Coccidioidomycosis
STUDY GROUP

Proceedings of the Fifty-Third Annual Coccidioidomycosis Study Group Meeting

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Proceedings of the Fifty-Third Annual Coccidioidomycosis Study Group Meeting

Meeting Number 53
April 4, 2009
California State University Bakersfield
Bakersfield, California

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Chairperson

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Meeting Program

7:30 a.m. **Registration and Coffee**
8:00 a.m. **Welcome and Announcements**

8:30-10:00 a.m. **Session 1: Ecology**
Moderator: Andrew Comrie

1. Soil moisture, climate change, and areas endemic for *Coccidioides*: Texas, a model for the southwestern U.S.? Fisher FS, Johnson SM, Bultman MW, Pappagianis D.

2. *Coccidioides* species, potential natural reservoir in Comarca Lagunera, Mexico. Galeana Pizaña JM, Martínez Verduzco GC, González-Martínez MR, Flores-Sánchez MA, Durán-Vázquez A, Castañón-Olivares LR.

3. An exploratory ecologic study on new building construction and incident coccidioidomycosis in Los Angeles County, 1992-2005. Guevara RE, Peterson A, Terashita D.

4. Climate factors related to coccidioidomycosis in the Tucson and Phoenix areas. Comrie A, Tamerius J, Glueck M.

5. Mapping *Coccidioides* growth and exposure: results from remote sensing. Yool S, Pianalto S, Stacy P

10:30-11:45 a.m. **Session 2: Epidemiology**
Moderator: Demo Pappagianis

6. Comparative study between two Mexican coccidioidins for skin testing. Aroch-Calderón A, Toriello C, Laniado-Laborín R, González-Martínez MR, Miranda-Mauricio S, Muñoz-Hernández B, Palma G, Manjarrez-Zavala ME, Pérez-Mejía A, Hernández–Navarez A, Flores-Sánchez MA, Aranda-Uribe, IS, Castañón-Olivares LR.

7. Comparison of coccidioidomycosis state surveillance and self-report survey cases in Tucson, Arizona. Tabor JA , O'Rourke, MK.

8. Epidemiology update of coccidioidomycosis in Arizona. Tsang C, Anderson S, Imholte S, Erhart L, Casimir B, Chen S, Christ C, Park B, Chiller T, Komatsu K, Sunenshine R.

9. Coccidioidomycosis risk factors identified from a telephone survey in Tucson, Arizona. J.A.Tabor and Mary Kay O'Rourke.

10. Investigation of an increased incidence of coccidioidomycosis in the northwest valley, metropolitan Phoenix. Chang L, Ahlquist A, Sunenshine R, Harris J, Imholte S, Tsang C, Anderson S, Erhart L, Schumacher M, Santana S, Nesset A, Komatsu K, Chen S, Chiller T, Park B.

11. Can a single test detection of coccidioidal immunoglobulin G antibody suffice for case counting of coccidioidomycosis? Wheeler C, Emery K, Delea M, Daily P, Kao A, Chiller T, Mohle-Boetani J, Vugia D.

12. Coccidioidomycosis among community-acquired pneumonia patients visiting urgent care and emergency departments in Tucson, Arizona. McCotter O, Sunenshine R, Wright M, Erhart L, Chiller T, Guerrero R, de Boer M, Anderson S, Waterman S, Komatsu K, Harris R, Park B.

2:15-3:30 p.m. **Session 3: Clinical Coccidioidomycosis**
Moderator: Royce Johnson

13. Analysis of coccidioidal meningitis at a referral medical center in southern Arizona. Drake KW, Adam RD.

14. Post transplantation reactivation of coccidioidomycosis despite azole therapy and prophylaxis. Blair JE, Kusne S.

15. Coccidioidomycosis and HIV. A cohort analysis in the age of potent antiretroviral therapy. Ampel NM, Masannat F.

16. Diagnosis and medical management of granulomatous pericarditis due to *Coccidioides immitis* in a dog. Davidson A, MacDonald K.

17. Nikkomycin Z update. Hoover SE, Nix DE, Galgiani JN.

18. Morbidity and Mortality of Central Nervous System Coccidioidomycosis. Khurana J, Sharma P, Suharirene S, Ghafarizadeh B, Sandhu G, Heidari A, Johnson R, Einstein H.

4:00-5:30 p.m. **Session 4: Basic Laboratory Science and Diagnostics**
Moderator: Suzanne Johnson

19. Diversity of microbial communities in surface soil samples of Kern County, CA, with emphasis on the detection of potential antagonistic microbes to *Coccidioides immitis*. English L, Boehning R, Chen J, Lauer A.

20. Cytokine patterns and memory induced by mature dendritic cells loaded with T27K, a coccidioidal antigen preparation. Ampel NM, Nesbit L, Johnson SM, Pappagianis D.

21. Multilocus genotyping among clinical and environmental *Coccidioides* spp. isolates in Mexico. Muñiz-Salazar R, Baptista-Rosas RC, Luna-Isaac JA, González-González GM, Castañón-Olivares LR, Bazan-Mora E, González Martínez MR.

22. Detection of *Coccidioides* antigenemia requires dissociation of immune complexes. Durkin M, Wheat LJ.

23. SNP-based genotyping of coccidioidomycosis. Driebe E, Sheff K, Pearson T, Colvin J, Beckstrom-Sternberg S, Barker B, Rounsley S, Keim P, Engelthaler DM.

24. Clinical impact of false positive IgM serological results in coccidioidomycosis. Kuberski T, Herric J, Pappagianis D.

5:30-6:00 p.m. **Session 5: Case Presentations**
Moderator: Rafael Laniado-Laborín

Presentations by Drs. Rafael Laniado-Laborin and Chitra Damodaran.

Poster Session

1. Molecular cloning and expression of two β-N-acetylglucosaminidase enzymes of *Coccidioides posadasii*. Lunetta JM, Johnson SM, Pappagianis D.

2. Coccidioidal fungemia. Keckish D, Blair JE, Vikram HR.

3. The spectrum and presentation of disseminated coccidioidomycosis. Adam RD, Elliott SP, Taljanovic MS.

4. Coccidioidal meningitis in the post-fluconazole era. Mathisen G, Shelub A, Troung J, Wigen C.

5. Fungus involved in Posada's case (1892). Canteros CE, Toranzo A, Suarez-Alvarez R, Davel G, Castanon-Olivares LR, Napoli J, Malbrán CG.

6. Genetic diversity among *Coccidioides* spp Isolates from Mexico and Angentina. Duarte-Escalante E, Canteros CE, Castañón-Olivares LR, Toranzo A, Ibarra-Camou B, Reyes-Montes MR

7. Differentiating *C. posadasii* and *C. immitis* in real-time. Sheff K, Driebe E, York E, Waddell V, Barker B, Keim P, Engelthaler DM.

8. Treatment of disseminated coccidioidomycosis with voriconazole. Adam RD.

9. A case-control study of distance to new construction as a risk factor for coccidioidomycosis in Antelope Valley, CA, 2004-2006. Peterson A, Guevara RE, Terashita D.

ABSTRACT 1: Soil Moisture, Climate Change, and Areas Endemic for *Coccidioides* - Texas, a Model for Southwestern U.S.?

Frederick S. Fisher[1], Suzanne M. Johnson[2], Mark W. Bultman[3], Demosthenes Pappagianis[2]

With the exception of the extreme northern part of the Texas panhandle all of the soils in the State may be classified as thermic or hyperthermic; the two soil temperatures where most, perhaps all, *Coccidioides* growth sites are located. Soil moisture regimes are arranged in north to south belts with moisture increasing from west to east. Rainfall ranges from 200mm at El Paso to 1200mm at the eastern boundary of the State. This uncomplicated pattern of the two main factors that define the endemic zone of *Coccidioides* makes Texas the ideal place to test hypotheses concerning the effects of climate change on the distribution of *Coccidioides* in the soil. The eastern extent of the *Coccidioides* endemic zone in Texas is currently approximated by the 900mm precipitation line with the easternmost *Coccidioides* soil separation site located near the town of Beeville.

Climate change predictions for Texas suggest increased temperatures, reduced precipitation, seasonal changes in precipitation, and greater storm severity, all of which will result in increased soil erosion and greater transportation distances for dust. These changes will affect vegetation types, density, and distributions. The soils will respond with important changes in water infiltration rates, salt movement, microorganisms, and soil aggregation. The range and distribution of burrowing animals (which are probably the most effective *Coccidioides* soil dispersal agents) will change with the extinction of some, as food supplies decrease.

Maps (Reheis, M and Rademaekers, J., USGS, 2003) of predicted dust generation in the southwest U.S. show that the area of greatest dust generation lies in extreme eastern New Mexico and much of the southern part of the Texas panhandle. This area includes the *Coccidioides* endemic zone along the Pecos river valley and also endemic areas in the southwestern part of the panhandle of Texas. Windborne dust (along with *Coccidioides* arthroconidia) generated by severe storms in these areas may very well reach heavily populated regions in central Texas (e.g., Dallas/Ft. Worth, Midland, Abilene, Waco) thereby subjecting several million people to possible infection and disease. Also, with increasing aridity, the *Coccidioides* endemic zone in Texas will likely migrate eastward as soil moisture decreases.

ABSTRACT 2: *Coccidioides* Species, Potential Natural Reservoir in Comarca Lagunera, Mexico

Galeana Pizaña JM[1], Martínez Verduzco GC[1], Durán-Vázquez A[2], González-Martínez MR[3], Flores-Sánchez MA[2], Castañón-Olivares LR[2]

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[2]Facultad de Medicina, Universidad Nacional Autónoma de México. México
[3]Departamento de Microbiología. Facultad de Medicina, Universidad Autónoma de Coahuila, Torreón. México

The Comarca Lagunera, is a region located in the north-center of Mexico, it is integrated by 16 distrites. The cutaneous surveys made in the region, they have demonstrated that the *Coccidioides* infection prevalence in the human, varies from 30 to 93%; also, there are several publications associated to the report of new cases of illness. For the above mentioned, the Comarca Lagunera has been considered traditionally, like an endemic area of this mycosis. The isolation of *Coccidioides* spp. from natural sources, made in USA show that the fungi had been able to be recovered of regions with environmental conditions related to pluvial precipitation, temperature, orientation and elevation of the land, as well as the type and soil use.

The goal of our research, is to locate in the Comarca Lagunera the *Coccidioides* spp development points, using a space analysis. Particularly in this work we present, as preliminary results, the potential areas that could be the fungi natural reservoires. The Comarca Lagunera is a region divided in 77 topographical maps (escale 1:50,000). With founded in discoveries made by diverse authors, we elaborate a database with the following parameters and values: pluvial precipitation (00-00 mm), temperature (-0.5°C at 30°C), soil pH (≥7.5) and soil texture (same or bigger to 50% of sand); those characteristics were used spacely as layers, that were analyzed using the software ARC GIS version 9.2.

An interpolation was made (IDW) specifically about the soils, which together with the pluvial precipitation and temperature data, were submitted to Boolean algebra. Actually, the results obtained reveal approximately 131,728.5 acres in which exist the possibility of harboring the natural habitat of *Coccidioides* spp. The evaluated surface would correspond to 27 polygons inside the Comarca Lagunera. We must to introduce the values assigned to the orientation and elevation of the land, as well as the soil use. Of course, the obtained results have their support in that entire information published by outstanding investigators and experts in the topic.

Finally, the validity of the designed pattern, will be to check in field the obtained predictions.

ABSTRACT 3: An Exploratory Ecologic Study on New Building Construction and Incident Coccidioidomycosis in Los Angeles County, 1992-2005

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Background: Coccidioidomycosis is caused by the airborne spores of *Coccidioides immitis* and *Coccidioides posadasii*. These fungi grow in loose soil of arid to semiarid environments with low to moderate rainfall, mild winters, and long hot seasons. Increased coccidioidomycosis has been known to follow events of soil disturbance including earthquakes, dust storms, archaeological digs, and construction.

Methods: Coccidioidomycosis incidence rates using cases passively reported to the Los Angeles County (LAC) Department of Public Health were calculated annually for 1992-2005, analyzed by geographic area, and plotted in Microsoft Excel against new building permit data for 1992-2005 from the United States Census Bureau to apply linear regression lines to measure correlations as R2.

Results: A sharp increase in coccidioidomycosis incidence rate occurred in LAC in 2003-2005. Compared to other geographic areas in LAC, Antelope Valley (AV) and West Valley (WV) health districts had substantial increases in average annual incidence of coccidioidomycosis comparing 1992-2002 to 2003-2005 (17 to 159 cases/million population/year in AV, and 18 to 37 cases/million population/year in WV). New building construction in these two health districts increased over consecutive years since at least 2001, and for 1996-2005, correlations between new building construction and coccidioidomycosis incidence rate measured at R^2=0.9522 and 0.6819 for AV and WV, respectively.

Conclusion: Increases in coccidioidomycosis incidence rates in AV and WV accounted for the 2003-2005 rate increase in LAC. New building construction has a strong positive correlation with coccidioidomycosis incidence rate in AV, and a fairly strong positive correlation with coccidioidomycosis incidence rate in WV.

ABSTRACT 4: Climate Factors Related to Coccidioidomycosis in the Tucson and Phoenix Areas

Andrew Comrie, James Tamerius, Mary Glueck

University of Arizona
School of Geography & Development

This work aims to further our understanding of climate-related factors and their role in coccidioidomycosis outbreaks in Arizona. We have developed extensive data quality controls and pre-processing techniques for effective evaluation of a set of climate variables against coccidioidomycosis case rate data for Pima and Maricopa counties, 1995-2006. Climate variables included temperature (min, max, mean), humidity (min, max, mean), precipitation, wind speed (mean, max) & direction, vapor pressure deficit, solar radiation, and dust (PM-10). We performed data reduction via principal components analysis of the climate data, and obtained two key underlying components reflecting (1) moisture/precipitation and (2) temperature. Leading variables in these two components were employed in a lag correlation analysis of case rates adjusted for exposure date and for trend and variance corrections resulting from data recording issues. Analyses confirmed the utility of seasonal precipitation as the key driving variable for coccidioidomycosis cases in Arizona. In particular, a strong, simple and consistent climate effect emerges that is consistent with the “grow & blow” hypothesis. In the growth phase, October through December precipitation is significantly linked to coccidioidomycosis 6-18 months later. High or low precipitation in this period leads to corresponding high or low case rates across most seasons in a lagged time window centered on a year later. In the dispersion phase, concurrent monthly precipitation is negatively correlated with coccidioidomycosis. Thus, a wet or dry month leads to corresponding low or high case rates at the same time, presumably because of soil wetting reducing dust and dispersion. These results further refine and confirm that seasonal climate fluctuations, especially Fall-Winter precipitation, explain much of the coccidioidomycosis case rate variability about the trend from 1995-2006.

ABSTRACT 5: Mapping *Coccidioides* Growth and Exposure: Results from Remote Sensing

Stephen R. Yool, F. Scott Pianalto, Patrick Stacy

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Seasonal patterns of coccidioidomycosis and remotely-sensed normalized difference vegetation index (NDVI) are consistent with fungal spore dispersion in the dry seasons (Figure 1). Results of lagged regression modeling suggest moist soil resulting from winter precipitation may be associated with increased incidence up to a year later in all three counties (Figure 2). Surface disturbance detected using remotely-sensed data timeseries appears to track incidence for a majority of recent years (Figure 3).

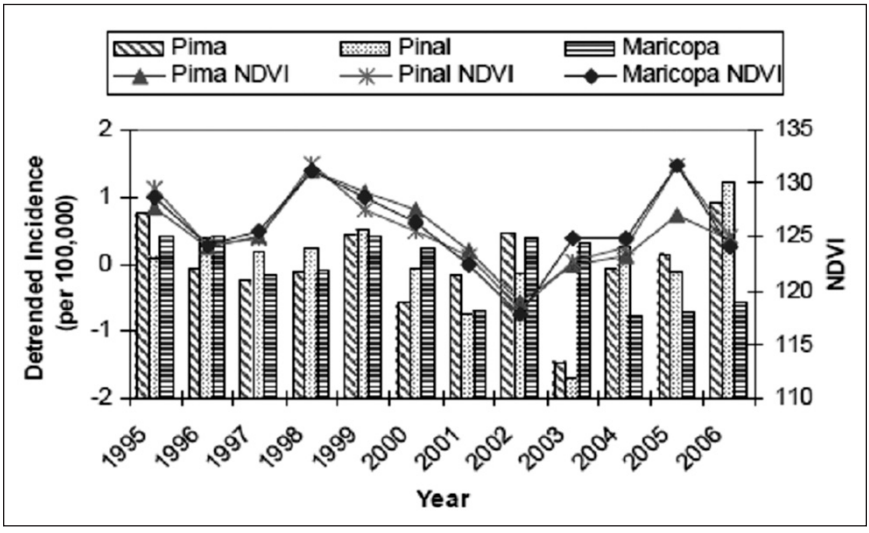

Figure 1. NDVI vs. incidence suggests spore dispersion during dry (low NDVI) periods. High NDVI periods would tend to confine spores to the surface, decreasing incidence.

(Continued)

ABSTRACT 5 *(Continued)*: **Mapping *Coccidioides* Growth and Exposure: Results from Remote Sensing**

Stephen R. Yool, F. Scott Pianalto, Patrick Stacy

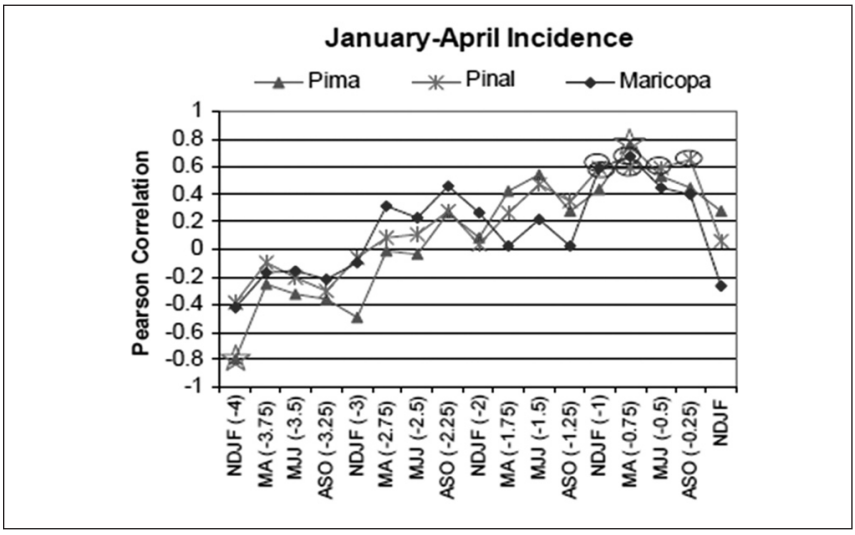

Figure 2. Lagged NDVI response precedes incidence up to one year.

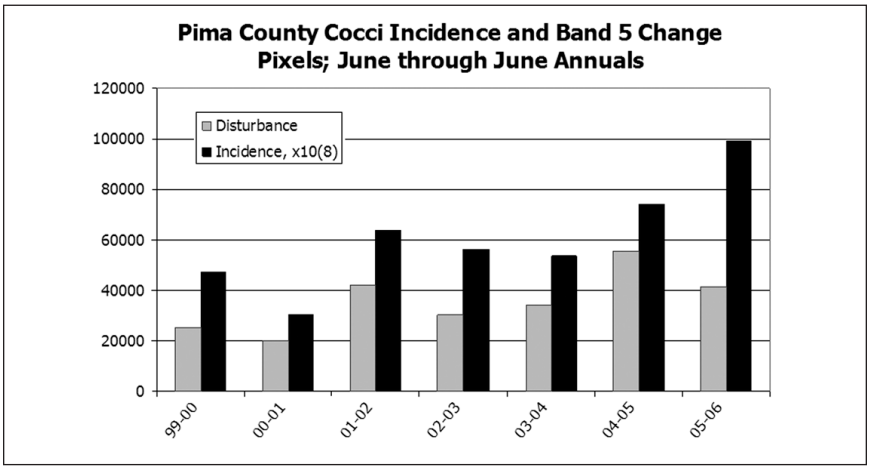

Figure 3. Remotely-sensed surface disturbance vs. incidence for Pima County, AZ. Magnitude incidence tracks magnitude disturbance in a majority of years.

ABSTRACT 6: Comparative Study Between Two Mexican Coccidioidins for Skin Test

Aroch-Calderón A[1]; Toriello C[1], Laniado-Laborín R[2]; González-Martínez MR[3]; Miranda-Mauricio S[4]; Muñoz-Hernández B[5]; Palma G[5]; Manjarrez-Zavala ME[5]; Pérez-Mejía A[1]; Hernández–Navarez A[1]; Flores-Sánchez MA[1]; Aranda-Uribe IS[1]; Castañón-Olivares LR[1]

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At National University of Mexico (UNAM) coccidioidin antigen is elaborate following the development requirements set, place by the Ministry of Health, Mexican national institution which in turn, supports the antigen homonym sold by the company named Birmex.

To know the immunological properties of UNAM-coccidioidin, we developed a comparative study of skin reactions produced between UNAM-coccidioidin *vs.* BirMex-coccidioidin.

Antigens were used simultaneously by intradermal route and prior consent, in each of 184 individual volunteers from different States of Mexico. Also, a questionnaire was used to capture demographic, clinical and epidemiological data.

It was noted that 76 persons were positive to UNAM-coccidioidin, while for BirMex-coccidioidin 88 were positive (not found significant differences (p<0.05 IC 95%); but the statistical analysis in the induration measure, reveals significant differences: indurations from 5 to 14 mm occurs more frequently with UNAM-coccidioidin, while indurations of 14 mm or more, are most frequent caused by BirMex-coccidioidin (p>0.05 IC 95%).

So far, the clinical, biochemical and immune analysis, demonstrates that the UNAM-coccidioidin, is an useful biological reagent because: 1) that meets the requirements edited in the Farmacopea Mexicana and 2) that can be reliably used in epidemiological surveys.

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.

ABSTRACT 8: Epidemiology Update of Coccidioidomycosis in Arizona

Tsang C[1], Anderson S[1], Imholte S[1], Erhart L[1], Casimir B[1], Chen S[1,2], Christ C[1], Park B[2], Chiller T[2], Komatsu KK[1], Sunenshine RH[1,2]

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[2]Centers for Disease Control and Prevention, Atlanta, GA

Background: Coccidioidomycosis (Valley Fever) is an emerging fungal disease endemic to the southwestern United States, Central and South America. Sixty percent of nationally reported cases occur in Arizona, where physician and laboratory reporting is mandatory. Arizona Department of Health Services (ADHS) initiated enhanced coccidioidomycosis surveillance to characterize the knowledge, treatment, and impact of coccidioidomycosis in affected individuals.

Methods: ADHS contacted every tenth Arizona coccidioidomycosis case reported from January 2007 to February 2008 and interviewed them with a standardized questionnaire. Results from enhanced surveillance were compared with data from the Behavioral Risk Factor Surveillance System (BRFSS), an annual population-based telephone survey about health behaviors and opinions.

Results: Of 5,696 cases reported, 493 were interviewed. Cases waited a mean of 44 (median 11) days from symptom onset before seeking medical care. A mean of 3 (median 2) provider visits occurred before coccidioidomycosis diagnostic testing was ordered. Forty-four percent were seen in an emergency room and 41% were hospitalized overnight for their illness. Twenty-six percent saw their doctor at least ten times during their illness. Individuals reported that symptoms lasted a mean of 193 (median 109) days. Cases diagnosed with coccidioidomycosis lived in Arizona for an average of 16.3 (median 12) years compared to people who answered the BRFSS survey, who lived in Arizona for an average of 26.4 (median 22) years ($p < 0.01$). Only 5% of case-patients heard about valley fever from their doctors, whereas 11% of the general public heard about it from their doctors. Cases with valley fever knowledge prior to seeking healthcare were more likely to be diagnosed earlier than those who were not familiar with the disease [79 days vs. 282 days, respectively ($p=0.04$)] and were 1.8 times (CI: 1.01-3.21, $p = 0.045$) more likely to request testing for coccidioidomycosis from their physicians. Coccidioidomycosis accounted for $59 million in hospital charges in Arizona in 2007.

Conclusions: Coccidioidomycosis has a significant impact on the economy, healthcare system, and quality of life of Arizonans. Physician and patient education to improve timeliness of coccidioidomycosis diagnostic testing may allow for earlier recognition of complicated disease.

ABSTRACT 9: Coccidioidomycosis Risk Factors Identified from a Telephone Survey 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, raising concerns about a possible epidemic and its causes. A cross-sectional telephone survey of 5,460 households in greater Tucson, Arizona, was conducted in 2002-2003 to evaluate inherent, socio-economic, and environmental risk factors of coccidioidomycosis. Two demographic strata assuring recruitment of a minority population and three landscape strata controlled for differences in community-level exposures. The household response rate was 41% and information was obtained for 14,105 individuals. Educational attainment and location of residence by landscape and demographic strata confounded risks of symptomatic coccidioidomycosis due to age, race, and cigarette smoke exposure. Odds ratio for being Hispanic compared to non-Hispanic White increased from a bivariate 0.3 to a multivariate 0.8 when controlled for susceptibility factors and community-level exposure and indicates the risk of coccidioidomycosis for being Hispanic is similar to being non-Hispanic White. This study supports findings of other studies that people older than 44 years or exposed to cigarette smoke are at greater risk of symptomatic coccidioidomycosis. Differential misclassification of subjects' exposure to *Coccidioides* arthroconidia and susceptibility to disease can be partially controlled by sampling stratification of landscape types and demographics following a landscape ecology approach.

ABSTRACT 10: Investigation of an Increased Incidence of Coccidioidomycosis in the Northwest Valley, Metropolitan Phoenix

Loretta V. Chang, A. Ahlquist, R Sunenshine, J.Harris, S.Imholte, C.Tsang, S.Anderson, L.Erhart, M.Schumacher, S.Santana, A.Nesset, K.Komatsu, S.Chen, T.Chiller, B.Park

Background: The fungal disease coccidioidomycosis is a common, though underdiagnosed source of morbidity in Arizona. It causes up to 29% of community-acquired pneumonias, 31 missed workdays per case, and $86 million in hospital charges statewide annually. In 2007, Arizona surveillance data indicated the age-adjusted coccidioidomycosis incidence in the NWV was more than twice that of the rest of metropolitan Phoenix.

Methods: To investigate reasons for this increase, we conducted a study with five components. First, we evaluated provider knowledge, attitudes, and practices using data from a recent provider survey. Second, we examined residents' health-seeking behaviors, using enhanced surveillance. Third, to estimate lab testing frequency, we calculated the positive-to-ordered *Coccidioides* test ratio from a large commercial laboratory. Fourth, the incidence among dogs was estimated as a human surveillance proxy using veterinary laboratory test data. Finally, we examined the impact of transient winter residents on reported incidence using home security data.

Results: Compared to other areas of metropolitan Phoenix, NWV providers were more likely to attend continuing medical education (RR= 2.6, 1.7-4.0) and to provide coccidioidomycosis counseling (RR=1.7, 1.3-2.0). NWV patients were tested earlier after disease onset (79 vs 170 days; p=0.06) and with fewer symptoms (RR=1.2, 1.1-1.4). No difference in the positive-to-ordered test ratio existed. Incidence among NWV dogs was lower (0.8% vs. 3.4%, p<0.001). Security data indicated that 20% of the NWV population are transient non-residents, compared to 5% statewide.

Conclusions: Our findings suggest that the higher reported coccidioidomycosis rate in the NWV may not accurately reflect a higher true incidence. The higher rate is likely due to a combination of increased awareness and testing, and an inflated numerator from transient non-residents.

ABSTRACT 11: *Can a Single Test Detection of Coccidioidal Immunoglobulin G Antibody Suffice for Case Counting of Coccidioidomycosis?*

Charlotte Wheeler[1], Kirt Emery[2], Maryann Delea[3,4], Pam Daily[5], Annie Kao[4], Tom Chiller[6], Janet Mohle-Boetani[1], Duc Vugia[1]

[1]California DPH, [2]Kern County DPH, [3]CDC/CSTE Applied Epidemiology Fellowship, [4]County of San Diego HHSA, [5]California Emerging Infections Program, [6]Centers for Disease Control and Prevention

Background. The Council of State and Territorial Epidemiologists (CSTE) coccidioidomycosis (cocci) surveillance case definition has clinical and laboratory components. In 2008, CSTE changed the definition to allow a single detection of coccidioidal immunoglobulin G (cocci IgG) antibody to serve as laboratory confirmation of infection. We performed a study to determine the predictive value positive (PVP) of a single cocci IgG and the practicality of a case definition that permits case counting by IgG alone, without clinical information.

Methods. We conducted enhanced surveillance from July 1–December 31, 2007 in three California areas: (1) *Coccidioides*-endemic Kern County (Kern), (2) mildly-endemic San Diego County (San Diego), and (3) non-endemic Alameda, Contra Costa, and San Francisco Counties (northern counties). Laboratories submitted positive cocci IgG reports; each laboratory determined its own criteria for a positive result. We obtained patient clinical information from physicians or by review of medical records. We defined a confirmed case of cocci as a positive cocci IgG in the context of symptoms meeting CSTE clinical criteria (see Box). We defined PVP as the number of persons with confirmed cocci divided by all persons with positive cocci IgGs.

Results. We identified positive cocci IgG results in 434 individuals: 369 (85%) from Kern, 44 (10%) from San Diego, and 21 (5%) from northern counties. The PVP for a single cocci IgG was 92% and did not differ significantly among areas (Kern: 92%, San Diego: 95%, northern counties: 90% [P = 0.7]). San Diego and northern county patients were older than Kern patients (median age 53 and 51 respectively vs. 38 years [P < 0.0001]) and more likely to be hospitalized (78% and 70% vs. 27% [P < 0.0001]). IgG was confirmed by immunodiffusion or complement fixation in 366 (92%) of confirmed cases. Of 27 (6%) confirmed cases that were tested by Elisa immunoassay (EIA) alone, physicians doubted a cocci diagnosis in seven (26%).

(Continued)

ABSTRACT 11 *(Continued)*: ***Can a Single Test Detection of Coccidioidal Immunoglobulin G Antibody Suffice for Case Counting of Coccidioidomycosis?***

Charlotte Wheeler[1], Kirt Emery[2], Maryann Delea[3,4], Pam Daily[5], Annie Kao[4], Tom Chiller[6], Janet Mohle-Boetani[1], Duc Vugia[1]

Conclusions. The cocci IgG PVP was high (≥90%) but may depend on clinical practice, laboratory test, and a region's endemicity. In our study's mildly and non-endemic areas, cocci was detected in populations known to have serious illness (older and hospitalized). Physicians doubted approximately one-quarter of positive IgG results obtained by EIA alone. The PVP of a single positive cocci IgG in a young, healthy person in a mildly- or non-endemic area, and the PVP of cocci IgG by EIA alone, may need further study.

Council of State and Territorial Epidemiologists
Clinical Case Definition for Coccidioidomycosis

An illness characterized by one or more of the following:

- Influenza-like signs and symptoms (e.g., fever, chest pain, cough, myalgia, arthralgia, and headache)
- Pneumonia or other pulmonary lesion, diagnosed by chest radiograph
- Erythema nodosum or erythema multiforme rash
- Involvement of bones, joints, or skin by dissemination
- Meningitis
- Involvement of viscera and lymph nodes

ABSTRACT 12: Coccidioidomycosis Among Community-Acquired Pneumonia Patients Visiting Urgent Care and Emergency Departments in Tucson, Arizona

McCotter O, Sunenshine R, Wright M, Erhart L, Chiller T, Guerrero R, de Boer M, Anderson S, Waterman S, Komatsu K, Harris R, Park B.

Abstract presented at meeting,
not available for publication.

ABSTRACT 13: Analysis of Coccidioidal Meningitis at a Referral Medical Center in Southern Arizona

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Purpose: Coccidioidal meningitis is associated with significant morbidity and mortality in endemic regions of the southwest Unites States and northwest Mexico. Since the prognosis can depend on the early recognition and treatment of the disease, it is important to be familiar with the varied clinical manifestations, risk factors associated with meningeal involvement, diagnostic challenges and therapeutic modalities.

Methods: We performed a retrospective analysis of 72 cases with meningeal disease seen from 1996 to 2007 at a referral medical center in an endemic region.

Results: The most common symptoms at presentation were headache, fever, nausea and vomiting. Those who were immunocompromised (HIV/AIDS and Chronic Steroids) were at increased risk; however, diabetics were not at increased risk. There was a preponderance of males (2:1) and people of Hispanic, African and Asian (esp Pacific Islanders) descent. Serology and culture were frequently negative on presentation. Imaging was also unhelpful in about one third of cases. The most frequent complication included hydrocephalus which frequently required VP shunting. Most were treated with fluconazole , and prognosis was good for a majority of those who remained on lifelong treatment.

Conclusions: Coccidioidal meningitis remains a diagnostic challenge. However, the diagnosis can be made with a high level of suspicion and appropriate diagnostic testing. In some patients the diagnostic testing must be repeated multiple times to confirm the diagnosis. In those whose disease is caught early and who remain on lifelong treatment, the prognosis is typically good.

ABSTRACT 14: Disseminated Coccidioidomycosis Prior to Transplantation is a Risk Factor for Reactivation After Solid Organ Transplantation

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Division of Infectious Diseases
Mayo Clinic in Arizona

Transplant recipients who become infected with *Coccidioides* spp have high rates of morbidity and mortality. Most coccidioidal infections in the organ transplant recipients are reactivated infection acquired prior to transplantation. Risk factors for such reactivated infection include a documented history of coccidioidomycosis or a positive serology at or just prior to organ transplantation. Azoles have markedly decreased the risk of such reactivation. We recently encountered and presented 2 patients who received azole prophylaxis for the prevention of reactivated coccidioidomycosis following transplantation, yet had active coccidioidal disease in their post-transplantation follow up. We subsequently analyzed our experience with the use of azoles to suppress coccidioidal infection post transplant. The courses of 58 patients whose coccidioidal illness predated their solid organ transplantation were reviewed. 55 patients had no reactivation on azoles, while 3 patients had reactivation of coccidioidal infection following transplantation. 1 patient had occult, active but untreated, pulmonary coccidioidomycosis at the time of transplantation, and despite early institution of antifungal therapy, had disseminated infection following organ transplantation. Two other patients had disseminated coccidioidomycosis of varying control prior to transplantation, with subsequent post transplantation reactivation. When compared with patients whose coccidioidal illness remained quiescent (n=55), the cohort (n=3) whose infection reactivated despite azole prophylaxis, had no significant differences in race, age, sex, type of organ transplanted, presence of diabetes mellitus, CMV mismatch, components of immunosuppression or a history of rejection. The cohort whose infection reactivated despite the use of azole prophylaxis were more likely than their non-reactivated counterparts to have disseminated coccidioidomycosis prior to transplantation ($p=0.01$). Patients with disseminated coccidioidomycosis prior to transplantation appear to be at increased risk of reactivated coccidioidal infection following organ transplantation.

ABSTRACT 15: A Cohort Analysis Among HIV-Infected Persons Living in the Coccidioidal Endemic Region in the Age of Potent Antiretroviral Therapy

Masannat FY and Ampel NM

Division of Infectious Diseases and the Valley Fever Center for Excellence of the University of Arizona; and the Southern Arizona Veterans Affairs Medical Center, Tucson, AZ

Coccidioidomycosis is an important opportunistic infection in those with HIV-1 infection living in the coccidioidal endemic region. Prior to the advent of potent antiretroviral therapy (pART), nearly 25% of persons attending a clinic within the endemic region developed active coccidioidomycosis over a period of 41 months. pART results in a rise in peripheral blood CD4 T lymphocytes and in immune reconstitution, so it would be presumed that the incidence and severity of coccidioidomycosis among those with HIV-1 infection might have declined. However, there are no studies regarding this. To determine the effect of pART on coccidioidomycosis, a retrospective analysis among persons attending an HIV clinic located within the coccidioidal endemic region was undertaken. Over a 5-year period, 29 cases among a total of 257 patients were found to have active coccidioidomycosis (11.3%) and 12 (4.7%) of these patients were diagnosed with active coccidioidomycosis during the period of analysis. Among patients with coccidioidomycosis, those with less severe disease were significantly more likely to be on pART and to have an undetectable plasma HIV level than those with more severe disease (for both, $P < 0.01$). Sixteen of the those with coccidioidomycosis either received no antifungal therapy or had it discontinued during the course of their follow-up. All did well and no deaths were attributable to coccidioidomycosis. Cases of coccidioidomycosis had significantly lower peripheral blood CD4 T lymphocyte counts than those without (285 ± 42 vs 477 ± 21, $P = 0.003$). These data demonstrate a decline both in the severity and incidence of coccidioidomycosis during pART era and these effects appear to be directly attributable to effective treatment of HIV-1.

ABSTRACT 16: Diagnosis and Medical Management of Granulomatous Pericarditis due to *Coccidioides immitis* in a Dog

Davidson AP, MacDonald KM

School of Veterinary Medicine, University of California, Davis VCA Animal Care Center of Sonoma, Rohnert Park, CA

In April 2008 a 6 year old female dog was evaluated for lethargy, anorexia and cough. The dog had been rescued two weeks previously from northwestern Mexico. On exam, the dog was thin, with poor body condition (BCS 3 of 9), irregular heart rate of 120, respiratory rate 52, and mildly increased respiratory effort. The mucous membranes were pale and dry. Splenomegaly was present without lymphadenomegaly. Gait was normal. Laboratory studies revealed a mild anemia (PCV 36%) and hyperglobulinemia. Two-view thoracic radiographs showed a markedly enlarged and globoid cardiac silhouette and a generalized interstitial pulmonary pattern. Echocardiogram (echo) showed a large pericardial effusion, causing tamponade of the right atrium and right ventricle. A hyperechoic, oscillating soft tissue density at the heart base measured 2.47 x 1.5 cm^2 . The right atrial free wall was hyperechoic and thickened, suggesting an infiltrative process. No heartworms were seen and there was no evidence of pulmonary hypertension. Pericardiocentesis yieldied 400 ml of cloudy yellow fluid, with suppurative and granulomatous cytology. Culture grew Actinomyces spp. Paired serum and pericardial fluid samples were positive for precipitin (IgM) suggesting a recent coccidioidal infection with dissemination. The dog was placed on fluconazole, 75 mg po q 24h and amoxicillin. After 20 days, no increase in the pericardial effusion was seen on repeat echo. At the 3 month evaluation, the dog had improved attitude and weight. There was no pericardial effusion, and the soft tissue density at heart base measured 1.45 x 0.7 cm^2. The fluconazole was continued. Six months into therapy the granuloma at the heart base had resolved. Serology was positive only with a concentrated sample. At 11 months, the dog had an unremarkable physical examination, and serology was negative.

Veterinary recommendations for the management of infectious granulomatous pericarditis traditionally include both antimicrobial therapy and subtotal pericardectomy; such an approach is needed because of a high rate of recurrent life threatening tamponade requiring multiple pericardiocenteses, and poor antimicrobial penetration into pericardial fluid. Constrictive pericarditis with right heart failure are eventually secondary to chronic pericarditis. Financial constraints precluded thoracotomy in this dog, whose good response to medical therapy suggests that infectious pericarditis is not always a surgical disease.

ABSTRACT 17: Nikkomycin Z Update

Susan Hoover, John Galgiani and David Nix

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We present the preliminary results from the first human trials of nikkomycin Z for coccidioidomycosis. Nikkomycin Z is a chitin synthase inhibitor that demonstrates a very low minimum inhibitory concentration against *Coccidioides* spp in vitro. Animal studies by Hector and coworkers have demonstrated sterilization of the lungs of experimentally infected mice. We are conducting two clinical trials: a Phase I/II trial in patients with early, uncomplicated *Coccidioides* pneumonia, and a Phase I safety trial in healthy volunteers. Both trials use a rising dose strategy and a 14-day treatment period. Both patients and healthy volunteers receive intensive clinical and laboratory monitoring, with frequent blood sampling for pharmacokinetics on days 1 and 14. Pneumonia patients also undergo serial chest imaging and sputum cultures. At the time of this presentation, 6 pneumonia patients and 8 healthy volunteers had completed the study, with no significant adverse events or laboratory abnormalities in either group. Nikkomycin Z appears to be well tolerated in humans. We expect the healthy volunteer study to be completed by the end of 2009. Remaining challenges include evaluation of efficacy and improving drug production methods.

ABSTRACT 18: Risk Factors of Morbidity and Mortality Associated with Coccidioidomycosis Meningitis

A. Heidari, S. Suady, J. Khurana, B. Ghafarizadeh, R. Johnson, H. Einstein – Kern Medical Center UCLA, Bakersfield, CA

Background. Several risk factors have been studied for severe forms of coccidioidomycosis (CM) disease. Previously, diabetes found to be an independent risk factor for severe pulmonary CM. Subsequently, poor glycemic control in diabetics shown to be a risk factor for dissemination of CM. Diabetics shown to have higher mortality with CM overall. However, the risk factors associated with morbidity and mortality of CM meningitis in particular have not been clear.

Method. This is a retrospective chart review study of the patients with the diagnosis of CM meningitis from CM clinic at Kern Medical Center (San Joaquin Valley California) from January 1, 2000 to December 31, 2008.

Results. 129 cases were included and evaluated. Of these, average age at the time of diagnosis was 34.5 years, 70.5% were male, 65% were Latinos, 16% were African Americans, 13% were Caucasians and 2% Filipino. Most common presenting symptoms were: headache (80%), vomiting (44%), fever (39.5%), followed by Nausea (30%), Chills (27%), night sweats (14%), and weight loss (13%). During the course of the disease 47% had hydrocephalus (12% at presentation), 34% had stroke, 21% had arachnoiditis, 18% had seizures and 11% had cranial nerve palsies. In general, 46.5% underwent ventriculoperitoneal shunting. Males had significantly higher risk of developing stroke (OR 3.0; 95% CI: 1.1-8.5; P = 0.015). Excluding 36 patients who were lost to follow up the mortality rate was 15.5% overall. Patients with diabetes (n=29, average age of 41.1 years at the time of diagnosis) had significantly higher risk of developing stroke (OR 2.6; 95% CI: 1.0-6.6; P = 0.023), difficulty in ambulation (OR 4.3: 95% CI: 1.5-12.5: P = 0.001), altered mental status (OR 3.2; 95% CI: 1.1-8.8; P = 0.01) and significantly higher rate of mortality (OR 3.6; 95% CI: 1.1-11.1; P = 0.008). In comparison with non-diabetics initial cerebrospinal fluid analysis revealed significant higher average protein levels (296 to 133 mg/dl) and higher average number of cell count (1219 to 683 /cmm).

Conclusion. Diabetes is a major risk factor for mortality and morbidity of CM meningitis. Males with CM Meningitis have higher risk of developing stroke.

ABSTRACT 19: Diversity of Microbial Communities in Surface Soil Samples of Kern County, CA, with Emphasis on the Detection of Potential Antagonistic Microbes to *Coccidioides Immitis*

LeAnn English, Ryan Boehning, Jeffrey Chen, and Antje Lauer

California State University Bakersfield (CSUB), Bakersfield, CA, USA

The ecology of *Coccidioides immitis*, the 'Valley Fever Fungus' is poorly understood. Especially the interactions between *C. immitis* and other microbes in loamy sands have never been investigated in detail. This project focuses on the detection of *C. immitis* in loamy sands around Bakersfield in Kern County, CA, over a period of a year with molecular methods such as DNA extraction, Polymerase Chain Reaction (PCR), and Denaturing Gradient Gel Electrophoresis (DGGE). Ten sampling sites were investigated with 3 different depths: 0-2 cm, 6-10 cm, and 18-22 cm with specific primer pairs for *C. immitis* (ITS spacer region) and primer pairs for all fungi and bacteria based on the 16S and 18S rDNA gene. *C. immitis* was found sporadically in several of our sampling sites, in different depths. At none of the sites *C. immitis* was detectable throughout the year. DGGE analysis revealed that the overall diversity of fungi in the soils was very low, compared to the high diversity of bacteria in all samples and depths. Through comparing DGGE profiles from sites that have the same or very similar physical and chemical properties, but differ in the presence of *C. immitis*, potential antagonistic microbes to this fungal pathogen can be detected by identifying specific DGGE bands. A proposal has been submitted to NSF for a collaborative project with the Medical Center of the University of Mexico (UNAM) to isolate these antagonistic microorganisms. Challenge assays on nutrient media will be performed to determine anti-*C. immitis* properties.

Our project will add significantly to our understanding of the diversity of the soil microorganisms in loamy sands where *C. immitis* can be found and might lead to the discovery of anti-*C. immitis* microorganisms that can be used as probiotics in the future to reduce or eliminate *C. immitis* through antagonistic effects.

ABSTRACT 20: Cytokine Patterns and Memory Induced by Mature Dendritic Cells Pulsed with T27K, a Coccidioidal Antigen Preparation

Nesbit L, Johnson SM, Pappagianis, D, and Ampel NM

Division of Infectious Diseases and the Valley Fever Center for Excellence of the University of Arizona; and the Southern Arizona Veterans Affairs Medical Center, Tucson, AZ; and the Department of Microbiology and Immunology, the University of California at Davis, Davis, CA

We have previously demonstrated that lymphocytes from donors without coccidioidal immunity can be activated when using mature dendritic cells pulsed with the coccidioidal antigen preparation T27K (mDCT27K). However, that study did not examine release or intracellular expression of T-helper type 1 (Th1) cytokines. In the present study, we wished to determine if polyfunctional T lymphocytes expressing intracellular Th1 cytokines occur in the peripheral blood of naturally immune donors and if mDCT27K could induce this property on cells obtained from non-immune donors. Peripheral blood mononuclear cells (PBMC) were derived by density gradient centrifugation. The cytokines interleukin-2 (IL-2), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) were measured in supernatant of PBMC incubated with 20 µg/ml T27K for 48 hr. For determination of polyfunctionality, intracellular flow cytometry was performed after 48 hr of incubation with T27K and also anti-CD28, anti-CD49d and brefeldin A to assess for the simultaneous expression of IL-2, IFN-γ and TNF-α. Among CD4 lymphocytes from immune donors, a median of 137 cells out of 400,000 events were polyfunctional compared to 11 cells from non-immune donors. Incubation with mDCT27K did not increase the number of polyfunctional CD4 T lymphocytes for either non-immune or immune donors (for all, $P > 0.05$), although they did increase the supernatant concentrations of both IL-2 and IFN-γ for non-immune donors (for both, $P < 0.01$). Memory for polyfunctional T lymphocytes was assessed based on CD45RO positivity and expression of CCR7 using PBMC incubated with T27K and the mitogen PHA. While PHA induced a predominance of CCR7 cells, a mixture of CCR7+ and CCR7- CD4 and CD8 lymphocytes were noted when PBMC were incubated with T27K (for both, $P = 0.03$ compared to PHA). These data demonstrate that while mDCT27K can induce the release of Th1 cytokines, they do not increase the number of polyfunctional T lymphocytes seen in naturally immune donors and which are presumed to represent protective cellular immunity.

ABSTRACT 21: Multilocus Genotyping Among Clinical and Environmental *Coccidioides spp.* Isolates in Mexico

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Coccidioides immitis is believed to occur solely in an area comprising California and adjacent parts of northern Mexico, while *C. posadasii* can be found throughout the rest of the American southwest and Texas, Mexico, Central America, and northern South America 1,2. However, the current status of CM in Mexico is not widely known. In the present study we determined by molecular methods the species and the genotype of C*occidioides spp.* of clinical samples from various states of Mexico. A total of 47 *Coccidioides spp.* clinical isolates obtained from Universidad Nacional Autonoma de Mexico were inoculated in GYE 2x at 28 °C /5 d. Before DNA extraction, cultures were inactivated at 100 °C/15 min. DNA genomic was extracted using DNA kit Ultraclean MicrobialMobi. Specific identification and typing of the isolates was done by DNA sequencing analysis of fragments amplified by polymerase chain reaction (PCR) from the ribosomal RNA coccidioidal gene using oligonucleotides Coi9-1F/Coi9-1R developed by Umeyama 3 and by ten microsatellite loci developed by Fisher 4. PCR products from ribosomal gene showed a product of 720bp for *C. immitis* and 634bp for *C. posadasii*. PCR products of 720 pb were sequenced and aligned with those from *C. immitis* Ci45815 (*Accesion number* IFM45815) and 634 pb PCR products with C. posadasii Cp45810 (*Accesion number* IFM45810) using Clustal X5. Both types of PCR products showed a complete identity with *C. immitis* and *C. posadasii* sequences respectively. PCR products from microsatellites were resolved by electrophoresis of 2% agarose gels, and visualised by staining with gelstar under UV illumination. The size of each PCR product was determined by separation on a capillary based sequencer (ABI 3100 Genetic Analyser; Applied Biosystems). The inclusion of size standards (GS500;Applied Biosystems) allowed sizing of alleles by GeneMarker Software 1.71 (Softgenetics®). In total, 43 samples were identified as *C. posadassi* while 4 were C. immitis. The number of alleles per microsatellite loci for *C. posadasii* samples varied from one (locus ACJ) to ten (locus GA37), while for *C. immitis* the number of alleles per microsatellite loci for varied from one (loci KO1 and KO3) to 4 (locus GA37). Clinical samples of *C. posadasii* obtained from Coahuila and Monterrey were grouped in one clade, while some of samples which there are no origin data were grouped into the Coauhulia and Monterrey clade and the rest were grouped into a different clade. *C. immitis* samples were genetically distant from *C. posadasii* clade. Two of four samples of *C. immitis* came from Baja California. Our results are consistent with previous studies that *C. posadasii* has the widest distribution in Mexico.

ABSTRACT 22: Detection of *Coccidioides* Antigenemia Requires Dissociation of Immune Complexes

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During development of the MVista *Coccidioides* antigen assay, spike and recovery experiments were performed to assess the matrix effect of different body fluids. *Coccidioides* galactomannan was added to serum, urine, BAL, and CSF that were negative for *Coccidioides* antigen. Galactomannan concentration was measured in the MVista *Coccidioides* antigen assay. The concentration in the spiked specimen was 1.60 ng/mL in CSF, 1.10 ng/mL in BAL, 1.33 ng/mL in urine but undetectable in serum. To determine if dissociation of immune complexes would permit detection of antigenemia, 300 µL of spiked or negative serum and 100 µL 0.1 M EDTA were mixed and heated at 100°C for 6 minutes, then centrifuged at 10,000 x g for 10 minutes. The optical density of the negative control was 0.020, the untreated serum was 0.079, and the EDTA-heat treated serum was 0.845. These findings suggest that EDTA heat treatment is necessary for detection of *Coccidioides* antigenemia.

ABSTRACT 23: SNP-Based Genotyping in *Coccidioides*

Driebe E, Sheff K, Pearson T, Colvin J, Beckstrom-Sternberg S, Barker B, Rounsley S, Fang R, Keim P, Engelthaler DM

The Translational Genomics Research Institute, Northern Arizona University, and Applied Biosystems

Advanced genotyping systems should include phylogenetically informative markers. Single nucleotide polymorphisms (SNPs) have been shown to be more stable and more phylogenetically informative than other molecular markers, such as microsatellites. Comparative SNP analysis has proven to be remarkably effective at defining population structure as well as establishing molecular profiles or "fingerprints". There are challenges, however, with using SNPs in recombining organisms, (i.e., *Coccidioides*) as a result of character state conflicts or homoplasy, arising from convergence, reversals and/or lateral gene transfer. These challenges can be overcome through the use of large data sets and appropriate algorithms. Here we describe our progress in developing a comparative whole genome SNP genotyping system for *Coccidioides*. We analyzed 14 available Coccidioides whole genomes and identified 8,412 SNPs within *C. posadasii*. Our initial multiplexed SNP screening system was Applied Biosystems' GenPlex platform and allowed us to screen 78 SNPs across 96 strains of *C. posadasii*. Phylogenetic analysis showed clear subpopulations, tied to geographic location, with significant phylogenetic separation between geographic groups. However, our limited number of SNPs did not allow the resolution necessary to clearly define phylogenetic branching and provide informative genotyping. Our future plans include: more whole genome sequencing to improve SNP discovery; using larger SNP sets (N=1000+), and screening across larger repositories of strains (400+).

ABSTRACT 24: Clinical Impact of False Positive IgM Serological Results In Coccidioidomycosis

Tim Kuberski, MD, Judith Herrig, MT (ASCP) and D. Pappagianis, MD

The Premier® *Coccidioides* enzyme immunoassay is a commercially available serological test used for the diagnosis of coccidioidomycosis. It is used for the detection of both IgM and IgG by enzyme immunoassay (EIA) for antibodies directed against Coccidioides. Early studies on the Premier® assay suggested it may be subject to false positive IgM results. Patients with a false positive IgM EIA for coccidioidomycosis are thought to be experiencing acute infection, but actually may not have coccidioidomycosis. The clinical observation has been made that the IgM false positive results with the Premier® EIA test may distract from a correct clinical diagnosis.

A sample of 17 sera from patients with a IgM positive and IgG negative serological result using the Premier® *Coccidioides* EIA were studied to estimate the frequency of this observation and correlate the results with clinical findings. A split serum sample on the 17 patients was sent to a reference laboratory at the University of California School of Medicine, Davis, California to compare serological results. The medical records of the 17 patients were then reviewed several months after the serum samples were obtained and a consensus diagnosis made as to the presence or absence of coccidioidomycosis before the reference laboratory results were known.

There were 3 patients (18%) felt to have an acute *Coccidioides* infection by chart review. Only one of these was found to have an IgM positive and IgG negative EIA result by both the clinical and reference laboratories supporting the diagnosis of pneumonia and acute coccidioidomycosis. The two other patients had pneumonia, but it was indistinguishable from either coccidioidomycosis or other community acquired pneumonia; however, their positive IgM and negative IgG was not confirmed by the reference laboratory. Of the 17 patients, one had a positive IgG test at the reference laboratory and was considered a case of coccidioidomycosis, making a total of only 4/17 who had coccidioidomycosis. Of the 17 study patients, seven (41%) had a diagnosis of pneumonia, five (29.5%) with fever of unknown origin (FUO) and five patients (29.5%) with other diagnoses.

The clinical laboratory performed 2,139 EIA serologies for coccidioidomycosis in 2008; of these, there were 104 (5%) with a positive IgM and negative IgG result. Extrapolating that 13/17 (76%) were falsely positive in this sample suggests that 79 patients in 2008 may not have had acute coccidioidomycosis. If the estimated 76% false positive IgM rate can be confirmed it would have a significant impact and would suggest that the EIA IgM test as it is currently performed is clinically not very useful.

ABSTRACT 25: Most Unusual Cases of Coccidiodomycosis – Mediastinal Fibrosis Causing Superior Vena Cava Obstruction Secondary to Coccidiodomycosis

Chitra Damodaran MD
Infectious Disease, Loma Linda VA Medical Center, CA

Case report: 27 year old Hispanic male living in Mentone, CA served in Irag from 2003-2004, was diagnosed in November 2005 with pulmonary Coccidiodomycosis, by BAL. Cocci titer was 1:8. CT chest showed focal consolidation in the R upper lobe with numerous foci of ground glass opacification in the peribronchial region and extensive mediastinal and hilar adenopathy. CT guided biopsy of supra hilar lymphnode revealed granulomatous reaction with fibrosis and chronic inflammation. He was started on Fluconazole but after 2-3 months he stopped taking it and lost for follow up. He continued to have chest pain on the right side. Restarted Fluconazole in June 2006 for about 5-6 months.

November 2008 presented with 2 month history of dyspnea on exertion, right sided chest pain, night sweats. Headache for 1 month. Felt pressure at the R ear with" popping" sensation. His face turned purple when he was playing basketball and had to sit down. Felt dizzy intermittently. Visual changes noted with multiple colors at the periphery of the eyes. No cough or hemoptysis. CT chest showed 4 cm mass encasing the SVC with greater than 90% stenosis of the SVC and multiple right upper chest collateral flow seen. He was admitted November 25, 2008, to Loma Linda VA Hospital.

On exam there was facial edema and swelling of the right side chest with collaterals visible.

Cocci titer CF:

12/2/08	1:2
11/21/08	1:4
6/5/08	1:4
12/27/05	1:4
11/8/05	1:8

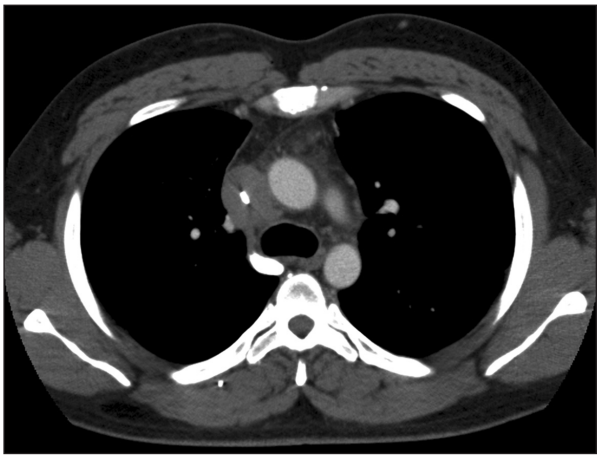
Hospital course: He was started on iv Heparin.and Liposomal amphotericin B. He underwent CT guided biopsy of the mass encasing the SVC by IR. Biopsy showed fibrotic scar tissue with chronic inflammation consistent with fibrosing mediastinitis. PAS stain was negative for fungal elements. After the pathology report he was switched to Fluconazole. The shortness of breath improved after starting Heparin. On December 8, 2008, he underwent stent placement at the SVC stenosis. Postoperatively the dyspnea and facial edema improved but still had some headaches. Collaterals were less prominent.

(Continued)

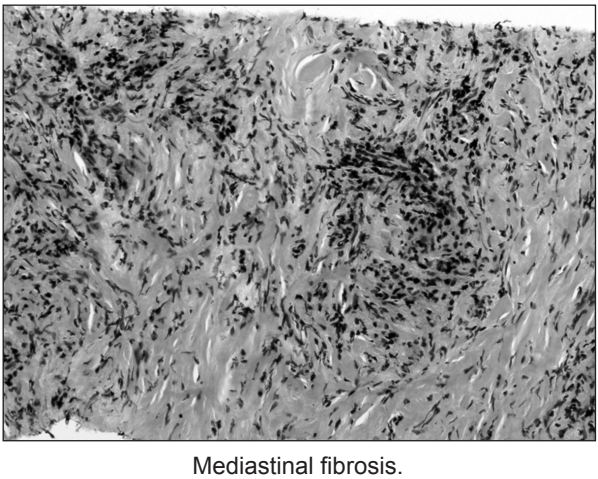
ABSTRACT 25 *(Continued)*: **Most Unusual Cases of Coccidiodomycosis – Mediastinal Fibrosis Causing Superior Vena Cava Obstruction Secondary to Coccidiodomycosis**

Chitra Damodaran MD
Infectious Disease, Loma Linda VA Medical Center, CA

Comments: Mediastinal fibrosis has not been commonly described in Coccidiodomycosis. There was one case report presented as an abstract at the 2008 Coccidiodomycosis focus group meeting. This entity may be more common than currently recognized and need to be considered.



4 cm mass encasing the SVC causing obstruction.



Mediastinal fibrosis.

POSTER ABSTRACT 1: Molecular Cloning and Expression of Two β-N-Acetylglucosaminidase Enzymes of Coccidioides Posadasii

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β-N-acetylglucosaminidase activity has been detected in the protective subcellular coccidioidal T27K vaccine as well as in two protective subcomponents (GFI and AE3). These findings led to the consideration of the enzyme as a potential vaccine candidate and experiments were initiated to characterize the source of the β-N-acetylglucosaminidase enzyme activity. Molecular cloning experiments resulted in the isolation of two full-length cDNAs that encode for two predicted proteins (CpHex1 and CpHex2) that share homology with fungal β-N-acetylglucosaminidases (NAGs). The CpHex1 cDNA encodes a 595 amino acid polypeptide with a calculated molecular mass of 68 kDa that shares high homology with the NAGs from *Aspergillus nidulans*, A*spergillus oryzae* and *Penicillium chrysogenum*. The CpHex2 predicted protein consists of 603 amino acids, has a calculated molecular mass of 68.5 kDa and shares homology with the β-N-acetylglucosaminidases from *Paracoccidioides brasiliensis* and *Trichoderma spp*. Both deduced proteins have conserved features characteristic of the glycosyl hydrolase family 20 β-N-acetylglucosaminidases including the amino acid pair (aspartate and glutamate) that is required for catalytic activity. Despite the structural similarities, the CpHex1 and CpHex2 predicted proteins share only 23% identity with each other and have dissimilar homologies sharing more identity with other fungal β-N-acetylglucosaminidases and the human and mouse β-N-acetylhexosaminidases than with each other. A phylogenetic analysis of selected fungal NAG proteins placed CpHex1 in a group with the NAGs from *A. nidulans, A. oryzae, P. chrysogenum* and *Candida albicans* and CpHex2 in a cluster with NAGs from *P. brasiliensis* and *Trichoderma spp*. RT-PCR analysis of the transcripts encoding CpHex1 and CpHex2 showed that the CpHex1 transcript was more abundant than the *CpHex2* transcript during SE phase and that the temporal pattern of the *CpHex1* transcript correlated with enzyme activity. The addition of N-acetylglucosamine to SE phase cultures induced expression of the *CpHex1* transcript and caused an increase in β-N-acetylglucosaminidase activity. A native β-N-acetylglucosaminidase enzyme was purified from SE phase spherules and culture supernatant and identified as CpHex1 by mass spectrometric analysis.

POSTER ABSTRACT 2: Coccidioidal Fungemia

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Coccidioidomycosis is an endemic fungal infection in the Southwestern United States. Sites of dissemination commonly include the skeletal system, skin, and central nervous system (CNS). *Coccidioides* fungemia (CF) is an uncommon manifestation of disseminated infection. The microbiology database at Mayo Clinic Hospital, Phoenix, AZ, was explored to identify all episodes of CF between 1998 and 2008. Medical records of identified cases were reviewed in detail. We subsequently conducted a comprehensive review of the English language literature from 1967 to 2008 to identify and describe previously reported cases of CF. Six cases of CF were identified at MCH between 1998 and 2008. Three of our patients had diabetes mellitus (DM), 2 were receiving steroids, and the remaining patient had chronic vasculitis. Three patients (50%) died. Review of the literature is currently ongoing and, to date, 70 additional cases have been identified. Out of the 76 cases of CF (including cases at MCH), mean age was 42.7 years. Thirty eight (56%) were Caucasian, and 86% were male. Thirty-seven (49%) had HIV infection, 18 (24%) were immunosuppressed (10 received chemotherapy, 5 had organ transplantation), 6 (8%) had DM, and 2 (2.6%) were pregnant. Thirty patients had isolated pulmonary involvement, 12 had CNS infection, 11 each had skeletal and liver lesions, and 7 had cutaneous involvement. In addition to blood, *Coccidioides sp.* was isolated from sputum and/or bronchoalveolar lavage in 49 patients (65%). Fifty two patients (68%) died. Majority of cases originated in Arizona (79%). Coccidioidal fungemia is a marker of severe disseminated *Coccidioides* infection. Majority of patients with CF were immunosuppressed, and the disease was associated with high mortality.

POSTER ABSTRACT 3: The Spectrum and Presentation of Disseminated Coccidioidomycosis

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Purpose. Extrapulmonary dissemination of *Coccidioides* species is associated with significant morbidity and mortality. The clinical manifestations vary widely according to the host, the severity of illness, and location of dissemination. The morbidity and mortality can be reduced by early recognition and treatment, which in turn depends on understanding the spectrum and presentation of disease.

Methods. We performed a retrospective analysis of 150 cases with extra-pulmonary non-meningeal disease seen from 1996 to 2007 at a referral medical center in an endemic region.

Results. Hematogenous dissemination was associated with high mortality and occurred primarily in immunocompromised patients, but only 30% of patients with more limited forms of dissemination were immunocompromised. In keeping with prior studies, there was a preponderance of males (nearly 2:1) and people of African or Asian (especially Pacific Islanders) descent. In contrast, Hispanics and diabetics were not at increased risk. Serology was frequently negative in immunocompromised patients, but the diagnosis could be established by isolation of the organism in culture, or in histologic or cytologic specimens.

Conclusions. Although coccidioidomycosis is a great imitator, the diagnosis can usually be made readily if a high level of suspicion is maintained and appropriate diagnostic testing is performed. In most patients, that will include serologic testing in addition to cultures and histology or cytology of appropriate samples.

POSTER ABSTRACT 4: Coccidioidal Meningitis in the Post-Fluconazole Era

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Since the early 1980s, the management of coccidioidal meningitis (CM) has changed considerably with a shift from intrathecal amphotericin B to azole-based therapies. This paper is the first, large-scale study comparing the clinical presentation and management of *Coccidioides immitis* meningitis in the azole era (post-1980) to a cohort of patients from the pre-azole era. We reviewed 30 CM cases seen at three Los Angeles hospitals between the years 1993 to 2008 (2008 cohort) and compared them to 31 patients (1980 cohort) described by Bouza et al.(Medicine [Baltimore] May; 60(3):139-72, 1981). The demographics and clinical presentation of patients in the current cohort were similar to the previous study except for a higher prevalence of Hispanic patients (2008: 53% vs. 1980: 6%) and a greater percentage of patients with underlying, predisposing clinical conditions (2008: 66% vs. 1980: 32%). Ten patients in the 2008 cohort had HIV/AIDS, a condition not reported in the earlier study. Laboratory findings were similar between the two groups except for a lower incidence of peripheral leukocytosis and eosinophilia in the current group. There were marked differences in drug treatment between the two eras. In the 2008 cohort, a total of 29 patients received fluconazole therapy; 13 of these patients were treated with fluconazole monotherapy and 16 patients received a combination of fluconazole and intravenous amphotericin B. Although almost all the patients (29/31) in the 1980 cohort received intrathecal amphotericin B, only three patients in the 2008 study received amphotericin B via this route. With respect to complications of CM, a similar percentage of patients in either cohort developed complications such as stroke and hydrocephalus. The 2008 cohort (40%) had similar mortality when compared to patients in the 1980 study (39%) with both groups experiencing significant impairment of activities of daily living (ADL). Although recommended as first-line therapy for CM, azole-based therapies are not curative and do not necessarily prevent complications associated with the disease. *Coccidioides* meningitis remains a serious illness with a high rate of morbidity and mortality. Immunocompromised individuals, especially those with HIV/AIDS, are especially at risk for CM and represent a greater share of the overall population with this condition. Despite the clear advantages of azole treatment in CM, new therapeutic approaches are needed in order to provide definitive cure and reduce the need for long-term suppressive therapy.

POSTER ABSTRACT 5: Fungus Involved in Posada's Case (1892)

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In 1892 Alejandro Posadas described the first worldwide case of coccidioidomycosis in a patient named Domingo Escurra. A preserved necropsy piece from the patient's remains is conserved in the Museum of Pathology of the Medical School, Buenos Aires University. Paraffin-embedded specimens obtained from this piece served to identify the fungus involved in the case. Histological slices from different lesion sites were submitted to a genus-specific immunohistochemical staining in order to select the more suited areas in terms of abundance/integrity of fungal spherules and endospores. Fungal DNA was amplified from selected deparaffinated slices using a nested PCR designed to amplify a segment of the gen *Ag2/PRA* and differentiate *C. immitis* from *C. posadasii*. This PCR was also applied to two reference strains (*C. immitis* M38-05, C. *posadasii* 1-NL) and isolates obtained from four recent coccidioidomycosis cases occurred in Argentina. Amplified products were submitted to sequencing of both DNA strands. The obtained sequences were edited, aligned and compared with *C. posadasii* (Access Nº AY536446, strain Silveira) and *C. immitis* (Access N° AY536445) deposited in GeneBank. DNA sequences from Escurra's lesions were 100% homologous to the recent Argentinean cases and the reference strain 1-NL. A single point C→G difference in position 1228 was observed with respect to sequence of strain *C. posadasii* Silveira. For the first time, *Coccidioides* DNA is recovered from a museum piece which is more than 100-years-old. Our results confirm that the original case of Posadas's disease was caused by the recently described *C. posadasii*.

POSTER ABSTRACT 6: Genetic Diversity Among *Coccidioides spp* Isolates from Mexico and Argentina

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Coccidioidomycosis is an endemic disease in the Americas. Currently, the two recognized etiologic agents *Coccidioides immitis* and *C. posadasii*, are distinguishable only through molecular techniques. Preliminary studies suggest the existence of intra-species diversity, but ignore the distribution of genotypes in different geographical areas in Latin America.

For to know the genetic relationships between *Coccidioides spp* isolates, we analized 11 Argentine's clinical isolates and 15 from Mexico with AFLP technique using four primer combinations : Eco+AA:*Mse*CTC; *Eco*+AA:*Mse*+CAT; *Eco*+AA:Mse+CTG and Eco+AA:*Mse*+CTG.

With the polymorphic patterns obtained, a dendrogram was generated in which we appreciate two defined groups corresponding to each species.

Analysis of results show that isolates from Argentine's patients generated a monomorphic pattern among them, probably originated from a single clone, unlike the isolates from Mexico who had higher genetic diversity.

Poster Abstracts Presented But Not Submitted

POSTER ABSTRACT 7:
Differentiating *C. Posadasii* and *C. Immitis* in Real-Time
Sheff K, Driebe E, York E, Waddell V, Barker B, Keim P, Engelthaler DM

POSTER ABSTRACT 8:
Treatment of Disseminated Coccidioidomycosis with Voriconazole
Adam RD

POSTER ABSTRACT 9:
A Case-Control Study of Distance to New Construction as a Risk Factor for Coccidioidomycosis in Antelope Valley, CA, 2004-2006
Peterson A, Guevara RE, Terashita D

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	





• Publishing courtesy of Mayo Clinic in Arizona •