. Johnson, D. Pappagianis

Meeting Program

- 10:20 a.m.** ***Coccidioides* Cupin Protein 21 Immunoreactivity: Native versus Recombinant**
S.M. Johnson, E.L. Carlson, L.A. Nesbit,
N.M. Ampel, D. Pappagianis
- 10:35 a.m.** **VT-1161 Reduces Fungal Burden and Improves Survival in Murine Respiratory *Coccidioidomycosis***
L.F. Shubitz, H. Trinh, L. Lewis, J.N. Galgiani,
E.P. Garvey, W.J. Hoekstra, W.R. Moore, R.J. Schotzinger
- 10:50 a.m.** **Dendritic Cell-Based Vaccine Against *Coccidioides* Induces Antibody Response**
A. Conkleton, C. King, S. Awasthi
- 11:05 a.m.** **Diagnostics**
Moderator: Demo Pappagianis
- 11:05 a.m.** **Evaluation of *Coccidioides* Antigen Detection by Enzyme Immunoassay for Quantification of Fungal Burden in Murine Models of *Coccidioidomycosis***
L.J. Wheat, E. Kirsch, J. Capilla, K.V. Clemons,
D.A. Stevens, S. Sokamoto, J. Fierer
- 11:20 a.m.** **Comparative *Coccidioides* Serologic Results as Indicated by EIA and Immunodiffusion**
J. Einstein, D. Pappagianis
- 11:35 a.m.** **Posters and Authors**
- 12:15-12:45 p.m.** **Lunch**
- 12:45 p.m.** **Open Business Meeting Part 2**
- 1:00 p.m.** **Diagnostics (*continued*)**
Moderator: Demo Pappagianis
- 1:00 p.m.** **A Phase II Study for the Safety and Stability of *Coccidioidin* in Sensitized Subjects Undergoing Repeated Testing**
R.F. Hector, N.M. Ampel
- 1:15 p.m.** **Measuring *Coccidioides* Cellular Immunity by a Simple Blood Test in a *Coccidioidomycosis* Clinic**
L.A. Nesbit, S. Chavez, S.M. Johnson, D. Pappagianis,
J.N. Galgiani, N.M. Ampel
- 1:30 p.m.** **Clinical *Coccidioidomycosis***
Moderator: Rafael Laniado-Laborin

Meeting Program

- 1:30 p.m. **Suggested Algorithms for Therapy and Follow-Up of Coccidioidomycosis in Patients on Biologic Response Modifiers or Disease-Modifying Antirheumatic Drugs**
S. Knowles, S. Taroumian, J. Lisse, N.M. Ampel, E. Gall, B. Vaz, J.N. Galgiani, S.E. Hoover
- 1:45 p.m. **Two Cases of Coccidioidomycosis Disseminated to the Spine and Literature Review of Preferred Management**
H. Boro
- 2:00 p.m. **The Prevalence of Immunity to *Coccidioides* Among Healthy Arizona Residents Without a History of Symptomatic Doccidioidomycosis**
D. Lake, Y. Ruiz, S. Duffy, T. Pitta, M. Clarkson, T. Vaughn, J.E. Blair
- 2:15 p.m. **PTHrP and Hypercalcemia in Disseminated Coccidioidomycosis**
J. Fierer, D.W. Burton, P. Haghighi, L.J. Deftos
(requested not to publish in proceedings)
- 2:30 p.m. **Evaluation of Vitamin D Levels in Patients with Coccidioidomycosis: A Case Control Study**
A. Heidari, J. Dickey, N. Jhangiri, B. Ghafarizadeh, G. Peterson
- 2:45 p.m. **Clinical and Immunological Aspects of Bronchoalveolar Lavage Fluid in Acute Pulmonary Coccidioidomycosis**
L.A. Nesbit, K.S. Knox, S. Chavez, S.M. Johnson, D. Pappagianis, N.M. Ampel
- 3:00-3:30 p.m. **Break**
- 3:30 p.m. **Unusual Case of Coccidioidomycosis**
- 3:40 p.m. **Symposium: Coccidioidal Meningitis**
Moderator: Tony Catanzaro
Treatment of Experimental CM: Karl Clemons
Treatment with Azoles in 2012: Glen Matheson
Treatment with Amphotericin in 2012: Royce Johnson
Neurosurgical Aspects of CM: Mike Lemole
- Discussion**
- 5:15 p.m. **Adjourn**
- 7:00 p.m. **Dinner**

Poster Session

Cutaneous Coccidioidomycosis in Four Mexican Patients

M. Arce-Ramirez, E. Bazan-Mora, M.R. Reyes-Montes,
E. Duarte-Escalante, L.R. Castanon-Olivares

Disseminated Coccidioidomycosis Mimicking Cancer of the Female Genital Tract

Y. Wu, M. Skinner, N. Samad, T. Kuberski

The Dispersal of *Coccidioides spp.*: Microns to Kilometers

F.S. Fisher, S.M. Johnson, M.W. Bultman, D. Pappagianis

Development of a Coccidioidomycosis Case History Form in Kern County, 2011

K. Emery, M. MacLean, M. Lancaster, S. Joy, M. Habib, P. McMasters

Coccidioidomycosis Cases Continue to Rise in Kern County During 2011

K. Emery, M. Lancaster, V. Oubsuntia, C. Jonah, P. McMasters

The Sensitivity of Diagnostic Testing for Acute Coccidioidomycosis in Solid Organ Transplant Recipients

N. Mendoza, J.E. Blair

Detection of Antigen in the Cerebrospinal Fluid in Patients with *Coccidioides* Meningitis

D. Bamberger, L. Proia, L. Ostrosky-Zeichner, M. Ashraf,
E.P. Scully, F.M. Marty, L.J. Wheat

***Coccidioides spp.* Search in Soil and Air of the Comarca Lagunera Region, Mexico**

L.R. Castanon-Olivares, M.G. Pizana,
G.M. Verduzco, R. Gonzalez-Martinez, et al.

Detection of a Coccidioidal Peptide in Plasma from Patients with Active Coccidioidomycosis

S. Duffy, J.E. Blair, K. Antwi, S. Johnson, M. Orbach,
A. Mandel, T. Pitta, D.F. Lake

Diagnostic Value of Adenosine Deaminase Levels in Lymphocytic Pleural Effusions Caused by Coccidioidomycosis

G.R. Thompson III, D. Bays, S. Sharma, M. Davis,
R. Libke, D. Pappagianis

**Coccidioidomycosis Surveillance in Arizona:
Comparison of 2007 and 2011 Data**
Corey Benedum; Clarisse Tsang, MPH

Arizona Department of Health Services

Background: Coccidioidomycosis (Valley Fever) is an emerging fungal disease endemic to the southwestern United States, Central and South America. Since 2009, Arizona has seen a drastic increase in the rate of reported coccidioidomycosis cases. This increase has been found to correlate with a change in enzyme immunoassay (EIA) reporting in which a major commercial laboratory began reporting positive EIA tests without confirmation by immunodiffusion testing in accordance with Arizona reporting rules. The Arizona Department of Health Services (ADHS) launched an investigation to better understand the impact of EIA testing on coccidioidomycosis surveillance.

Methods: Coccidioidomycosis cases reported from February through December 2011 were compared to the 2007 data from the same timeframe. All cases identified by positive EIA test results with no confirmatory testing (EIA alone) were removed to form a new dataset for 2011. The data derived from this dataset was then compared to the 2007 data.

Results: The total amount of coccidioidomycosis cases examined from the above timeframe in 2011 was 14,127 (239 cases per 100,000 population). Seventy-eight percent of the cases had only positive EIA tests. Looking at 2007 data, there were 4,459 cases (73 per 100,000) compared to 3,094 (49 per 100,000) for 2011 when excluding the EIA alone data. Looking at age, we see that for the 2011 data, the median age was 47 (mean 47). Interestingly, the median age was 52 (mean 51) for 2007, which is similar to the median age for the 2011 data excluding the EIA alone results (median 52, mean 50). The 2011 data showed the majority of cases to be in females (58%, males 42%) whereas the 2007 data (46% female, 54% male) was similar to the 2011 data excluding the EIA alone results (45% female, 55% male).

Conclusions: Based upon the results, the reporting of positives identified only by EIA significantly alter the 2011 data by shifting the demographics of coccidioidomycosis in Arizona to be mostly in females and in a younger population than what was previously reported. It is highly probable that EIA testing alone is causing an increase in coccidioidomycosis reporting. It is important to have rapid medical testing to identify coccidioidomycosis in patients but these tests should continue to be confirmed by immunodiffusion tests to avoid false positive results.

Skin Test with Coccidioidin in 13 Mexican Sites

LR Castañón-Olivares¹; A Higuera-Iglesias²; M Arce-Ramírez³; R Cervantes-Olivares⁴;
R Cicero-Sabido⁵; C Contreras-Pérez⁶; R Fernández⁷; GM González-González⁸;
MR González-Martínez⁹; S Hernández-Campos¹⁰; R Laniado-Laborín¹¹; ME Manjarrez-Zavala²;
S Miranda-Mauricio¹²; MG Moctezuma-Zárate¹³; B Muñoz-Hernández²; R Padilla¹⁴; G Palma²;
A Pérez-Mejía¹; E Ramírez⁵; A Rendón⁸; Y Romo-Lozano¹⁵; E Ruiz-Baca¹⁰; C Toriello¹

¹Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM),
Distrito Federal (DF), México;

²Instituto Nacional de Enfermedades Respiratorias,
Secretaría de Salud (SS), DF;

³Clínica Derma-Care, Tijuana, Baja California;

⁴Facultad de Medicina Veterinaria y Zootecnia, UNAM;

⁵Hospital General de México, SS, DF;

⁶Laboratorio de Micología. Instituto de Diagnóstico y
Referencia Epidemiológicos, SS, DF;

⁷Hospital General "Manuel Gea González", SS, DF;

⁸Hospital Universitario de la Universidad Autónoma de Nuevo León,
Monterrey, N.L.;

⁹Unidad Médica de Atención Especializada IMSS Torreón, Coahuila;

¹⁰Facultad de Medicina y Nutrición de la Universidad Juárez del Estado de
Durango y Hospital General de Durango, SS.;

¹¹Hospital General, SS, Tijuana, Baja California;

¹²ISSSTESON, Ciudad Obregón, Sonora;

¹³Universidad Autónoma de San Luis Potosí;

¹⁴Laboratorio Estatal de Salud Pública, Mexicali, Baja California;

¹⁵Universidad Autónoma de Aguascalientes. email: lrcastao@unam.mx

As shown by Smith and Whiting (1948), approximately 60% of subjects infected with *Coccidioides spp.* are asymptomatic and infection can only be demonstrated by serology or a positive skin test (IDR) to antigens of the fungus.

The prevalence of infection with *Coccidioides* in Mexico, has been reported in skin survey with either coccidioidin and / or spherulin in Baja California, Sonora and Coahuila, all considered within the endemic area, but the prevalence is unknown in the rest of the country. The latest survey, reaction to coccidioidin at a national level, was reported in 1966 by Gonzalez Ochoa.

In order to determine the prevalence of infection with *Coccidioides spp* and update data on the prevalence in Mexico, we designed a multicenter skin testing survey with coccidioidin.

Coccidioidin antigen was prepared in the Laboratorio de Micología Básica, UNAM; testing was carried out in 13 different sites, at eight Mexican states. All testing was conducted at hospitals in every site, and included in-patient and out-patient population, medical and paramedical staff, medical students and other hospital personnel.

(Continued)

Skin Test with Coccidioidin in 13 Mexican Sites (Con't)

LR Castañón-Olivares¹; A Higuera-Iglesias²; M Arce-Ramírez³; R Cervantes-Olivares⁴;
R Cicero-Sabido⁵; C Contreras-Pérez⁶; R Fernández⁷; GM González-González⁸;
MR González-Martínez⁹; S Hernández-Campos¹⁰; R Laniado-Laborín¹¹; ME Manjarrez-Zavala²;
S Miranda-Mauricio¹²; MG Moctezuma-Zárate¹³; B Muñoz-Hernández²; R Padilla¹⁴; G Palma²;
A Pérez-Mejía¹; E Ramírez⁵; A Rendón⁸; Y Romo-Lozano¹⁵; E Ruiz-Baca¹⁰; C Toriello¹

Subjects were interviewed with a questionnaire that included demographic, socioeconomic and clinical items, followed by skin testing with 0.1 mL of coccidioidin through the intradermal route. Test was considered positive, when an induration equal to or greater than 5 mm in diameter was present 48 h after administration of the antigen.

A total of 992 subjects were tested, of which 293 were positive, yielding an overall prevalence of 29.5%.

The subjects age ranged from 10 to 86 years, with a median age of 37 years; 68% of the subjects with a positive skin test were in the age group between 20 to 49 years.

The highest rate of infection (67%) was reported in Ciudad Obregon, Sonora, significantly higher than that of the other sites.

Our result is similar to that reported by some authors in the United States which on average show a prevalence of 24%.

According to the Fiese criteria of coccidioidomycosis endemicity, we can conclude, based on published clinical cases, isolation of *Coccidioides* from the soil, proven *Coccidioides* infection in domestic mammals and high prevalence of infection in skin surveys, that the state of Sonora can be considered as a definitive endemic area for coccidioidomycosis in Mexico.

**PCR Survey for *Coccidioides* spp. from
Presumed Highly Endemic Soils**

*Suzanne M. Johnson¹, Erin L. Carlson¹,
Demosthenes Pappagianis¹, Richard F. Hector²*

¹University of California, Davis;

²University of California, San Francisco

Soil samples of varying quantities were collected during summer 2011 from sites within 2 miles of the Pleasant Valley State Prison (PVSP) and near Avenal State Prison (ASP) to ascertain whether DNA consistent with *Coccidioides* spp. could be detected. Samples were obtained primarily from undisturbed soil or from rodent burrows. Thirty five of 50 samples collected near PVSP and 8 of 25 collected near ASP were of sufficient quantity for this study. Using standard flotation techniques, the samples were processed for arthroconidia enrichment and then the DNA was isolated. A nested PCR amplifying a region of the rRNA followed by a diagnostic PCR designed to amplify a portion of the internal transcribed spacer 2 (ITS2) region of *Coccidioides* spp. was performed. PCR products of the final amplification were gel purified and sequenced for identification.

Preliminary analysis of 25 samples collected near PVSP has yielded 2 positive samples. While the methods hold promise for the detection of *Coccidioides*, they remain labor-intensive. Additional studies are planned to determine the method's sensitivity and specificity.

A Strategy for Optimization of Detection of *Coccidioides* in Soil Samples

*James L Beebe¹, Colin Khoushabian²,
Matthew Applebaum², Ryan Hendrickson²*

¹San Luis Obispo County Public Health Laboratory;

²California Polytechnic University, San Luis Obispo, CA

Background: Many studies of methods to detect *Coccidioides* in soil samples have been reported that have employed solid medium culture, animal inoculation, and PCR, as well as combinations of these three methods. Each method has advantages and disadvantages, although current detection methods have been burdened with labor-intensive and time-consuming animal studies and solid medium culture. Analysis of large number of soil samples – a practical necessity to fully characterize the uneven distribution of *Coccidioides* in soil-- is hampered by the lack of an optimal method.

Methods: We present a strategy to develop an optimal non-animal-based detection scheme using the combination of soil preparation, spore concentration, PCR and liquid selective-enrichment medium cultivation methods.

Results: We have established methods for PCR detection, soil DNA preparation, DNA extraction, *Coccidioides* inoculum standardization, selective-enrichment liquid medium (SLO medium) cultivation with ATP-based growth measurements in Biosafety level 3 conditions. We propose to test soil and human clinical *Coccidioides* isolates for growth in SLO medium to determine optimal growth conditions for PCR detection--before visible growth is observed--in an effort to determine the most sensitive method of detection with growth confirmation.

Conclusion: Development and implementation of a labor-sparing, but sensitive method of detection of *Coccidioides* with performance confirmed by culture confirmation, will allow more extensive soil characterization efforts in endemic areas.

Population Genetics and Genomics of *Coccidioides*

Bridget M. Barker¹, Joe Tabor²,
Lisa Shubitz², Marc J. Orbach²

¹Montana State University; ²University of Arizona

Coccidioides is a soil-dwelling dimorphic fungus endemic to semi-arid areas of the Americas. In 2002, the previously monotypic genus was formally recognized as two closely related species. Additionally, both species are divided into at least two populations: northern and southern California for *C. immitis*, and Arizona/Mexico and Texas/South America for *C. posadasii*. To further investigate the population genetics of *C. posadasii*, we have analyzed variable microsatellite markers within a collection of isolates obtained from infected humans and other mammals, and from environmental strains, all collected in the Tucson basin of Arizona. Data show that soil isolates are superior at determining the range of the species and populations, likely due to the fact that both patients and infectious spores may travel long distances. Additionally, the strains that infect animals, or cause more severe disease symptoms, are not phylogenetically distinct from the environmental strains. Nor is there any population-based separation among strains differing in virulence. Therefore, all soil isolates must be considered as reservoirs of inoculum for mammalian infections. Work is ongoing to collect soil samples from other regions to determine the population structure of *Coccidioides* throughout its range. This should include sampling transects from Arizona to southern California to determine if the species are allopatric or sympatric. Additionally, genomic analysis of sequenced strains of *C. posadasii* and *C. immitis* reveals insights into the population biology of these organisms. Strains were chosen for sequencing based on phylogenetic and geographic distribution, virulence and source (i.e. clinical or environmental). Work completed to date shows sequence similarity between species is ~97%, whereas similarity within species is ~99%, which is typical of species that have diverged sometime in the past 10 million years. Additionally, there is strong evidence for hybridization and introgression that occurred sometime after the speciation event. Genome comparisons identified regions from each genome that had a closer match to the opposite species than its own. For most of the *C. posadasii* strains, there were few regions that had a closer match to *C. immitis*. This analysis further revealed one fragment that contains a common border toward the centromere of the chromosome, and a variable border toward the telomere. This conserved region was further evaluated in our larger collection of isolates. Approximately half of the *C. immitis* isolates contain the *C. posadasii* fragment, and the majority of those are from the southern California and Mexico populations. Genes found in this region are being investigated for transcriptional changes in response to the morphological shift from arthroconidia to spherule. These results suggest that hybridization has occurred between species, and at least one fragment appears to have introgressed in the southern California *C. immitis* population, but not into the northern California population. Understanding the population structure of an environmentally acquired pathogen may allow for better prediction of disease outbreaks and provide a framework for understanding differences in disease outcomes and drug resistance.

Expression, Purification, and Immunoreactivity of *Coccidioides posadasii* Heat Shock Protein 10

Erin L. Carlson, Suzanne M. Johnson, Demosthenes Pappagianis

University of California, Davis

Infection with the fungus *Coccidioides* confers lifelong immunity, making the disease a reasonable candidate for vaccine-mediated control. T27K, a vaccine prepared from disrupted spherules, has been shown to protect mice exposed to lethal levels of *C. posadasii*. Examination of fractions made from processing T27K by gel filtration and anion exchange has led to less complex vaccines yet still capable of inducing immunoprotection in mice. Mass spectrometric analysis of one of the protective subfractions identified only three proteins, one of which was Heat Shock Protein 10 (HSP10). Here we describe the expression, purification and immunoreactivity of recombinant HSP10.

The coding sequence for HSP10 was amplified from a cDNA library prepared from *C. posadasii* strain Silveira endosporeulating spherules. The PCR product was cloned and expressed in *E. coli* and then purified over a nickel sepharose column using the vector encoded 6x His tag. All amino acids associated with the vector tag were removed by Sumo Protease. Purity of the final product was ascertained by SDS-PAGE and its identity was checked by mass spectrometry. Immunoreactivity of human serum was measured using an indirect ELISA. Three pools of human serum were examined: complement fixation positive (CF+), precipitin positive (PPTN+), and negative. Antibodies were detected with an anti-human IgG or IgM antibody conjugated to HRP.

When the purified product was viewed by SDS-PAGE, a single band migrating at the predicted size of 11 kDa was seen. Mass spectrometry confirmed its identity to be HSP10. Reactivity of pooled human serum detected with the anti-IgG secondary antibody was much greater than that detected with the anti-IgM antibody. Additionally, the CF+ pool showed the highest reactivity. Thus, it appears that the antibody reacting with the recombinant is primarily of the IgG isotype.

The production of a recombinant protein capable of conferring protection against *Coccidioides* would benefit endemic regions greatly. Given its origin and recognition by positive human serum, the protective efficacy of recombinant HSP10 needs to be explored. Finally, an expansion of the current ELISA to include individual serum should be performed to determine if there is a correlation between CF titer and IgG reactivity.

***Coccidioides* Cupin Protein 21 Immunoreactivity: Native versus Recombinant**

*Suzanne M. Johnson¹, Erin L. Carlson¹, Lance A. Nesbit²,
Neil M. Ampel², Demosthenes Pappagianis¹*

¹University of California, Davis; ²University of Arizona, Tucson

We previously identified a 21 kDa protein containing a cupin domain, the *Coccidioides* cupin protein (CCP21), in a fraction of the coccidioidal vaccine T27K that stimulated *Coccidioides* immune human blood cells. Molecular methods were used to prepare a recombinant version of this protein that was used to hyperimmunize rabbits. The following study compares the immunoreactivity of the recombinant protein with that of the native coccidioidal derived protein.

The recombinant protein was expressed in *E. coli* and purified using immobilized nickel chromatography. Native coccidioidal protein was isolated from the T27K using an antibody column of anti-CCP21 polyclonal rabbit serum. Reactivity with serum pools prepared using specimens containing the coccidioidal precipitin (IgM) antibody, complement fixation (IgG) antibody, or without detectable antibodies were measured using the enzyme-linked immunosorbant assay. Wells were coated with 100 ng of protein and both anti-IgG and anti-IgM HRP secondary antibodies were used. The immunostimulation of human immune and non-immune cells was measured following incubation with the native or recombinant protein. Immunostimulation was demonstrated by the release and subsequent detection of IL-2.

The native CCP 21 showed reactivity with both precipitin positive and complement fixation positive human serum. This reactivity ranged between 3.1 to 6.3 times greater that of the negative pooled serum. The highest reactivity was measured with precipitin positive serum detected with the anti-IgM secondary antibody. The recombinant protein was less reactive and the pooled positive serum only slightly higher than the pooled negative serum. The native CCP21 also specifically induced release of IL-2 from immune cells which was approximately 20 times greater than that released from immune cells incubated with the recombinant.

The observed immunoreactivity of the recombinant CCP21 was much lower than that of the native. Previous experiments indicated that the native CCP21 is glycosylated. The expressed protein lacks this modification and its absence may explain the difference in immunoreactivity. To our knowledge this study is the first direct comparison of immunoreactivity between recombinant and native proteins of *Coccidioides*.

VT-1161 Reduces Fungal Burden and Improves Survival in Murine Respiratory Coccidioidomycosis

*Lisa F. Shubitz¹, Hien Trinh¹, Lourdes Lewis¹, John Galgiani¹,
Edward P. Garvey², William J. Hoekstra²,
William R. Moore², Robert J. Schotzinger²*

¹*Valley Fever Center for Excellence, The University of Arizona, Tucson, Arizona;*

²*Viamet Pharmaceuticals, Raleigh, North Carolina*

VT-1161 is a CYP51 inhibitor that has shown efficacy in murine models of candidiasis and cryptococcosis. In this study, VT-1161 was tested in a respiratory model of coccidioidomycosis in mice. In addition to its antifungal activity, VT-1161 has a very long half-life and can be detected in murine serum several days after dosing has been discontinued.

Methods: Swiss-Webster mice were infected with a lethal dose of 500 spores of *C. posadasii*, strain Silveira, intranasally and treatment was begun on day 5. Mice were orally gavaged once daily with 10 mg/kg or 50 mg/kg of VT1161 in 20% CremaphorEL (CrEL), 25 mg twice daily of fluconazole, or CrEL only. Mice were treated for 7 days and half the mice (n=8) were sacrificed 1 day after discontinuing treatment and the other half of the mice (n=8) were observed for 14 additional days and sacrificed. Mice were weighed and scored grossly for disease; lungs were weighed and quantitatively cultured; and spleens were cultured *in toto* to determine dissemination. Plasma levels of VT-1161 were determined at 1 and 14 days after last dose.

Results: By averages, all groups lost weight during the drug treatment period, possibly due to stress of the gavage procedure. Mice in the sham treatment group lost the most weight and appeared ill, with all 16 animals being euthanized for moribundity by day 14 post-infection. Mice treated with 10 mg/kg VT-1161 lost more weight than mice in either the 50 mg/kg VT-1161 group or the fluconazole treatment group. At one day post-treatment, mice treated with either sham or 10 mg/kg VT-1161 had significantly higher lung weights ($P<0.005$), higher gross disease scores, and more weight loss than mice treated with fluconazole or 50 mg/kg VT-1161. However, colony forming units in the 10 mg/kg group were not significantly higher than for fluconazole and the 50 mg/kg groups; the latter two groups looked similar and were statistically indistinguishable for all parameters. At two weeks, mice treated with 50 mg/kg VT1161 had grossly better lungs with near normal lung weights (0.3g) and small granulomas ≤ 2 mm in size compared to mice treated with fluconazole or 10 mg/kg VT-1161, which both had 50-100 percent abnormal lung tissue. Mean lung CFU for the 50 mg/kg VT-1161 group was significantly lower than fluconazole ($P=0.006$) or 10 mg/kg VT-1161 ($P=0.038$). At two weeks, mice treated with 50 mg/kg VT1161 had grossly better lungs with near normal lung weights (0.3g) and small granulomas ≤ 2 mm in size compared to mice treated with fluconazole or 10 mg/kg VT-1161, which both had 50-100 percent abnormal lung tissue.

(Continued)

VT-1161 Reduces Fungal Burden and Improves Survival in Murine Respiratory Coccidioidomycosis (Con't)

*Lisa F. Shubitz¹, Hien Trinh¹, Lourdes Lewis¹, John Galgiani¹,
Edward P. Garvey², William J. Hoekstra²,
William R. Moore², Robert J. Schotzinger²*

Mean lung CFU for the 50 mg/kg VT-1161 group was significantly lower than fluconazole ($P=0.006$) or 10 mg/kg VT-1161 ($P=0.038$). At two weeks, nearly all mice treated with 10 mg/kg VT1161 or fluconazole had fungal growth in the spleen, while only one mouse in the 50 mg/kg VT-1161 group had a single colony growing from the spleen. Subjectively, while the mice in the fluconazole and low dose VT1161 groups were failing and would be expected to die with the condition of the lungs, the 50 mg/kg VT-1161-treated mice were resolving the infection and thriving clinically.

Conclusions: VT-1161 at 50 mg/kg effectively prevented death and led to a reduction in fungal burden compared to fluconazole over a three week period of time that included 7 days of treatment and 14 days of additional observation. Fungal burden in the mice was significantly reduced ($P<0.001$) in that period of time and mice were clinically thriving with small lung lesions. VT-1161 at 10 mg/kg produced a response similar to fluconazole at 50 mg/kg, with progression of disease and similar clinical condition, lung weights, and disease scores after withdrawal of medication. VT-1161 merits further investigation with possible longer treatment times and optimization of doses to determine if fungal sterility can be achieved.

**Dendritic Cell-Based Vaccine Against
Coccidioides Induces Antibody Response**
Alexandra Conkleton, Caatherine King, Shanjana Awasthi

*Department of Pharmaceutical Sciences,
University of Oklahoma Health Sciences Center, Oklahoma City, OK*

Background: Valley Fever or coccidioidomycosis is an infection caused by soil-borne, highly-virulent fungus: *Coccidioides posadasii* or *immitis*. Infection with *Coccidioides* can be life-threatening in certain groups of individuals. An effective treatment is not available. A number of studies have shown that the cell-mediated immunity is protective. Thus, vaccine development against *Coccidioides* infection is of contemporary interest. In this study, we evaluated a primary dendritic cell (DC)-vaccine, developed in our lab, for its ability to induce *Coccidioides* -antigen-specific antibody response in an extremely-susceptible BALB/c mouse model.

Methods: The DC-vaccine was prepared by non-virally transfecting the primary bone marrow-derived DCs with *Coccidioides* -Ag2/PRA-cDNA (protective epitope). Mice were intranasally immunized with Dc-vaccine on days 0 and 7. Immunized mice were sacrificed on days 6, 29, and 45. At the time of necropsy, blood was collected from immunized mice. Serum samples were subjected to ELISA for detecting Ag2/PRA-specific IgG and its isotypes.

Results: We found that the total IgG antibody titers were significantly increased at all time points after DC-vaccination as compared to controls. Increased levels of IgG2a, IgG2b and IgG3 isotypes contributed to increased IgG titers. However, the IgG1 titer remained unaffected.

Conclusions: This is first evidence of DC-vaccine-induced antibody response against *Coccidioides*. The IgG2a, IgG2b and IgG3 isotypes are indicative of Th1 response. These findings strengthen our broad hypothesis that the primary DC-vaccine induces protective Th1 response against *Coccidioides* infection.

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Evaluation of *Coccidioides* Antigen Detection by Enzyme Immunoassay for Quantification of Fungal Burden in Murine Models of Coccidioidomycosis

L. J. Wheat, E. Kirsch, J. Capilla, K. V. Clemons,
D. A. Stevens, S. Sokamoto, J. Fierer

Background: Alternatives to quantitative culture for measurement of fungal burden and experimental coccidioidomycosis are desirable. Antigen detection has proven useful in other animal models of systemic fungal disease. *Coccidioides* antigen detection could be useful in research employing murine models of coccidioidomycosis.

Methods: Intravenous model. Five outbred CD-1 male mice were infected with *C. immitis*, Silveira strain, 40 arthroconidia/mouse. On day 13, mice were euthanized and organs were removed and homogenized. Homogenates were diluted for CFU determination, and after centrifugation for 5 minutes at 1,100 g., the supernatants were filtered through a 0.45µm syringe filters and filtrates were immediately stored at -80° C. Serums were pooled for antigen testing undiluted and homogenates were tested after 1:10 dilution.

Intrathecal model. Same as above except 25 arthroconidia were injected into the lumbar subarachnoid space, and mice were euthanized 9 days later.

Intranasal model. Fifteen C57BL/6 (sensitive) mice were infected with 75 arthroconidia of the RS strain of *C. immitis* inside a biosafety hood in a BSL laboratory. Mice were sacrificed 14 days later and their lungs and spleens were removed for quantitative mycology. Blood was obtained by cardiac puncture from some mice at time of sacrifice, allowed to clot and the serum was filter sterilized. Tissues were homogenized in 0.5 mL of sterile saline using TissueLyser and then serially diluted in saline. Appropriate dilutions were plated onto Sabouraud's agar and incubated at room temperature for 5 days. Colony counts were log transformed.

Intraperitoneal model. Four DBA/2 (resistant) and four C57BL/6 mice were infected with 1000 arthroconidia and lungs and spleens were removed 14 days later and processed as above. A 1:8 dilution of serum and 1:10 and 1:100 dilution of organ supernatants were tested for *Coccidioides* galactomannan antigen in the MiraVista Quantitative *Coccidioides* Antigen Enzyme Immunoassay.

Results: Results are summarized in the table. Antigen concentration correlated positively with quantitative culture: intravenous model (all-lung, spleen, kidney, liver), $r=0.754$; intranasal model-serum, $r=0.875$; spleen, $r=0.820$; lung, $r=0.848$.

(Continued)

Evaluation of *Coccidioides* Antigen Detection by Enzyme Immunoassay for Quantification of Fungal Burden in Murine Models of Coccidioidomycosis (Con't)

*L. J. Wheat, E. Kirsch, J. Capilla, K. V. Clemons,
D. A. Stevens, S. Sokamoto, J. Fierer*

	Intravenous, 40 Arthro- conidia	Intrathecal, 25 Arthro- conidia	Intranasal, 75 Arthro- conidia	Intraperitoneal, 1000 Arthroconidia	
Infected	CD-1 mice	–	C57BL/6	C57BL/6 (sensitive)	DBA 2J (resistant)
Lung	5/5 (100%) 6.8 ng*	5/5 (100%) 0.3 ng	13/13 (100%) 8.2 ng	4/4 (100%) 2.1 ng	4/4 (100%) 1.0 ng
Spleen**	5/5 (100%) 0.6 ng	5/5 (100%) 0.4 ng	13/15 (87%) 5.6 ng	4/4 (100%) 3.2 ng	3/4 (75%) 0.3 ng
Serum	Pool 8.2 ng	Not done	10/10 (100%) 1.2 ng	Not done	Not done
Uninfected					
Lung	0/5 (0%). 0 ng	Not done	Not done	0/6 (0%) 0 ng	–
Spleen	0/5 (0%) 0 ng	Not done	Not done	0/6 (0%) 0 ng	–
Serum	Pool 0 ng	Not done	Not done	0/6 (0%) 0 ng	–

*Positive/total (%) mean ng/ml

**Kidney rather than spleen was used for the intrathecal model

Summary: Antigen concentration in the blood and tissues correlates well with quantitative culture of organ homogenates in murine models of coccidioidomycosis.

Conclusion: Quantification of antigen offers an alternative to quantitative culture for assessment of fungal burden in murine models of coccidioidomycosis.

Comparison of Coccidioidal Serologic Results Obtained by Enzyme Immunoassay (EIA) and Immunodiffusion (ID)

Jessica Einstein, Wendy Tsang

*Demosthenes Pappagianis
University of California, Davis*

Serologic tests for coccidioidomycosis (CM) have been used for about 100 years; and for 70 years have had a history of practical utility and general reliability for diagnosis and management of CM. The tests currently used are complement fixation (CF) (generally useful for detection and quantitative determination of CF (IgG (G)) antibody), immunodiffusion (ID) useful for detection of "precipitin" (IgM(M)) and CF (IgG), EIA for detection of M and G, and latex particle agglutination for detection of M.

There have been a number of reports on the comparative merits of EIA and ID some indicating putative false positive results especially for IgM detected by EIA. In the present report, we present data on sera from 1645 patients whose sera were tested by EIA in other institutions and by ID in our laboratory. The figures represent the results of these comparisons.

Summary/Conclusions

90% of sera positive for M by EIA were negative for M by ID.

Of Indeterminate reactions for M by EIA 99% were found to be negative by ID.

Approximately 25% of sera Indeterminate for G were positive by G by ID.

56% of sera positive for G by EIA were positive for G by ID.

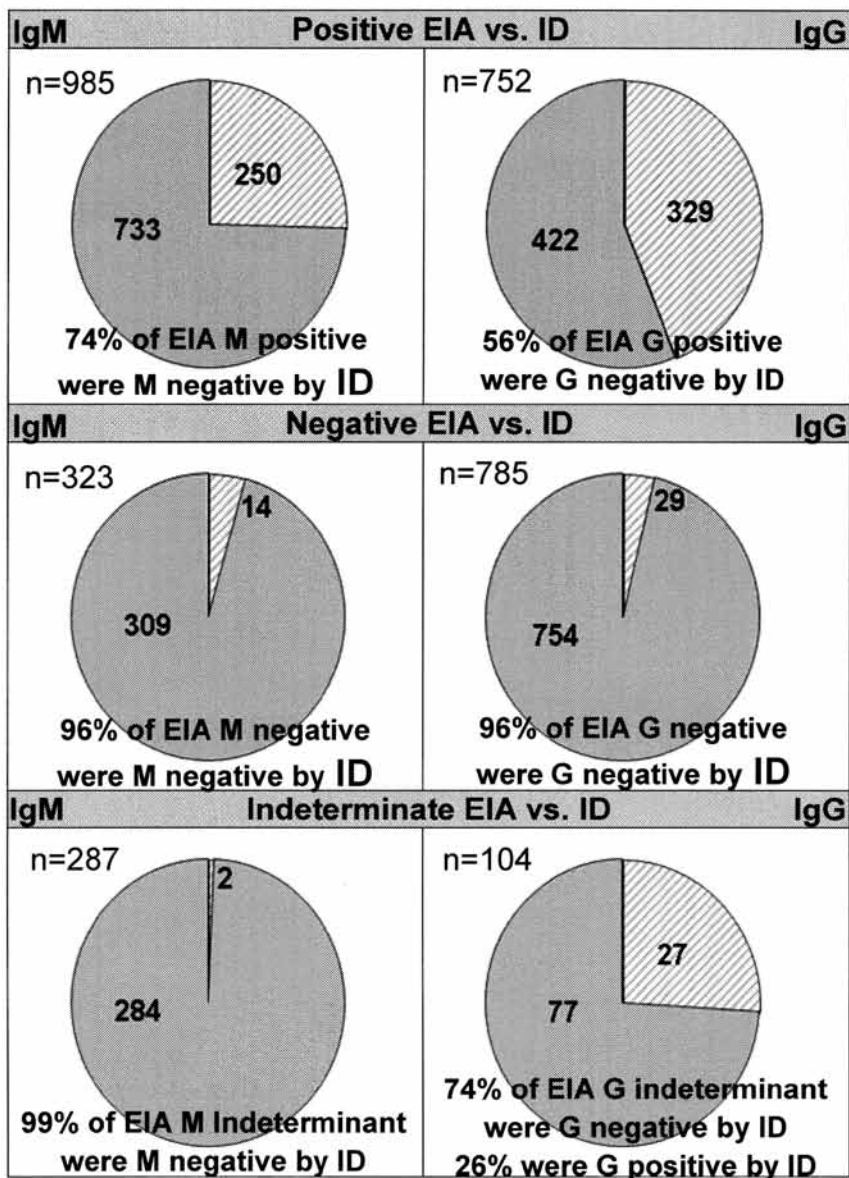
A few of the positive for G by EIA were positive patients from whom these sera were obtained later converted to ID positive.

Only a few who remained negative by ID have had cultural or histopathologic confirmation of CM but additional such verification is still needed.

(Continued)

**Comparison of Coccidioidal Serologic Results Obtained
by Enzyme Immunoassay (EIA) and Immunodiffusion (ID)**
(Con't)

Jessica Einstein, Wendy Tsang



A Phase II Study of the Safety and Stability of Coccidioidin in Sensitized Subjects Undergoing Repeated Testing

*Richard F. Hector, George Rutherford,
Demosthenes Pappagianis, Neil M. Ampel, M.D.*

*The University of California at San Francisco;
University of California, Davis;
Southern Arizona Veterans Affairs Health Care System (SAVAHCS);
University of Arizona*

Background: Delayed-type hypersensitivity (DTH) testing is an established method for determining coccidioidal cellular immunity, a protective response to infection. We have previously reported on the usefulness of an archived lot of mycelial-based coccidioidin for DTH testing. We now review its safety, stability and potency when repeatedly injected intradermally among a healthy, coccidioidal immune cohort over time.

Methods: In a combination of six studies, healthy subjects between 18 and 59 years received 0.1 mL of 1:55.8 diluted coccidioidin in phenol or vehicle into the volar surface of the forearm. Induration was assessed from 2-72 hours after placement. A finding of ≥ 5 mm of induration in any axis at the site of the skin test at any time was considered positive (Ampel et al, Mycopathologia 2006; 161:67). A sum of induration was determined by measuring induration in two-axes and adding the result.

Results: A total of 169 subjects entered into six studies were found to be coccidioidin positive. The proportion of positive DTH reactions was greater at the 48 hr interval compared to the 24 hour intervals ($p < .001$). Nine subjects had repeated testing with coccidioidin. When the results of these nine subjects were grouped across all six studies and analyzed by Kruskal-Wallis, there were no significant differences in the sum of induration in response to coccidioidin over time ($P = 0.71$). The coccidioidin dilution was manufactured in 2004 and results for stability submitted for release testing in November 2009 demonstrated no significant changes.

Conclusions: Coccidioidin was well tolerated at the dilution administered to normal sensitized subjects over all study periods. Reading induration at 48 hours led to more positive results than at 24 hours. Coccidioidin was found to maintain its potency over a 3.5 year period of testing and repeat testing did not lead to any increase in induration over time.

Measuring Coccidioidal Cellular Immunity by a Simple Blood Test in a Coccidioidomycosis Clinic

*Lance A. Nesbit, Suzette Chavez, Suzanne M. Johnson,
Demosthenes Pappagianis, John N. Galgiani, Neil M. Ampel*

*Southern Arizona Veterans Affairs Medical Center (SAVAHCS);
Valley Fever Center for Excellence, University of Arizona;
Department of Microbiology/Immunology,
the University of California at Davis*

Background: Measurement of cellular immunity in coccidioidomycosis could have important implications regarding whether antifungal treatment is warranted and when to stop such therapy. However, there is no currently available way to measure this in the United States. We applied an already established in vitro whole blood assay (Ampel et al. Clin Diagn Microbiol 2002, 9:1039) to patients visiting a coccidioidomycosis clinic located in the endemic area. This assay relies on the increased expression of CD69 on CD3⁺ T-lymphocytes after antigen stimulation.

Methods: Patients attending the coccidioidomycosis clinic at SAVAHCS were asked to participate in the study, which was approved by the Research Office of SAVAHCS and the IRB of the University of Arizona. Patients donated 5 mL of blood into a lithium heparin tube. Aliquots of 0.5 mL were placed into 10 mL conical polypropylene tubes and were incubated in 5% CO₂ at 37° C alone or with 20 µg/mL T27K, ruptured spherule supernatant (SS), or the superantigen SEB. At the end of 24 hours, red blood cells were lysed, the remaining cells were incubated with labeled CD3 and CD69 antibodies and 50,000 events assessed by flow cytometry after gating on lymphocytes. The mean fluorescent intensity (MFI) and percent (%) above threshold for CD69 was determined. An MFI >150 above control and >1% above control to T27K or SS was considered indicative of an expression of coccidioidal cellular immunity.

Results: To date, six patients have been studied; three demonstrated a cellular immune response to T27K and four to SS. Two had primary pulmonary pneumonia (one receiving methotrexate) and one had a pulmonary cavity. The two patients who did not respond included a patient with primary pulmonary disease who also had HIV infection and a patient with multisite dissemination. The latter also did not respond to SEB.

Conclusions: These data on a small cohort indicate that an in vitro assay using two different coccidioidal antigen preparations readily determines the presence of coccidioidal cellular immunity. Additional subjects will be tested and repeat testing will further determine the utility of this assay.

Suggested Algorithms for Therapy and Follow Up of Coccidioidomycosis in Patients on Biologic Response Modifiers or Disease-Modifying Antirheumatic Drugs

*Susan Knowles MD¹, Sara Taroumian MD¹, Jeffrey Lisse MD¹,
Neil M. Ampel MD², Eric Gall MD³, Berchman Vaz MD, PhD⁴,
John N. Galgiani MD⁵, Susan E. Hoover MD, PhD¹*

¹University of Arizona College of Medicine;

²Southern Arizona VA Health Care System;

³Arizona Arthritis Center;

⁴Catalina Pointe Rheumatology;

⁵Valley Fever Center for Excellence, Tucson, Arizona;

contact: shoover@deptofmed.arizona.edu

Background: Coccidioidomycosis can be a challenging problem in patients who are taking biologic response modifiers (BRMs) or disease-modifying antirheumatic drugs (DMARDs) for treatment of arthritis. Few studies have addressed the approach to management of these patients after the infection is diagnosed and treated.

Methods: We conducted a retrospective study of patients diagnosed with coccidioidomycosis while receiving BRMs and/or DMARDs at a University- or Veterans' Administration-based rheumatology clinic between 2007 and 2009. Chart review focused on management of BRM, DMARD, and antifungal therapy.

Results: We identified 44 patients with coccidioidomycosis. Rheumatologic treatment included BRM alone (11), oral DMARD alone (8), or combination therapy (25). Coccidioidomycosis manifestations included asymptomatic positive serologies (6), pulmonary coccidioidomycosis (29), and disseminated disease (9). After the diagnosis of coccidioidomycosis, ten patients had no change in immunosuppressive therapy, 26 patients had all BRM and DMARDs stopped, and 8 patients had BRM stopped but DMARD therapy continued. All but 3 patients had antifungal therapy initiated for 3 months or longer. Follow-up data were available for 38 patients. BRM and/or DMARD therapy was continued or resumed in 33 and none have had subsequent dissemination or complications from coccidioidomycosis. Sixteen also continued on concurrent antifungal therapy. Four patients were not retreated with BRM/DMARD due to remission of their rheumatic disease. Follow-up for patients who stopped antifungals or were never treated was 3 to 96 months (median 30 months).

Conclusions: Resuming treatment of rheumatic disease with a BRM and/or DMARD after coccidioidomycosis appears to be safe in some patients based on our series. Lifelong antifungal therapy may not be necessary in all patients. We propose an algorithm to guide management of these patients both at the time of diagnosis of coccidioidomycosis, and subsequently.

Coccidioidomycosis Disseminated to the Spine: Case Reports and Review of Literature

Herbert Boro, MD

Kaiser Permanente, Fresno California

Case I: 1990

This is a 27 y.o. A.A. male who was referred immediately postoperatively March 8, 1990 by his neurosurgeon. The patient underwent decompressive laminectomies (without instrumentation) at T1-3 March 7 and repeated April 13, 1990 for severe cord compression and left leg weakness. He became paraplegic but fully recovered with 4.5 grams of I.V. amphotericin B deoxycholate (ABC) therapy. Fluconazole was applied for several more years.

Case II: 2010

This is a 29 y.o. A.A. male who was referred for coccidioidomycosis of the RLL with multiple skeletal sites of dissemination. He had been placed on fluconazole 600 mg., then 1000 mg. daily without improvement. In March, 2010 a soft tissue lateral view of the neck showed retropharyngeal thickening with bony destruction of C6; the MR scan showed an inflammatory process from C5-T3 with dimensions of 10 by 4.7 by 3.2 cm. The C5 body showed lateral destruction, and the bodies of C6-7 showed diffuse enhancement. The patient received ABC 3 grams perioperatively, for April 22 anterior C6-7 corpectomies with instrumented fusion followed by April 27, 2010 posterior instrumented fusion of C5-T2. Itraconazole was applied indefinitely.

Literature Review

The indications for neurosurgical intervention are similar for coccidioidomycotic, tubercular and bacterial pathogenesis of vertebral osteomyelitis. **Debridement** is indicated for destruction of bone and adjacent soft tissue when the degree of disease is large and/or medical management fails to control the process. **Corpectomy with instrumentation** is indicated when there are neurologic signs, severe kyphotic deformation and instability of the vertebrae. **Medical management** for coccidioidomycosis is usually undertaken with I.V. amphotericin B perioperative followed by azole therapy. The duration of azole therapy depends on the patient's immune status, other sites of dissemination, serial complement fixation titers and interval examinations. Many physicians would continue azole therapy lifelong when instrumentation has been placed at the site of infected bone.

The Prevalence of Immunity to *Coccidioides* among Healthy Arizona Residents without a History of Symptomatic Coccidioidomycosis

Lake D¹, Ruiz Y², Duffy S², Pitta T²,
Clarkson M², Vaughn T², Blair JE²

¹Arizona State University;

²Mayo Clinic Hospital, Phoenix, AZ

Background: Coccidioidomycosis is asymptomatic in approximately 60% of persons who acquire this fungal infection from endemic areas. Prior coccidioidal infection can be assessed *in vivo* by delayed type hypersensitivity to *Coccidioides* antigens (skin testing), or *in vitro* by assessing the lymphocyte activation response to *Coccidioides*. The presence of such immunity is indicative of immunity from future reinfection.

Methods: As part of a study of the incidence of coccidioidomycosis among healthy Mayo Clinic in Arizona employees, we sought to exclude the population who had previously (but unbeknownst to them) contracted coccidioidal infection. Immunocompetent persons without a history of clinical coccidioidomycosis, a positive skin test or positive serology were recruited. A questionnaire was administered and 10 milliliters blood drawn. 0.5 cc whole blood was incubated with formalin-fixed Coccidioidin (CDN-F) for 24 hours at 37°C. After the incubation, red cells were lysed and the remaining lymphocytes were stained with anti-CD3 and anti-CD69 antibodies. Flow cytometry was employed to assess activated CD69-positive lymphocytes from the CD3-positive population.

Results: 329 subjects were recruited and signed informed consent. 183 subjects were employed at the Phoenix campus, the site of the construction, and 146 control subjects were employed at the Scottsdale campus. 85% of subjects were female (correlating well with the ratio of female employees in general), mean age 47 years (18-76). 96% were indoor employees, and 72% were regularly active outdoors. 22 of 184 (12%) Phoenix campus employees and 19 of 145 (13%) Scottsdale campus employees were positive, indicating prior infection.

Conclusions: The presence of immunity to coccidioidomycosis was identified a substantial minority of healthy subjects who resided in the endemic area without any known history of coccidioidomycosis.

Evaluation of Vitamin D Levels in Patients with Coccidioidomycosis, a Case Control Study

Arash Heidari, M.D.¹, John Dickey, M.D.², Nooshin Jahangiri, M.D.³,
Bahareh Ghafarizadeh, M.D.², Greti Peterson, M.D.²

¹Infectious Diseases, Kern Medical Center/UCLA, Bakersfield, CA;

²Internal Medicine, Kern Medical Center/UCLA, Bakersfield, CA;

³Family Practice, Kern Medical Center/ UCI, Bakersfield, CA

Background: Replacement of inadequate serum Vitamin D 25OH total (VitD) levels have been recently the focus of infectious diseases providers. There is a body of evidence that shows an association between low VitD levels and increase in incidence of several infectious diseases such as tuberculosis. However, to our knowledge there has been no study that evaluated the VitD levels in patients with Coccidioidomycosis.

Methods: Retrospective, matched case-control study in population ≥ 18 years old from 1990 to 2010 at Kern Medical Center with measured VitD 25OH total levels in ng/ml. VitD deficiency defined as levels < 20 ng/ml and levels between 20 and 30 defined as VitD insufficient. Total of 118 cases selected who had positive diagnosis of Coccidioidomycosis by serology (97%), pathology, or cultures (31%). From these cases 35% (42) had pulmonary and 65% had at least one from of dissemination. Mean age of cases was 42.8 with majority being male (63%). Ethnicity was 69% Hispanics followed by 17% African American and 14% Caucasians. Controls were selected from the general patient population without any history or evidence of Coccidioidomycosis infection. Cases and controls were randomly matched (ratio: 1 to 4) by age, race and sex and evaluated for demographics and co-morbidities. Analysis was performed by SAS JMP.

Results: Numerical analysis indicated that mean VitD level was statistically lower in controls (22.5 ng/ml) compare to cases (25.9 ng/ml); $P=0.0016$. The prevalence of VitD insufficiency ($20 \leq \text{VitD} < 30$) was not statistically different between cases and controls; $\text{OR} = 1.5 \{ \text{CI}_{95} (0.9, 2.5) \}$ $P=0.05$. However, it was statistically higher in controls for VitD deficiency ($\text{VitD} < 20$); $\text{OR} = 1.8 \{ \text{CI}_{95} (1.1, 3.1) \}$ $P=0.009$. Logistical regression analysis showed diabetes (DM) and chronic kidney disease (CKD) as cofounders. Prevalence of DM $\text{OR} = 1.7 \{ \text{CI}_{95} (1.1, 2.7) \}$ $P=0.004$ and CKD $\text{OR} = 3.7 \{ \text{CI}_{95} (1.8, 7.7) \}$ $P=0.0001$ were statistically higher in controls compare to cases. After exclusion of DM and CKD from cases and controls no statistical difference between cases and controls for prevalence of VitD deficiency and insufficiency combined were found. $\text{OR} = 0.95 \{ \text{CI}_{95} (0.6, 1.5) \}$ $P=0.4$.

Conclusion: There is no association between low VitD levels and Coccidioidomycosis.

Clinical and Immunological Aspects of Bronchoalveolar Lavage Fluid in Acute Pulmonary Coccidioidomycosis

*Lance A. Nesbit, Kenneth S. Knox, Suzette Chavez,
Suzanne M. Johnson, Demosthenes Pappagianis, Neil M. Ampel*

*Southern Arizona Veterans Affairs Medical Center (SAVAHCS);
Pulmonary Division of the University of Arizona;
Department of Microbiology/Immunology,
the University of California at Davis*

Background: The peripheral blood cellular immune events during coccidioidomycosis have been previously described with the finding of polyfunctional T-lymphocytes responding to coccidioidal antigen. However, the local response to coccidioidal infection in the lungs among humans has not been previously examined. We have utilized methods initially developed for peripheral blood mononuclear cells to examine the cellular immune response in the bronchoalveolar lavage (BAL) compartment.

Methods: Subjects undergoing BAL for pulmonary abnormalities were eligible. The study was approved by the Research and Development Committee of SAVAHCS and the IRB of the University of Arizona. The BAL procedure instilled 300 mL of saline into the bronchus of the infiltrated lobe. The fluid was recovered by aspiration and the excess, usually 30-50 mL, was used for study. Cells were pelleted and assessed by cytology as well as incubated for 48 hr in serum-free media containing penicillin, gentamicin and amphotericin B with the coccidioidal antigen preparation T27K at 20 µg/mL. Intracellular cytokine production and cytokine release were performed as previously described (Nesbit et al, Infect Immun 2010; 78:309).

Results: Five subjects had an unequivocal diagnosis of acute pulmonary coccidioidomycosis. Of these, 3 had positive cultures for *Coccidioides* from the BAL fluid and the same 3 had >25% eosinophils in the lavage fluid. Assay for coccidioidal galactomannan was positive in only 1 of 4 tested. After incubation of BAL fluid cells with T27K, 3 of 4 had >10 polyfunctional CD4 cells and 4 of 5 had >150 pg/mL IL-2 and >10 pg/mL of IL-17. None of 4 subjects with pulmonary infiltrates due to other etiologies had elevated polyfunctional CD4 cells or elevated IL-2 or IL-17 in BAL cell culture.

Conclusions: BAL fungal culture and assessment for eosinophilia appear to be useful clinical methods for diagnosing coccidioidomycosis. Immunological assessment suggests that the presence of polyfunctional T-lymphocytes in addition to release of IL-2 and IL-17 in response to coccidioidal antigen may be helpful in establishing the diagnosis.

Pregnant with *Coccidioides*

Amber Noon, MD

Southern Arizona Infectious Disease Specialists

A 22-year-old female who is 19 weeks pregnant presents with headache, fevers, and photophobia for 10 days. She presented to her OB 7 days earlier with headache and earache and was given a prescription of amoxicillin. On presentation she was febrile to 100.9°F, hypotensive and tachycardic. On examination she was alert and oriented, with mild nuchal rigidity and noted photophobia. Her CSF revealed 325 WBC, 76% neutrophils, 0 RBC, protein of 54 and glucose of 30. She was started on broad spectrum antibiotics for presumed partially treated bacterial meningitis.

Her coccidioidal complement fixation from CSF was negative as was her fungal culture. She initially improved, but her headache and photophobia persisted. An MRI was performed and was normal. Her fever curve trended back up and subsequently developed focal neurological symptoms and suffered from a seizure. A repeat lumbar puncture was performed and revealed 229 WBC, 90% PMN, 225 RBC, protein 162, and glucose <20. Her serum coccidioidal immunodiffusion returned positive for IgG, titer by complement fixation was 1:64. Her coccidioidal complement fixation from repeat CSF was 1:8 and her cultures grew *Coccidioides immitis*. She was started on Fluconazole 800 mg intravenous daily and Liposomal amphotericin B at 5 mg/kg intravenous daily. A repeat MRI found scattered diffuse small acute infarcts consistent with arteritis and enhancement of the leptomeninges of the sylvian fissure, no hydrocephalus noted. Over the next week she developed cranial neuropathies and repeat MRI found evolving infarcts. Prednisone was added to her regimen for the arteritis and she has since improved.

This case illustrates many aspects of coccidioidal infections that are of interest and are still debated. First, this was an unusual presentation given the relatively acute onset of her symptoms and her initial cerebrospinal fluid analysis. Second, she was pregnant, in the second trimester, at the time of presentation, presenting treatment complications. Third, she was treated with high dose fluconazole and liposomal amphotericin B. Liposomal amphotericin B has been thought to have higher penetration into the CNS in animal studies. Lastly, she developed arteritis and was treated with steroids with clinical improvement. I believe this case illustrates many different concepts in management of complicated coccidioidal infections.

Cutaneous Coccidioidomycosis in Four Mexican Patients

*M Arce-Ramírez¹, E Bazán-Mora², MR Reyes-Montes²,
E Duarte-Escalante², LR Castañón-Olivares²*

¹Clínica Derma-Care, Tijuana, Baja California;

²Departamento de Microbiología, Facultad de Medicina, UNAM

The primary infection with *Coccidioides spp* involves the lungs and dissemination occurring in less than 1% of cases. Extra pulmonary disease usually affects the skin, central nervous system, bones and joints.

We present four cases of cutaneous coccidioidomycosis in patients attending a dermatology clinic in Tijuana, Baja California, Mexico in the period between 2009 and 2011.

All patients were males, aged between 23 and 58 years.

Lesions were located in the face, neck and trunk, and knees, and were characterized by scaly plaques, nodules, abscesses and fistule; only three cases had a history of previous lung infection.

The diagnosis of coccidioidomycosis was established by direct examination and culture of skin secretions and crusts. No serological tests were done and skin test with coccidioidin was applied only in three cases, with two positive and one negative results.

The cases were treated with amphotericin B and itraconazole orally, with relapses.

Currently we have isolated the DNA of *Coccidioides* cultures (primary and secondary isolates), to know their identification to species level with: 1) PCR as described by Umeyama et al. (2006) and 2) PCR designed by Bialek et al. (2004), sequencing a fragment of Ag2/PRA gene.

The presentation of these cases shows that the clinical diagnosis can be confused with other chronic infectious disease which can delay treatment and cause failed. This paper highlights the importance of clinical knowledge of this mycosis, to suspect and diagnose and thus have the actual incidence of this infection in our territory.

Disseminated Coccidioidomycosis Mimicking Cancer of Female Genital Tract

*Ying Wu, MD, Matthew Skinner, MD,
Nedall Samad, MD, Tim Kuberski, MD*

Maricopa Medical Center, Phoenix, Arizona

Abstract

The female genital tract is rarely involved by coccidioidomycosis. We describe a woman with disseminated coccidioidomycosis involving the female pelvic organs. She presented with menorrhagia, anemia, thrombocytopenia, uterine masses, hydrosalpinx, hydronephrosis, and elevated tumor markers CA19-9 and CA 125. She had no fever, chills or night sweats. She had no history of pneumonia and her chest X-ray was unremarkable. The clinical suspicion was that of a malignancy of the female genital tract because of the elevated tumor markers. At exploratory laparotomy a total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed because of the suspicion of a malignancy. However, subsequent pathology demonstrated coccidioidomycosis involving the round ligament, uterus, fallopian tubes and both ovaries. The abnormal CA 19-9 and CA-125 returned to normal after surgical resection.

Dissemination of *Coccidioides* to the female pelvic organs is rare. We identified 17 reported cases since the first report in 1929. Pelvic coccidioidomycosis can present with the clinical, radiographic, and laboratory features of a female genital tract malignancy. Increased CA-125 was reported in two of these cases; no previous case of pelvic coccidioidomycosis reported an elevated CA 19-9. As in our case, a diagnosis of coccidioidomycosis was made only after microscopy of the surgical specimen was performed, it was not suspected preoperatively. Treatment generally involves surgical excision followed by antifungal therapy. Female genital tract coccidioidomycosis is unusual, but in endemic areas it should be considered in the differential diagnosis in patients with a possible pelvic malignancy with elevated tumor markers. Infectious disease consultation is recommended for the management of female genital tract coccidioidomycosis.

The Dispersal of *Coccidioides* spp. - Microns to Kilometers

Frederick S. Fisher¹, Suzanne M. Johnson²,
Mark W. Bultman³, Demosthenes Pappagianis⁴

¹University of Arizona, Geosciences;

²University of California, Davis, School of Medicine;

³U.S. Geological Survey, Geologic Division, Tucson, Arizona;

⁴University of California, Davis, School of Medicine

Understanding the methods and agents of *Coccidioides* spp. dispersal both within and outside of its recognized endemic areas is fundamental to comprehending the spread of coccidioidomycosis throughout the southwestern United States. Physical and chemical weathering followed by wind and/or water erosion, and to a lesser extent mass wasting, are geomorphic processes that, acting alone, or in concert, cause the entrainment of soil borne *Coccidioides* arthroconidia in the atmosphere and in flowing water. Weathering is the wearing away or breakdown of rock material by chemical and/or physical means. The products of weathering are regolith (loose rock debris that cannot support rooted vegetation), soils (rock debris and clays that are capable of supporting vegetation), and ion bearing waters that migrate both upward and downward through the soil profile. It is in this surficial material that *Coccidioides* spp. are found. *Coccidioides* arthroconidia that are present in the regolith or soil are considered sediment and when being transported they will behave similar to other low density, silt-sized materials that are deposited when the transporting medium (wind or water) can no longer hold them in suspension. Effective dispersal is when viable arthroconidia being transported by wind; either enter the lungs of a host causing infection and the initiation of the parasitic life cycle of the organism; or when arthroconidia are incorporated into surficial material having the appropriate environmental conditions whereby they can initiate the saprophytic phase and grow as mycelia. Both the parasitic and the atmospheric environments are inhospitable habitats for the organism. The parasitic phase ends either with the death of spherules and endospores via the host's immune system, or with the death of the host. Dispersal may end at this point; however if the endospores find their way into the soil they may convert back to the mycelia form and grow until the soil is disturbed and arthroconidia are once again entrained by wind or water. Arthroconidia in the atmosphere and those deposited on the land surface are exposed to harsh conditions that may compromise their viability; such as high temperatures (>50° C), UV radiation, desiccation, saturated soils, clay rich soils lacking textural pore space (required for growth), and competition with existing vegetation and other microorganisms. *Coccidioides* dispersal is mainly "passive" (i.e. dependant for movement on agents other than themselves) such as wind, water, burrowing organisms (both animals and insects), and movement of infected hosts during parasitic phases of the *Coccidioides* life cycle. "Dependant" dispersal by growth of hyphae and mycelia is not a major factor in the distribution of *Coccidioides* in the natural environment. Because of these vastly different means of movement, the dispersal range of *Coccidioides* is as small as a few microns and as great as hundreds of kilometers.

Development of a Coccidioidomycosis Case History Form in Kern County, 2011

*Emery K, Mac Lean M, Lancaster M,
Joy S, Habib, M, McMasters P*

In 2011, the Kern County Public Health Services Department in conjunction with Kings, Tulare, Fresno, San Luis Obispo, and San Joaquin Counties, developed and field tested a coccidioidomycosis case history form. The new form was designed to collect patient demographic, clinical, underlying medical conditions, extrapulmonary spread, and risk factor information for acquisition of disease.

During November and December 2011, twelve health care providers completed 33 new case history forms selected randomly from the 407 coccidioidomycosis positive test results reported in October 2011. The profession of health care providers completing the form included physicians/nurses, hospital infection control practitioners, medical assistants, epidemiologists, and laboratorians. Depending upon the level of access to medical record information, the form completion time ranged from 2 to 53 minutes (median of 10 minutes).

This poster will present the feasibility of implementing this case history form in an endemic county, as well as, any changes that will be necessary in order to minimize the burden on health care providers and still collect meaningful information.

Coccidioidomycosis Cases Continue to Rise in Kern County During 2011

Emery K, Lancaster M, Oubsuntia V, Jonah C, McMasters P

In 2010, Kern County reported 2,051 cases of coccidioidomycosis for an annual incidence rate of 244.3 per 100,000 residents. This increase clearly signaled a change in the number of cases reported in Kern County since the 1991-1994 epidemic and the findings from 2010 were presented at the 2011 Coccidioidomycosis Study Group Meeting.

In 2011, the number of cases continued to increase and Kern County reported 2,734 cases for an annual incidence rate of 322.2 per 100,000 residents. The increase in 2011 represents a 32% increase in the number of cases and a 31% increase in the rate of disease per 100,000. This poster presents the epidemiology and the geographic distribution of the 2011 reported cases in Kern County.

The Sensitivity of Diagnostic Testing for Acute Coccidioidomycosis in Solid Organ Transplant Recipients

Neil Mendoza, MD¹, Janis Blair, MD²

¹Resident in Internal Medicine, Mayo School of Graduate Medical Education and ²Consultant in Infectious Diseases, Mayo Clinic, Scottsdale, AZ

Background: The diagnosis of coccidioidomycosis is challenging even when strongly suspected. Such a diagnosis may be more difficult in immunosuppressed patients such as transplant recipients due to the effect of antirejection treatments on a patient's ability to mount a serological response. We sought to characterize the utility of diagnostic tests for the diagnosis of acute coccidioidomycosis in solid organ transplant patients.

Methods: We conducted a search of all patients with reported coccidioidomycosis and cross referenced that list with the recipients of a kidney, liver, pancreas or heart transplants. Clinical records of the transplant recipients with acute coccidioidomycosis were subsequently examined, and the results of all coccidioidal diagnostic tests were tabulated, including: cultures (blood, respiratory secretions, cerebrospinal fluid, urine, other), histopathology and cytology (tissue or fluids), serology results [enzyme immunoassay (EIA), complement fixation (CF) and immunodiffusion (ID)], PCR, urinary antigen, and radiology.

Results: From January 1999 through August 2011, 2,246 organ transplant recipients had medical care provided at Mayo Clinic in Arizona. Of these, 239 had one or more positive coccidioidal diagnostic tests, 29 of whom had acute coccidioidomycosis. By EIA, IgG was positive in 13/25 (52%) patients and IgM was positive in 6/25 (24%). A second EIA increased the number of patients with a positive IgG to 15/25 (60%) and IgM to 7/25 (28%). By ID, IgG was positive in 9/23 (39%) patients and IgM was positive in 4/23 (17%). A second ID assay increased the number with positive IgM to 6/23 (26%), but the number of patients with positive IgG was unchanged. By CF, a titer $\geq 1:2$ was identified in 7/25 (28%) patients and increased to 10/25 (40%) with a second test. Peripheral eosinophilia was present in 5/23 (22%). Abnormalities were identified by chest radiograph and chest CT in 16/23 (70%) and 21/23 (91%) respectively. Diagnostic yields of other tests were as follows: culture [any respiratory specimen 9/15 (60%), tissue (2/6), pleural fluid (1/2)], biopsy [4/6 (66%)], cytology [1/6 (17%)], PCR [3/4 (75%)], urinary antigen [0/2].

Conclusion: Diagnostic tests to identify acute coccidioidomycosis in a transplant recipient appear to be insensitive. Serology, though commonly performed, was not sensitive, and improved minimally with repeated studies. Transplant recipients with suggestive symptoms may require a battery of diagnostic tests to secure a diagnosis.

Detection of Antigen in the Cerebrospinal Fluid in Patients with *Coccidioides* Meningitis

*David Bamberger, Laurie Proia, Luis Ostrosky-Zeichner, Madiha Ashraf,
Eileen P. Scully, Francisco M. Marty, L. Joseph Wheat*

*University of Missouri, Kansas City, MO;
Rush University Medical Center, Chicago, IL;
University of Texas Houston, Houston, TX;
Brigham and Women's Hospital, Boston, MA;
MiraVista Diagnostics, Indianapolis, IN*

Antigen detection complements antibody detection and cytopathology for diagnosis of coccidioidomycosis. In the setting of moderate to severe coccidioidomycosis, antigen was detected in the urine of 71% of patients (Durkin et al Clin Infect Dis 2008), and in milder cases antigen was detected in the serum of 73% of patients (Durkin et al Clin Vaccine Immunol 2009). Cross reactivity was noted in 11% of patients with other endemic mycoses, and specificity was 99% in cases with no other fungal infection. *Coccidioides* meningitis occurs in <5% of patients with coccidioidomycosis (Crum et al Medicine 2004) and the diagnosis may be difficult. Wet mount demonstration of spherules in the CSF is possible in <10% of cases and cultures are positive in less than one-third. The diagnosis usually is made by detection of anti-*Coccidioides* antibodies in the CSF, but this serologic test may be negative in 30-40% of cases (Mathisen et al. Medicine 2010). In this series of six patients with *Coccidioides* meningitis, antigen was detectable in the CSF, at the following concentrations: 0.2, 2.6, 4.6, 4.6, >8.2, and >8.2 ng/ml. Results of antigen concentration with other CSF diagnostic studies will be compared. Similar to other etiologies of fungal meningitis, antigen testing may be useful for the diagnosis of *Coccidioides* meningitis. Additional studies are needed to determine the diagnostic performance characteristic of *Coccidioides* antigen detection in the CSF for *Coccidioides* meningitis.

***Coccidioides spp* Search, in Soil and Air of the Comarca Lagunera Region, México**

**Laura Rosio Castañón-Olivares¹, Mauricio Galeana Pizaña²,
Guillermo Martínez Verduzco², Rocío González-Martínez³,
Jorge Talamantes⁴, Elva Bazán-Mora¹,
Edith Sánchez-Paredes¹, Irma Rosas-Pérez¹**

¹*Universidad Nacional Autónoma de México;*

²*Centro de Investigación de Geografía y Geomática*

“Ing. Jorge L. Tamayo”, CONACyT;

³*Universidad Autónoma de Coahuila;*

⁴*Department of Physics and Engineering,*

California State University Bakersfield. email: lrcastao@unam.mx

Background: Coccidioidomycosis is caused by two morphologically identical fungal species: *Coccidioides immitis* and *C. posadasii*. The work we present here has the following two parts to try to identify key environmental factors that define the habitat of *Coccidioides spp*:

- 1) We use Geographic Information Systems (GIS) to define the area of development and dispersal of *Coccidioides spp*,
- 2) We then try to corroborate the presence of *Coccidioides spp* in the places predicted.

Material and Methods: We chose the region called Comarca Lagunera, located in the north of México. We designed a predictive map of potential areas of development of *Coccidioides* using topological information resulting from the analysis of separate storage of different thematic layers (GIS). Twenty-seven areas were chosen randomly for sampling. From each site, we obtained 1 kg soil samples at 30 cm depth to develop an edaphological analysis. In addition, air samples were taken with a Burkard-two phases. All samples were grown in media for the isolation of fungi and a small sample of each substrate was reserved to perform DNA extraction.

Results: A cartographic model was obtained with an area of 53,000 Ha, which showed optimum environmental conditions for the recovery of *Coccidioides spp*. *Coccidioides spp* was not isolated from soil cultures. Instead, mainly fungi such as Mucorals and the genera *Candida*, *Penicillium* and *Malbranchea*. were isolated. The edaphological and molecular analysis are still in process.

Conclusion: At this moment, the results indicate that environmental characteristics reported as essential for the development of *Coccidioides spp* as saprobe, are insufficient to accurately identify the habitat of this organism.

Detection of a Coccidioidal Peptide in Plasma from Patients with Active Coccidioidomycosis

Stacy Duffy¹, Janis E. Blair², Kwasi Antwi¹, Suzanne Johnson³,
Marc Orbach⁴, Ale Mandel⁴, Tess Pitta², Douglas F. Lake¹

¹Arizona State University;

²Mayo Clinic in Arizona;

³University of California, Davis;

⁴University of Arizona

Abstract

Coccidioidomycosis, also known as Valley Fever, is a disease caused by the soil-dwelling fungus, *Coccidioides* sp. Coccidioidomycosis is difficult to diagnose because symptoms are similar to community acquired pneumonia. Current diagnostic tests rely on an antibody response to the fungus, but immune responses can be delayed causing false negatives. Detection of coccidioidal proteins or other components in blood would distinguish Valley Fever from other pulmonary infections and provide a definitive diagnosis.

Using mass spectrometry (LC-MS/MS) we examined a low molecular weight fraction of the plasma peptidome from patients with serologically confirmed Coccidioidomycosis. Mass spectra were searched using protein databases from *Coccidioides posadasii* str. *Silveira*. Spectra common among patients predicted a peptide, "PGLDSKSLACTFSQV" (PGLD). The peptide is derived from an open reading frame from a "conserved hypothetical protein" annotated with 2 exons from the *C. posadasii* genomic sequence. Spectra corresponding to the peptide were not detected in donors who had not been clinically diagnosed with Valley Fever. Annotation of the PGLD open reading frame (ORF) by the Broad Institute indicates that an intron exists between two exons in the ORF.

However, our sequence analysis of cDNA derived from pure polyadenylated mRNA demonstrates an unspliced transcript containing the PGLD nucleotide sequence. A monoclonal antibody generated against the PGLD peptide bound to a 16kDa protein in T27K coccidioidal lysate by western blotting; the same 16kDa band was not present in crushed spherule lysate. Since the size of the ORF is smaller than the 16kDa protein observed in western blotting analysis, it is likely that the parent protein is glycosylated. Studies are ongoing to further characterize the parent protein and monoclonal antibody.

**Diagnostic Value of Adenosine Deaminase Levels in Lymphocytic
Pleural Effusions Caused by Coccidioidomycosis**

*G.R. Thompson III, MD, D. Bays BS, S. Sharma DO,
M. Davis MD, R. Libke MD, D. Pappagianis MD, PhD*

Abstract not available for publication.

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	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
27	May 15, 1982	Los Angeles, CA	American Lung Association
28	March 20, 1983	La Jolla, CA	California Thoracic Society

Annual Meetings of the Coccidioidomycosis Study Group

No.	Date	Location	Held in Conjunction With
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, 2010	Surprise, CA	—
55	April 2, 2011	Davis, CA	—
56	March 24, 2012	Tucson, AZ	—

