Coccidioidomycosis
STUDY GROUP

Proceedings of the Fifty Second Annual Coccidioidomycosis Study Group Meeting

2008

April 5, 2008 • University of California San Diego • San Diego, California

Proceedings of the Fifty Second Annual Coccidioidomycosis Study Group Meeting

Meeting Number 52
April 5, 2008
University of California San Diego
San Diego, California

Antonino Catanzaro, M.D.
Chairperson

Janis E. Blair, M.D.
Secretary

Catalogued by the National Library of Medicine

Address editorial correspondence to:
Janis E. Blair, M.D.
Mayo Clinic Arizona
5777 East Mayo Boulevard
Phoenix, Arizona 85054

Meeting Program

7:30 a.m. **Registration & Coffee**

8:00 a.m. **Welcome and Announcements**

8:30 a.m. **Session I: Epidemiology, Environment, Fungal Biology**
Moderator: Tom Chiller

1. The *Coccidioides* Endemic Zone: Boundaries and Extremes
   F.S. Fisher, S.M. Johnson, M.W. Bultman, D. Pappagianis
2. Effects of Climate and Land Surface Conditions on Coccidioidomycosis Incidence in Arizona
   A. Comrie, J. Tamerius, M. Glueck, S. Pianalto, P. Stacy, S. Yool
3. Valley Fever in Persons with Community-Acquired Pneumonia in Arizona
   S.E. Hoover, W. Bannister, J.N. Galgiani
4. Cocci: Renewing Public Health's Commitment
   T. Chiller, B. Park
5. Update from the California Department of Public Health: Rising Rates of Coccidioidomycosis, Outbreak Investigations, and Special Projects
   C.Wheeler, A. Karon, K. Cummings, D. Vugia, J. Mohle-Boetani
6. Arizona Coccidioidomycosis Surveillance: Improving Assessment of Disease Burden and Impact
   R.H. Sunenshine, S. Anderson, L. Erhart, S. Chen, B. Park, B. Casimir, T. Chiller, K. Komatsu
7. Coccidioidomycosis: Knowledge, Attitudes, and Practices Among Health Practitioners – Arizona 2007
   S. Chen, S. Anderson, L. Erhart, K. Komatsu, R. Miramontes, B. Park, T. Chiller, R. Sunenshine
8. Antibody Seroprevalence Against *Coccidioides* spp. in Patients with Clinical Diagnosis of Tuberculosis from Ensenada, Baja California, Mexico
   R.C. Baptista Rosas

10:00 a.m. **Break**

10:15 a.m. **Session II: Vaccine and Treatment**
Moderator: Karl Clemons

9. *Saccharomyces* as a Vaccine Against Coccidioidomycosis
   J. Capilla, K.V. Clemons, H.B. Levine, D.A. Stevens
10. Isolation and Evaluation of a Glycoprotein From the Coccidioidal Vaccine T27K
    S.M. Johnson, N.M. Ampel, L.A. Nesbit, C.N. Miller, D.Pappagianis
11. Multiparametric Flow Cytometry to Assess Coccidioidal Antigens as Potential Vaccine Candidate
    L. Nesbit, N.M. Ampel
12. Comparison of ABLC and AmBisome for the Treatment of Coccidioidal Meningitis in a Rabbit Model
    K.V. Clemons, J. Capilla, A.J. Tong, M. Martinez, D.A. Stevens

Meeting Program

11:15 a.m. **Session III: Unusual Cases of Coccidioidomycosis**
Moderator: Rafael Laniado-Laborin

13. Disseminated Coccidioidomycosis Complicated by Splenomegally
    A. Heidari
14. A Chest Wall Problem: Empyema Necessitatis
    L.A. Rendon Perez
15. Cocci in the Heart
    S. Hoover
16. Most Unusual Cases of Coccidioidomycosis: LUL Mass and Mediastinal Fibrosis
    S. Kasperbauer

12:15 p.m. **Lunch**

- Coccidioidomycosis Vaccine Project: All interested parties will be meeting informally to discuss current status and future projects. Location to be announced.

1:15 p.m. **Business Meeting**

- Coccidioidomycosis Study Group Bylaws
- Next Year's Date
- Storage of Historic Cocci Publications

1:45 p.m. **Session IV: Diagnostics**
Moderator:

17. Proteomic Identification of Coccidioidal Antigens from Lung Fluid of Infected Mice: the Search for Diagnostic Markers of Valley Fever
    F.S.E. Helfrich, L.F. Shubitz, A. Hilderbrand, D.M. Magee, D.F. Lake, N.M. Ampel, V. Wysocki, J.N. Galgiani
18. Counting Cocci: Quantitation of *Coccidioides* Using Real-Time PCR
    E. Driebe, C. Liu, J. Bowers, M. Schmoker, C. Bosch, D.M. Engelthaler
19. Coccidioidal Serologic Findings with Body Fluids Other Than Blood Serum
    D. Pappagianis
20. Diagnosis of Coccidioidomycosis Using the MVista™ *Coccidioides* Antigen Enzyme Immunoassay (EIA)
    M. Durkin, P. Connolly, L.J. Wheat
21. Ultrasonography in the Evaluation of Canine Coccidioidomycosis
    A.P. Davidson, T.W. Baker

3:15 p.m. **Break**

3:30 p.m. **Session V: Clinical Coccidioidomycosis**
Moderator: Peter Kelly

22. A Prospective Cohort Analysis of the Decision to Treat or Not Treat Primary Coccidioidal Pneumonia
    N.M. Ampel, A. Giblin, J. Mourani, J.N. Galgiani

23. Continuous Intrathecal Amphotericin B (AB) for Relapsed *Coccidioides(C) immitis* Meningitis (M)
    C. Berry, et al.
24. Impregnation of a Ventriculoperitoneal Shunt with Amphotericin B Deoxycholate Used in Meningitis Due to Coccidioidomycosis
    T. Kuberski, V. Ianas

4:30 p.m. **Adjourn**

Poster Session

1. Agent-based Modeling of Physical Factors That May Control the Growth of *Coccidioides* in Soils
   M.D. Gettings, F.S. Fisher

2. A Fluorescent Microsphere Immunoassay for Detection of Antibodies to *Coccidioides* Species
   S.J. Wong, T. Victor, D. Pappagianis, V. Chaturvedi

3. The Utility of *Coccidioides* PCR in Clinical Specimen
   D. Vucicevik, J.E. Blair, et al.

4. Surgical Consideration in Pulmonary Coccidioidomycosis: 10 Year Experience and Review of the Literature
   W.J Halabi, D.E Jaroszewski, J.E.Blair, R. Wong, J.Parish, P.A DeValeria, L. Lanza, V. Trastek, F.A. Arabia

5. Evaluation of Environmental Samples Collected in Utah, Arizona and California for the Presence of *Coccidioides*
   S.M. Johnson, F.S. Fisher, C.N. Miller, D. Pappagianis

6. Community-Based Epidemiological Study of Valley Fever in Tucson, Arizona
   J.A. Tabor, M.K. O'Rourke, M.D. Lebowitz

7. Environmental Search for *Coccidioides* spp. in Soil Samples of Endemic Areas from Baja California, Mexico
   R.C. Baptista Rosas

8. Comparison of Two Coccidioidins in an Endemic Region in Mexico
   H.A. Avila, A. Calleros, V.E. Castañeda, E. Chávez, C. Escobar, N. Gallegos, J. González, J.A. Limón, J.E. Paniagua, D.M. Rosales, I.A. Zepeda, R. Laniado-Laborín

9. *In silico* Genome Analysis
   D.M. Engelthaler, et al.

ABSTRACT 1: The *Coccidioides* Endemic Zone: Boundaries and Extremes

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

The *Coccidioides* Endemic Zone

Fisher, F.S.[1], Johnson, S.M.[2], Bultman, M.W.[3], Pappagianis, D.[2]
[1]Univ. of AZ; [2]Univ. of CA, Davis; [3]U.S. Geological Survey

The most important factors that define the endemic zone (EZ) of *Coccidioides* are soil temperature and soil moisture. Clearly these factors are related to the ambient air temperature and amount of precipitation respectively. However many other local factors affect soil temperatures; such as soil color, vegetation types and densities, sun aspect, thermal conductivity, depth in soil profile, and the soil moisture content. All of the of the known *Coccidioides* growth sites where we have measured soil temperatures and also all of the mapped sites throughout the southwestern United States are within soils classified as hyperthermic arid, or thermic arid or thermic semi-arid (fig. 1). Our data suggest that soils, at depths of 20 - 30cm and within a temperature range of 10° to 40° C, are most favorable for the growth of *Coccidioides*.

Precipitation, mostly in the form of rain, is the main factor determining the moisture supply needed for *Coccidioides* growth. However, the amount of water that infiltrates the soil to depths where *Coccidioides* may be present depends on the amount lost to runoff, which is controlled by the topography of the ground surface. Infiltration also depends on the surface roughness, soil texture, preexisting water content, and the soil content of salt, clay, and organic material. Moisture in the upper 20 - 30 cm of the soil profile is essential for the growth of *Coccidioides*; however, the actual amount required, is unknown at this time. Other authors have suggested that soil humidities between 56% and 90% are sufficient for *Coccidioides* growth. On a regional scale, precipitation, across Texas from west to east, increases from 20 cm in the west to140 cm in the easternmost parts of the state. The highly EZ in Texas (fig.1), (where precipitation is 30 to 50cm), extends southeastward along the Pecos River valley to the junction with the Rio Grande River valley which in turn, is highly endemic to about 140 kilometers from the coast. The eastern boundary of the EZ in Texas is gradational and controlled by higher (>80-90 cm) soil moisture regimes in the central parts of the state. If the central part of Texas becomes drier, leading to lower soil moistures, the EZ may migrate further east into heavily populated areas (e.g. Dallas/Fort Worth) exposing well over six million people to possible infection.

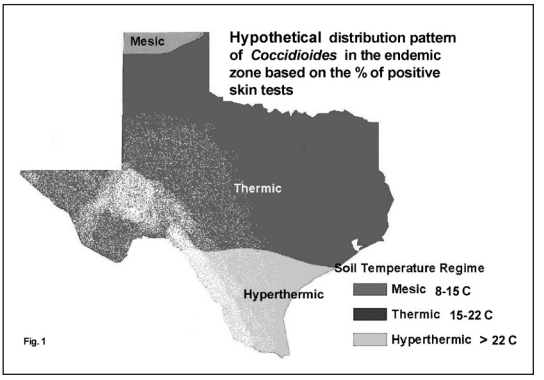


Fig.1

ABSTRACT 2: Effects of Climate and Land Surface Conditions on Coccidioidomycosis Incidence in Arizona

A. Comrie, J. Tamerius, M. Glueck, S. Pianalto, P. Stacy, S. Yool

Effects of Climate and Land Surface Conditions on Coccidioidomycosis Incidence in Arizona

Comrie A, Tamerius J, Glueck M, Pianalto S, Stacy P and Yool S
Department of Geography and Regional Development
University of Arizona, Tucson AZ 85721

Valley Fever (coccidioidomycosis) is a disease endemic to arid regions in the Western Hemisphere, and is caused by the soil-dwelling fungi, *Coccidioides immitis* and *Coccidioides posadasii*. Arizona is currently experiencing an epidemic with about 5000 cases annually. Our work seeks to further understanding of the relationships between surface disturbances, climate variability and other environmental factors that may interact to produce coccidioidomycosis outbreaks. Specifically, we are developing seasonal climate-based models and associated databases to improve basic knowledge of the disease ecology, and which will hopefully help anticipate coccidioidomycosis outbreaks and improve public health actions to mitigate them. These models describe the climate contribution to incidence and to epidemics, and they specify the covariability of atmospheric dust levels and coccidioidomycosis incidence. In final form, they enable seasonal forecasts of disease incidence by geographic area, utilizing satellite-derived spatial data on surface moisture, land cover change and disturbance along with observed climate records. Preliminary regression-based climate modeling results have shown that about 80% of the variance in seasonal coccidioidomycosis incidence for southern Arizona can be explained by precipitation and dust-related climate scenarios prior to and concurrent with outbreaks. Our results indicate that while climate, alone, cannot account for the recent broad increasing trend in incidence, precipitation and dust remain useful predictors. Furthermore, results confirm that climate and associated fluctuations in seasonal and year-to-year environmental factors account for much of the coccidioidomycosis incidence variability about the trend from 1995-2006.

ABSTRACT 3: Valley Fever in Persons with Community-Acquired Pneumonia in Arizona

S.E. Hoover, W. Bannister, J.N. Galgiani

Valley Fever in Persons With Community-Acquired Pneumonia in Arizona

Susan E. Hoover, MD[1], John N. Galgiani, MD[1], Wade Bannister, PhD[2]
[1]University of Arizona College of Medicine and Valley Fever Center for Excellence, Tucson, AZ; [2]Arizona State University Ira A. Fulton College of Engineering, Tempe, AZ

A small prospective study found that up to 29% of patients with community acquired pneumonia (CAP) syndromes in Tucson, Arizona were seropositive for coccidioidomycosis (valley fever). This finding led to a recommendation in 2006 by the Arizona Department of Health Services urging physicians to test patients with CAP for valley fever. Another small study, however, suggested that Arizona physicians rarely test such patients. We used Arizona Health Query, a large database of healthcare information on Arizonans, to examine diagnoses of CAP and valley fever among persons insured by Arizona Medicaid from 2004 to 2006. In total, there were approximately 125,000 CAP diagnoses and 3,500 valley fever diagnoses among approximately 3 million Medicaid enrollees. However, only 3.9% of persons with a diagnosis of CAP had valley fever testing (serology or fungal culture) within 90 days of the diagnosis. Interestingly, only 35% of persons with a diagnosis of valley fever had valley fever testing within 90 days. Among persons with CAP, 821 received a diagnosis of valley fever within 90 days of the CAP diagnosis (CAP-VF cohort). When compared with all persons with CAP, the CAP-VF cohort tended to be younger and had a greater percentage of males. Our data suggest that only a very small percentage of patients with CAP in Arizona are being tested for valley fever, and that physicians may be making the diagnosis of valley fever without supportive laboratory testing.

ABSTRACT 4: Cocci: Renewing Public Health's Commitment

T. Chiller, B. Park

Abstract presented but not submitted.

ABSTRACT 5: Update from the California Department of Public Health: Rising Rates of Coccidioidomycosis, Outbreak Investigations, and Special Projects

C..Wheeler, A. Karon, K. Cummings, D. Vugia, J. Mohle-Boetani

Coccidioidomycosis Surveillance for 1990–2007 Shows Increase in Cases 2001–2006

Wheeler C[1], Karon A[1,2], Cummings K[1], Vugia D[1], Mohle-Boetani J[1]
[1]California Department of Public Health, Richmond, California; [2]Centers for Disease Control and Prevention, Atlanta, Georgia

Background: Coccidioidomycosis cases have been physician-reportable in California for the past two decades. Additionally, California's local health departments (LHDs) are required to report disease outbreaks to the California Department of Public Health (CDPH).

Methods: We analyzed coccidioidomycosis case reports for 1990–2007 from the CDPH surveillance system. We estimated disease rates by using denominators derived from population estimates published by the California Department of Finance. We searched the CDPH surveillance system for 1990–2007 for coccidioidomycosis outbreak reports, and CDPH disease investigation files 2001–2007 for coccidioidomycosis outbreak investigation descriptions.

Results: After the epidemic of the early 1990s, rates of coccidioidomycosis declined to approximately 2.5 cases/100,000 population. Coccidioidomycosis rates then increased continuously during 2001–2006 (P<.0002 by linear regression) to 8.4/100,000 population. In 2007, the rate decreased to 7.7/100,000 population (Figure 1). During 2001–2006, a total of 77% of cases were reported from local health jurisdictions in the hyperendemic region of the San Joaquin Valley, and the 40–49-year age group was most affected (Figure 2).

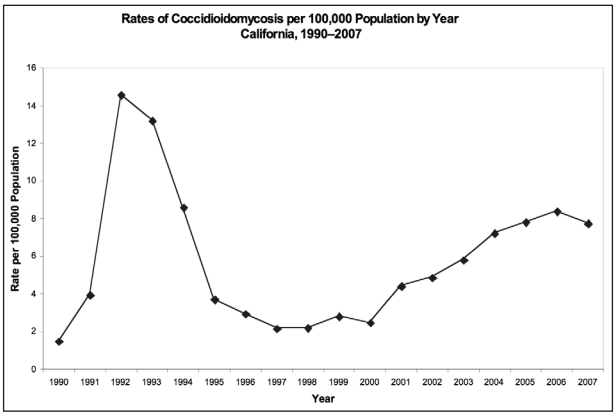
Outbreaks: No coccidioidomycosis outbreaks were reported through the CDPH surveillance system during 1990–2007. However, descriptions of four coccidioidomycosis outbreaks were identified in our disease investigations files for 2001–2007. All outbreaks occurred in known endemic areas and occurred in the following contexts: among attendees of a model airplane show in 2001; among inmates in correctional facilities during 2004–2005; and among a construction crew in 2007.

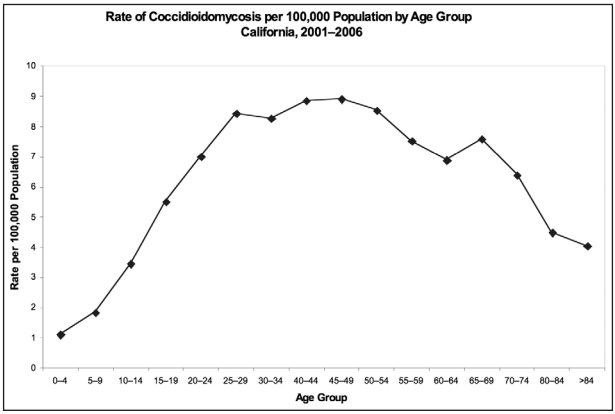
Summary: During 2001–2006, a >3-fold increase occurred in coccidioidomycosis rates, compared with the rate in 2000. The age group most affected was the 40–49-year group, and outbreaks occurred in endemic areas.

Public Health Actions: CDPH is evaluating surveillance methods for best estimating the burden of coccidioidomycosis. Laboratory reporting of coccidioidomycosis is being considered, and LHDs will be encouraged to report coccidioidomycosis outbreaks. CDPH is developing educational messages for persons at risk for serious illness and guidelines for construction crews working in endemic areas. California continues to support the development of a coccidioidomycosis vaccine.

ABSTRACT 5 *(continued)*: **Update from the California Department of Public Health: Rising Rates of Coccidioidomycosis, Outbreak Investigations, and Special Projects**

C..Wheeler, A. Karon, K. Cummings, D. Vugia, J. Mohle-Boetani





ABSTRACT 6: Arizona Coccidioidomycosis Surveillance: Improving Assessment of Disease Burden and Impact

R.H. Sunenshine, S. Anderson, L. Erhart, S. Chen, B. Park, B. Casimir, T. Chiller, K. Komatsu

Arizona Coccidioidomycosis Surveillance: Improving Assessment of Disease Burden and Impact

R.H. Sunenshine, S. Anderson, L. Erhart, C. Tseng, S. Imholte, S. Chen, B. Park, B. Casimir, T. Chiller, K. Komatsu
[1]Arizona Department of Health Services, Phoenix Arizona; [2]Centers for Disease Control and Prevention, Atlanta, GA

Background: Coccidioidomycosis (Valley Fever) is an emerging fungal disease endemic to the Southwestern US, Central and South America; 60% of known US cases occur in Arizona, where physician and laboratory reporting is mandatory. To improve case definition sensitivity, the Council of State and Territorial Epidemiologists (CSTE) voted in 2007 to include clinical cases diagnosed with a single rather than rising titer of coccidioidal immunoglobulin G. Arizona's simpler case definition is similar, but does not require correlation with clinical symptoms. To evaluate Arizona's coccidioidomycosis surveillance system, identify issues contributing to delayed or under-reporting, and assess its impact on Arizonans, we collected additional clinical and diagnostic data.

Methods: We telephoned every tenth coccidioidomycosis case reported to the Arizona Department of Health Services in 2007 to conduct interviews using a standardized questionnaire. If the case could not be reached after ≥3 attempts, the subsequent reported case was contacted.

Results: Of 4871 cases reported, 427 were successfully interviewed (87% of targeted sample). 97% of cases reported ≥1 symptom consistent with coccidioidomycosis at diagnosis [positive predictive value (PPV) 97%]and 95% met the CSTE case definition. Case-patients waited a mean of 51 days from symptom onset before seeking medical care. A mean of 3.0 provider visits occurred before providers ordered coccidioidomycosis diagnostic testing; 16% of case-patients reported initiating the request for testing themselves.

Of interviewed cases, 42% went to the ER for their illness and 40% were hospitalized overnight for the disease. Symptoms lasted a mean of 69 days. More than 64 million dollars were charged by hospitals for visits with a primary diagnosis of coccidioidomycosis in 2006 (>92 million dollars for primary and secondary coccidioidomycosis diagnoses). Three quarters of patients missed work and were unable to perform activities of daily living due to the disease with a mean of 33 workdays missed and about three months of interference with daily activities due to their illness.

Conclusions: Elimination of the requirement for clinical criteria from the coccidioidomycosis case definition simplifies surveillance, has little effect on PPV, and may facilitate implementation of mandatory coccidioidomycosis reporting in other endemic states, thereby improving assessment of disease burden. Significant delays in coccidioidomycosis diagnosis occur due to provider and patient practices. Coccidioidomycosis has a tremendous impact on Arizonan's healthcare system, economy, and quality of life.

ABSTRACT 7: Coccidioidomycosis: Knowledge, Attitudes, and Practices Among Health Practitioners – Arizona 2007

S. Chen, S. Anderson, L. Erhart, K. Komatsu, R. Miramontes, B. Park, T. Chiller, R. Sunenshine

Coccidioidomycosis: Knowledge, Attitudes, and Practices Among Health Practitioners — Arizona, 2007

Sanny Y. Chen[1,2], R. Miramontes[1], S. Anderson[2], L. Erhart[2], K. Komatsu[2], B. Park[1], T. Chiller[1], R. Sunenshine[1,2]
[1]Centers for Disease Control and Prevention, Atlanta, USA; [2]Arizona Department of Health Services, Phoenix, USA

Background: Coccidioidomycosis, the third most commonly reported infectious disease in Arizona, causes an estimated one-third of community-acquired pneumonias in Arizona, although <15% of pneumonia cases are tested for this disease. To direct future educational efforts, we assessed health practitioners' knowledge, attitudes, and practices (KAP) regarding diagnosis and treatment of coccidioidomycosis in Arizona.

Methods: A self-administered two-page survey was mailed to 7,978 health practitioners licensed by the Arizona medical, osteopathic, and nursing boards in October and December 2007. Two basic questions assessed general knowledge, and four clinical scenarios evaluated treatment practices. All questions were developed based on a comprehensive literature review, discussions with subject matter experts, and current Infectious Disease Society of America treatment guidelines. A multivariate logistic regression model was conducted to test predictors of at least 70% score on knowledge and treatment practices regarding coccidioidomycosis.

Results: Respondents were more likely to be physicians and from the Tucson area than nonrespondents. Of 1,823 (24%) who completed the survey, 53% were physicians and 52% were male. Mean age was 51 years (range: 29–87), and the mean number of years practicing medicine in Arizona was 13 (range: <1–59). Only 21% correctly answered all four treatment case scenarios. Approximately half reported "nearly always" testing patients presenting with community-acquired pneumonia for coccidioidomycosis; 32% reported "nearly always" treating any new diagnosis without evidence of comorbidities; and 45% reported "nearly always" treating any patient requesting treatment. Significant predictors of at least 70% score on knowledge clinical scenarios included always counseling patients after diagnosis (AOR= 4.4; 95% CI: 2.8-7.1), specializing in infectious disease (AOR= 2.4, 95% CI: 1.0-5.7), having received coccidioidomycosis CME credits in the last 12 months (AOR= 1.8, 95% CI: 1.2-2.6), and providing coccidioidomycosis educational materials to patients (AOR= 1.6, 95% CI: 1.1-2.3).

Conclusions: This KAP study, the first for coccidioidomycosis performed in the United States, had a low response rate. Multiple attempts to contact nonrespondents should be considered to increase generalizability. Despite the high incidence of coccidioidomycosis in Arizona, general knowledge and medical practices are inadequate, which underscores the need for a comprehensive education campaign to improve appropriate diagnosis and treatment of this disease in Arizona.

ABSTRACT 8: Antibody Seroprevalence Against *Coccidioides* spp. in Patients with Clinical Diagnosis of Tuberculosis from Ensenada, Baja California, Mexico

R.C. Baptista Rosas

Antibody seroprevalence against *Coccidioides* spp. in patients with clinical diagnosis of Tuberculosis from Ensenada, Baja California, Mexico

Baptista Rosas RC[1], Muñiz Salazar R[1], Salas Vargas SD[1], Arredondo Ozuna CA[1], Zimbrón Hernández MA[2], Riquelme M[3], Meza A[4]
[1]Molecular Epidemiology Program, School of Health Sciences UABC; [2]Department of Epidemiology, Ensenada General Hospital ISESALUD; [3]Department of Microbiology, Division of Experimental & Applied Biology CICESE; [4]PROMISE UABC

The current epidemiological impact of coccidioidomycosis (CM) in Northern Mexico is not known in detail. The main problem is the absence of epidemiological records of this important systemic fungal infection, since it is not mandatory a report for patients affected by the disease. In addition symptoms and clinical features of patients with CM are indistinguishable from tuberculosis (TB), which is also an endemic disease in Northern Mexico. Recently a series of reports have been published in Mexico where many cases of CM had been previously clinical diagnosed as TB, delaying treatment. We conducted serological screening in clinical samples of patients with previous diagnosis of TB with treatment failure or relapse in two reference hospitals in Ensenada, Baja California, Mexico.

We found a high prevalence (39%) of antibodies against *Coccidioides* spp. in clinical samples of patients with previous diagnosis of TB. Despite the absence of sputum smears staining or mycobacterium isolation, many cases were admitted for TB treatment under the current regulations. 15 positive samples resulted positive when tested with anti-*coccidioides* antibodies out of 38 clinical samples studied. 13 samples were positive to IgM, 1 sample positive to IgG and 1 sample positive to both antibodies. Despite the extensive literature on a high risk of infection and complications from disseminated CM in HIV-AIDS patients, we did not find any cases positive to HIV in this initial study. However, an obvious methodological bias suggests the probability that many immunosupressed patients with CM have reduced short-term survival.

ABSTRACT 8 *(continued)*: **Antibody Seroprevalence Against *Coccidioides* spp. in Patients with Clinical Diagnosis of Tuberculosis from Ensenada, Baja California, Mexico**

R.C. Baptista Rosas

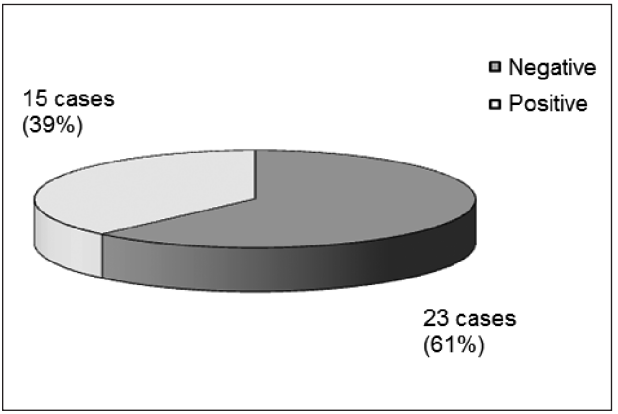


Figure 1. Proportion of cases before diagnostic serology to *Coccidioides* spp. with previous diagnosis of Tuberculosis.

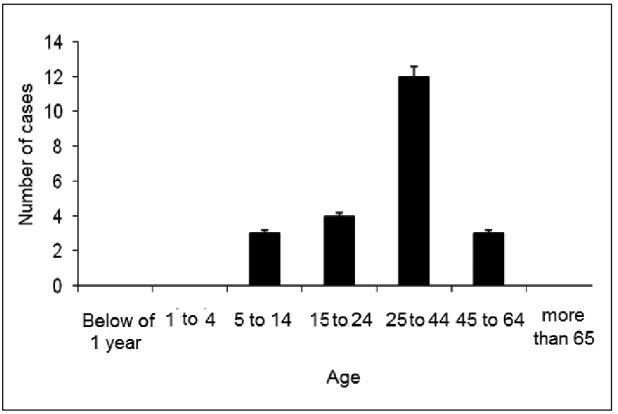


Figure 2. *Coccidioides* antibodies incidence per age group.

ABSTRACT 9: *Saccharomyces* as a Vaccine Against Coccidioidomycosis

J. Capilla, K.V. Clemons, H.B. Levine, D.A. Stevens

Saccharomyces Cerevisiae as a Vaccine Against Coccidioidomycosis

Javier Capilla[1-3], Karl V. Clemons[1-3], Hillel B. Levine[1], David A. Stevens[1-3]*
[1]California Inst. for Med. Res.,San Jose, CA, [2]Santa Clara Valley Med. Ctr., San Jose, CA, [3]Stanford Univ., Stanford, CA

Disseminated coccidioidomycosis is a serious infection even with appropriate therapy. We reported protection against aspergillosis in animals receiving heat-killed *Saccharomyces cerevisiae* (HKY) as a vaccine, and extended our studies to determine whether HKY is also protective against coccidioidomycosis. Male CD-1 mice received HKY (2.5 mg ± CpG or 5 mg) subcutaneously or by oral gavage (5 mg ± adjuvants sesame oil, CpG, MDP, MPL, BCG or IL-12) once a week for 3 or 4 weeks starting 21 or 28 days prior to intravenous infection with 260 or 300 arthroconidia. One group received live *Saccharomyces* orally (5 mg) weekly for 3 weeks. A positive control was a reference lot of the "gold standard" vaccine regimen, formalin-killed coccidioidal spherules (FKS) intramuscularly for 4 weeks. Survival was followed for up to 28 days and tissue burdens in surviving animals quantified. All 2.5 mg HKY sc regimens (2 experiments, 3 regimens) prolonged survival ($p \leq 0.005$) and reduced burdens from spleen, kidneys and lungs vs. nonvaccinated controls ($p \leq 0.015$); there were no significant differences between the groups. Survival of mice given HKY at 2.5 mg for 3 weeks was not significantly different from those given FKS, but was inferior to FKS in most comparisons of reduction of fungal burden in the organs. Five mg given sc or live *Saccharomyces* orally prolonged survival ($p = 0.005$ and 0.03, respectively), but did not affect fungal burden. HKY given orally with or without an adjuvant did not improve survival ($p = 0.057$ to 0.6 vs. nonvaccinated controls). We show vaccination with HKY given sc using different regimens and live *Saccharomyces* given orally are protective against a lethal coccidioidal infection. No improvement was found in combining HKY given sc with CpG or increasing dosing (up to 5 mg) or the number of doses. This novel heterologous protection offers a new approach to a vaccine against coccidioidomycosis.

ABSTRACT 10: Isolation and Evaluation of a Glycoprotein From the Coccidioidal Vaccine T27K

S.M. Johnson, N.M. Ampel, L.A. Nesbit, C.N. Miller, D.Pappagianis

Isolation and Evaluation of a Glycoprotein from the Coccidioidal Vaccine T27K

S.M. Johnson[1], N.M. Ampel[2], L.A. Nesbit[2], C.N. Miller[1], and D. Pappagianis[1]
[1]University of California, Davis and [2]University of Arizona, Tucson

Background. The coccidioidal vaccine T27K is a complex preparation derived from mature endosporulating spherules. This preparation engenders protection against lethal Coccidioides infection in the mouse model and specifically stimulates peripheral blood mononuclear cells (PBMC) from immune human donors as demonstrated by release of IL-2. Previous studies have shown this preparation to be highly glycosylated and following deglycosylation, the stimulatory capacity was reduced. We have therefore sought to isolate and evaluate the glycoproteins from the T27K.

Methods. Components of the T27K were reduced and denatured and then separated by continuous elutriation electrophoresis (CEE) using the BioRad Prep Cell. Fractions, 1 ml, were collected, samples of each electophoresed, and the gels stained with Gel Code Blue. Fractions containing the same molecular weight components as judged following electrophoresis were pooled. Glycoproteins were then isolated by affinity chromatography with conconavilin A (ConA) lectin. Presence of glycosylation was confirmed by Periodic Acid Schiff (PAS) staining following electrophoresis. One glycoprotein migrating at approximately 60 kDa was initially identified and isolated. This protein as well as T27K was incubated with immune and non-immune PBMC and the IL-2 released measured. The protein's identity was determined using mass spectrometry (LC MS/MS).

Results. Following CEE and ConA lectin affinity chromatography, a component migrating as a single band at approximately 60 kDa was obtained. This band stained with PAS and was terminally mannosylated as indicated by its binding to ConA. When immune cells were stimulated with this protein, IL-2 was specifically released. Mass spectrometry identified the protein as the heat shock protein 60.

Conclusions. A glycoprotein, identified using mass spectrometry as heat shock protein 60, was isolated from the T27K using CEE and ConA lectin affinity chromatography. This protein retained its stimulatory capacity when incubated with human immune PBMC. This protein represents a vaccine candidate and will be evaluated in the future.

ABSTRACT 11: Multiparametric Flow Cytometry to Assess Coccidioidal Antigens as Potential Vaccine Candidate

L. Nesbit, N.M. Ampel, S.M. Johnson, D. Pappagianis

Multiparametric Flow Cytometry to Screen Antigens as Potential Vaccines for Human Coccidioidomycosis

Lance Nesbit, B.S., Neil M. Ampel, M.D., Suzanne M. Johnson, Ph.D. and Demosthenes Pappagianis, M.D., Ph.D.
University of Arizona Valley Fever Center for Excellence and the Southern Arizona Veterans Affairs Health Care System, Tucson, AZ and the Department of Microbiology/Immunology at the University of California at Davis, Davis, CA

Background. The immune correlations generated by different antigens in T lymphocytes is not well understood. Recent evidence suggests that if an antigen induces a multicytokine response in T lymphocytes it has potential as a vaccine capable of inducing long-lived immune response. Of particular interest would be a vaccine candidate for coccidioidomycosis, which is a rapidly growing problem in the southwest United States. We explored the ability of T27K, a complex glycosylated antigen preparation, to induce multiple cytokine expression in human peripheral blood T lymphocytes.

Methods. Peripheral blood mononuclear cells (PBMC) from immune and non-immune donors were incubated with T27K for 48 hr and the simultaneous intracellular expression of IFN-γ, IL-2, TNF-α was determined using a multiple gating strategy on CD4+ lymphocytes. In addition, the surface expression of CCR7 and CD62L was assessed. Finally, mature dendritic cells (mDC) were generated using IL-4, G-CSF followed by TNF-α and PGE2 and used to present T27K to PBMC from non-immune donors.

Results. There was an increase in triple-cytokine positive (IL-2/IFN-γ/TNF-α) CD4+ lymphocytes from coccidioidal immune donors incubated with T27K compared to cells from non-immune donors and this was associated with release of IL-2 and IFN-γ. Using mature, monocyte-derived dendritic cells (mDC) as antigen-presenting cells, there did not appear to be an increase in the frequency of triple-positive CD4+ lymphocytes but there was an increase in IL-2 release by non-immune lymphocytes incubated with mDC. Among immune donors, the majority of cytokine-producing CD4+ lymphocytes were CCR7- and CD62L-, indicating an effector memory phenotype.

Conclusions. T27K, a known protective vaccine in mice, produces a cytokine pattern consistent with a protective immune response in humans. Through empirical cytokine analysis of other antigens, this system can be used to assess other possible vaccine candidates.

ABSTRACT 12: Comparison of ABLC and AmBisome for the Treatment of Coccidioidal Meningitis in a Rabbit Model

K.V. Clemons, J. Capilla, A.J. Tong, M. Martinez, D.A. Stevens

Comparative Efficacy of Abelcet and AmBisome Against Coccidioidal Meningitis in Rabbits

Karl V. Clemons[1-3], Javier Capilla[1-3], Ann-Jay Tong[1], Marife Martinez[1], Raymond A. Sobel[4,5], and David A. Stevens[1-3]
[1]California Institute for Medical Research, San Jose, CA; [2]Department of Medicine, Division of Infectious Diseases,Santa Clara Valley Medical Center, San Jose, CA; [3]Department of Medicine, Division of Infectious Diseases and Geographic Medicine, and [4]Department of Pathology, Stanford University, Stanford, CA; [5]Veterans Affairs Medical Center, Palo Alto, CA.

In separate previous studies we have shown that lipid complexed amphotericin B (ABLC) and liposomal amphotericin B (AmBi) are efficacious against coccidioidal meningitis in rabbits. Here, we compared ABLC and AmBi directly in a coccidioidal meningitis model. Male NZW rabbits were infected with 5 x 10^4 arthroconidia of *Coccidioides immitis* by direct cisternal puncture. Therapy began 5 days later with intravenous ABLC or AmBi at 7.5 or 15 mg/kg or sterile 5% dextrose water (D5W). Clinical assessments were done daily; CSF and blood were sampled at day 15 and at euthanasia. Survivors to day 25 were euthanatized, their CFU determined, and histology done on brain and spinal cord. Controls showed progressive disease, whereas animals treated with either dose of each drug showed few clinical signs of infection. All ABLC- or AmBi-treated rabbits survived, whereas 8 of 9 D5W-treated rabbits were euthanatized before day 25 ($P < 0.0001$). CFU in brain and spinal cord were 100 to 10,000-fold lower in ABLC- or AmBi-treated than in D5W-treated animals ($P < 0.0006$ to 0.0001). However, only two or fewer given a regimen of ABLC or AmBi were cured of infection in both tissues. Fewer ABLC-treated rabbits had any severity of meningitis compared to controls ($P = 0.015$ or 0.043 for ABLC at 7.5 or 15 mg/kg, respectively). Although AmBi regimens did not have fewer animals with meningitis than controls ($P > 0.05$), ABLC and AmBi were not different. In this model, ABLC and AmBi were equally and highly effective given intravenously, with few clinical signs, 100% survival, and significantly reduced fungal burdens. There appeared to be little benefit in using the 15 mg/kg dosage of either formulation. Neither ABLC nor AmBi showed significant advantage for treatment of coccidioidal meningitis. Further studies are required to determine the lowest effective dose of these formulations.

ABSTRACT 13: Disseminated Coccidioidomycosis Complicated by Splenomegally

A. Heidari

Disseminated Coccidioidomycosis Complicated by Splenomegally

Arash Heidari, M.D.

A 16 year-old female in her normal state of health noticed several skin lesions growing on her forehead, right eyelid and on the tip of her nose. The lesions' size rapidly increased without response to various antimicrobial agents given to her. The patient complained of constitutional symptoms including fever, chills, night sweats and 20 lbs weight loss over past four weeks. She was a student and lived with her family in San Joaquin Valley area in central California without any recent travel, prior illnesses, sick or animal contacts.

Physical examination was positive for high temperature up to 102 F, pale mucosa, splenomegaly and multiple cauliflower excoriated lesions over nose, right upper eyelid and forehead accompanied with several similar smaller lesions on her chest and back. Initial laboratory results showed leukocytosis of 26 with 49% eosinophils, normocytic anemia, hemoglobin of 6.1 with reticulocytosis. Chest radiograph had no infiltrate but three phase bone scan revealed areas of increased uptake in the right frontal and parietal bone. Coccidioidomycosis serology came back positive with complement fixation (IgG) titer of ≥1/512. Punch biopsy of her skin lesions showed granulomatous dermatitis with spherules with endospores confirming the diagnosis of disseminated disease. The patient was started on liposomal Amphotericin B. Consequently, her blood cultures from admission turned positive for coccidioidomycosis immitis. Fungmia persisted for almost 4 weeks despite amphotericin treatment. The patient's abdominal girth gradually increased and her levels of anemia worsened which made her transfusion dependant. Imaging studies revealed a massive splenomegaly with areas of infarct producing constant pain. Surgical evaluation deferred any interventions secondary to her overall condition. Bone marrow samples despite eosionphilia were essentially normal. Immunologic work up revealed decreased CD4+ T cells absolute count and percentile (236, 33%) and decreased natural killer cell's functional assay (3, 8-170). She was started on interferon gamma injections. Antifungal treatment changed to oral posaconazole and her fungemia quickly resolved. The patient's skin lesions resolved, her general condition and nutritional status improved and her anemia stabilized without any change in her spleen size. She was subsequently discharged on oral posaconazole and gamma interferon and followed up as an outpatient.

ABSTRACT 14: A Chest Wall Problem: Empyema Necessitatis

L.A. Rendón Pérez

A Chest Wall Problem: Empyema Necessitatis

Luis Adrián Rendón Pérez

A 36 year-old Hispanic male resident of Monterrey Mexico presented with a 4-week history of a skin lesion on his right lower chest wall. It started as a macule that became a pustule which opened and closed spontaneously several times leaving a scar. On the previous week to admission, he noticed a growing “mass” under the scar. He denied fever or cough. Examination showed the “mass” was hot and fluctuant but painless. The chest radiography showed a thin wall cavity on the RUL and a loculated pleural effusion. CT confirmed those findings and revealed a pleural-subcutaneous fistula with no pneumothorax. BAL smear for fungus was (-). Punction of the subcutaneous collection drained 300 ml of yellow pus. KOH stain showed abundant spherules and neutrophils. Pleural collection almost disappeared after the tap. Skin test, CF antibodies and bacteria cultures were all negative. Itraconazole 400 mg/day was started. At the third week of treatment the subcutaneous collection and the empyema were present again but about a third the original size. Patient was asymptomatic. *C. immitis* grew in both BAL and pus cultures. A second tap drained 100 ml of pus. The smear showed few spherules. Patient continued on itraconazole. By the second month, the subcutaneous collection had resolved and the pleural effusion had almost disappeared. Thereafter, he did very well even though the lung cavity remained after a year of therapy. He was doing well at the 2 years follow up visit.

ABSTRACT 15: Cocci in the Heart

S. Hoover

Cocci in the Heart

S. Hoover

A 64 year old black man with ischemic cardiomyopathy underwent an orthotopic heart transplant in August 2007. His past medical history was significant for valley fever "10 or 15 years ago." Following the transplant he received immunosuppression with mycophenolate and tacrolimus. No antifungal agents were prescribed.

Pretransplant serologies were as follows:

June 2007: Cocci IgM negative, IgG positive by immunodiffusion. Quantitative immunodiffusion negative.

August 2007: Same profile.

In September 2007 he was admitted with confusion, fever, and weight loss. Cocci IgG titer had risen to 1:64. A CT scan of the chest showed a new left-sided small pleural effusion and scattered ground glass opacities in the left lower lobe. Head CT was negative. No lumbar puncture was done. Fluconazole was started at 400 mg daily and the patient was discharged.

He was readmitted 2 weeks later with continued fatigue and weight loss, with no fever or neurologic symptoms. MRI of the brain was negative. Lumbar puncture was negative. Cocci titer was 1:16.

Heart biopsy showed the following (*see figure*):

1) No acute cellular rejection, Grade 0 (ISHLT WF 2004).

2) Granuloma due to coccidioidomycosis.

Comments: A solitary granuloma of epithelioid cells and giant cells contains a spherule with internal structure.

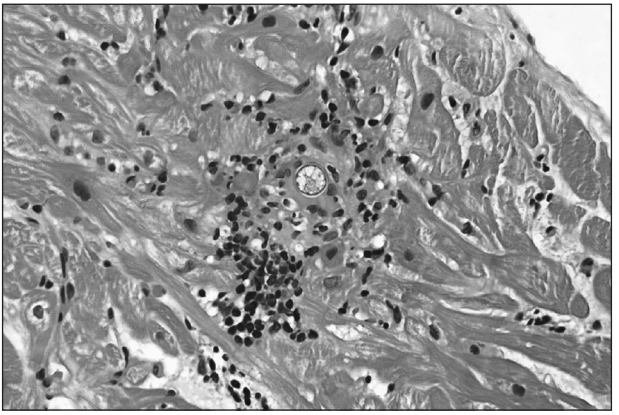
He received IV amphotericin B for 5 days and was discharged on voriconazole 400 mg po every 12 hours, which he continues to take. His titer in November 2007 was 1:32. As of February 2008, his cardiac graft continues to function well, and his fatigue has improved.

There are a few reports in the literature of coccidioidal infection of the heart found at autopsy in patients with overwhelming disease, including one heart transplant recipient (1). One case of apparent endocarditis has been published (2). We are not aware of any reports of *Coccidioides* discovered on a routine post-transplant heart biopsy, with such a good outcome.

References:

1. Vartivarian S et al. Am J Medicine 1987, 83:949.
2. La Via et al. Ped Inf Dis J 2005, 24:470.

ABSTRACT 15 *(continued)*: **Cocci in the Heart**
S. Hoover



ABSTRACT 16: Most Unusual Cases of Coccidioidomycosis: LUL Mass and Mediastinal Fibrosis

S. Kasperbauer

Most Unusual Cases of Coccidioidomycosis: LUL Mass and Mediastinal Fibrosis

Shannon Kasperbauer M.D.

This is a 22-year-old male from Pecos, Texas who reports a healthy childhood. In the summer of 2004 he was hospitalized with severe anemia. His CXR was notable for diffuse interstitial infiltrates of unknown etiology. A CT revealed left supra-hilar mass. This was biopsied in 9/04. All cultures were negative. The pathology noted abundant necrosis, scattered multi-nucleated giant cells and **positive spherules** with refractile walls and endospores. He began fluconazole, 200 mg a day for 1 month only. In 4/06 he is hospitalized again for CAP and fluconazole was restarted at 200mg daily. By 12/06 he developed chest wall pain and a CT reveals a LUL mass. In 5/07 he went to the OR for a left upper lobectomy. This was unsuccessful and instead, a biopsy was performed. The pathology showed hemorrhage with necrosis and surrounding fibrosis. No multinucleated giant cells or malignancy. He continued fluconazole at 200mg/day until he was hospitalized in 7/07 with hemoptysis. His CT of the chest showed progressive consolidation in the LUL with associated mediastinal fibrosis. He received posaconazole as an inpatient and then itraconazole for 6 weeks and stopped on 10/8/07. On 10/8/07 he restarted fluconazole at 800mg/day. He presently denies fevers, chills, cough, or shortness of breath. His only complaint is mild left anterior chest wall pain. A repeat CT 11/15/07 compared to 7/07: improvement in dense consolidative process involving the left upper lobe and lingual, no significant change in the mediastinal fibrosis. No evidence for cortical destruction of adjacent ribs. Mildly enhancing lymph nodes in the left axilla. Moderate pericardial effusion, unchanged. Elevated left hemi-diaphragm.

ABSTRACT 17: Proteomic Identification of Coccidioidal Antigens from Lung Fluid of Infected Mice: the Search for Diagnostic Markers of Valley Fever

F.S.E. Helfrich, L.F. Shubitz, A. Hilderbrand, D.M. Magee, D.F. Lake, N.M. Ampel, V. Wysocki, J.N. Galgiani

Proteomic Identification of Coccidioidal Antigens from Lung Fluid of Infected Mice: the Search for Diagnostic Markers of Valley Fever

F.S.E. Helfrich[1], L.F. Shubitz[2], A. Hilderbrand[1], D.M. Magee[3], D. F. Lake[3], N.M. Ampel[2], V. Wysocki[1], J.N. Galgiani, MD[2]
[1]Chemistry Department, University of Arizona; [2]Valley Fever Center for Excellence, Southern Arizona VA Health Care System and University of Arizona; [3]The Biodesign Institute, Arizona State University.

In the southwestern United States and especially Arizona, coccidioidomycosis (Valley Fever) is an important cause of community acquired pneumonia and less common but even more severe complications occur in both humans and pets. Current clinically available serologic tests for Valley Fever are highly specific but often insensitive in early infections. An alternative approach is to detect fungal proteins present before antibodies develop during infection. Bronchoalveolar lavage fluid (BALF) and lung homogenate (LH) samples were collected from mice infected with *Coccidioides posadasii*. Multidimensional protein identification technology (MudPIT) and C-18 reverse phase liquid chromatography tandem mass spectrometry were utilized for the analysis of coccidioidal proteins in the BALF and LH samples. Data analyzed by SEQUEST, X-Tandem, and Scaffold identified several proteins specific for Coccidioides, some of which are distinctly dissimilar to proteins in mammals or other genera of fungi. Additional work is planned to map epitopes that are recognized by serum antibodies from infected mice and patients. Proteins identified by this approach may be useful targets for clinically useful early markers of Valley Fever infections.

ABSTRACT 18: Counting Cocci: Quantitation of *Coccidioides* Using Real-Time PCR

E. Driebe, C. Liu, J. Bowers, M. Schmoker, C. Bosch, D.M. Engelthaler

Counting Cocci: Quantitation of *Coccidioides* Using Real-Time PCR

Elizabeth Driebe, Cindy Liu, Jolene Bowers, Michelle Schmoker, Cedric Bosch, Paul S. Keim, David M. Engelthaler
TGen North, The Translational Genomics Research Institute, Flagstaff, AZ

Coccidioidomycosis is an increasingly common infection in the desert southwest resulting in thousands of severe illnesses and dozens of deaths each year. There are no vaccines available and existing antifungal drugs are only used in extreme circumstances. Currently available diagnostics include serology and direct culture, both of which are time consuming and produce inconsistent results. A quantitative real-time PCR (qPCR) assay could help physicians better diagnose and manage patients and may be useful for drug evaluation studies to monitor changes in fungal load. Additionally, a qPCR assay would reduce the need for culturing infectious conidia and managing cumbersome select agent regulations in clinical labs. We developed a novel real-time qPCR assay (CocciQuant) for *Coccidioides* spp., targeting the ITS2 region. In order to validate the assay, we first cloned a quantitative standard, verified the standard by sequencing, calculated the copy number, and used dilutions of the standard to evaluate the sensitivity and kinetics of the CocciQuant assay. We also tested our assay for specificity by screening it across 110+ target isolates and 178 non-target species isolates. To investigate CocciQuant's usefulness with clinical specimens, we performed spiking experiments on normal flora sputum samples and extracted DNA from putative positive and negative sputum samples. CocciQuant was determined to have a PCR efficiency of 115-125%, a detection threshold of <5 genome equivalents/ul, and a quantification range of 102 to 109 copies/ul. Our results suggest that this will be an assay for quantifying *Coccidioides* fungal load *in vitro*, *in vivo*, and possibly *in enviro*.

ABSTRACT 19: Coccidioidal Serologic Findings with Body Fluids Other Than Blood Serum

D. Pappagianis

Coccidioidal Serologic Findings with Body Fluids other than Blood Serum

Demosthenes Pappagianis
School of Medicine, University of California, Davis, CA

Serologic tests are helpful in diagnosis and prognosis of coccidioidomycosis (coccy). Smith et al (1950 Am. J. Hyg. 52:1, Table) demonstrated clearly, the value of serologic tests with serum, cerebrospinal, pleural and peritoneal fluids, and they tested synovial fluid. We have routinely tested these fluids that can provide information on the course of coccy involving the related anatomical sites despite reservations expressed by some (2007 J. Clin. Microbiol. 45:26).

The coccidioidal complement fixation (CF) titer of the fluid may be similar to or different from the titer of the concomitant serum. Immunodiffusion (Figure) can demonstrate presence of coccidioidal antibody in fluids and can provide support for a diagnosis of coccy of the related anatomical site. Positive coccidioidal serologic results cannot rule out possible coexistent other disease. However, testing of synovial, peritoneal, or pleural fluids (always along with serum) can provide serologic evidence of coccy even in the absence of recognized prior primary coccy.

Table. Serologic testing using serum and peritoneal fluid.

Test result	Peritonitis (5 patients)		Pleural effusion (13 patients)	
	Serum	Peritoneal Fl.	Serum	Peritoneal Fl.
(+) precipitin (IgM)	3	3	8	6 of the 8
(+) complement fixation (IgG)	5*	5*	13**	13**

**Titer equal in 4; 1 with titer greater in serum; **Titer equal in 3; 10 with titer greater in serum.*

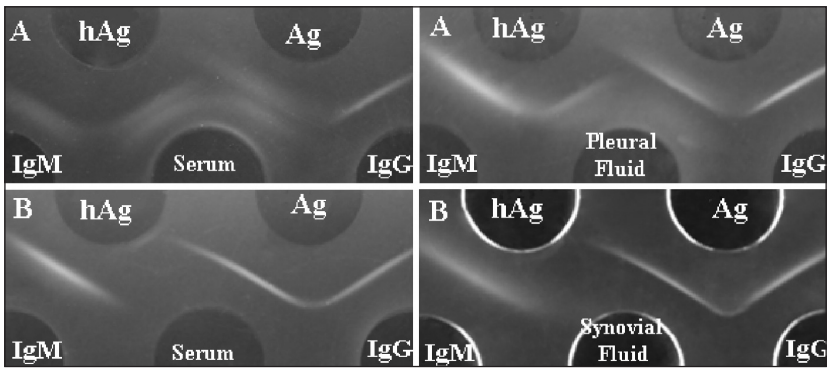


Figure. hAg = heated antigen, reactive with IgM; Ag = unheated antigen, reactive with IgG and IgM; IgM = "precipitin" (IgM) postive control serum; IgG = CF (IgG) positive control serum.

ABSTRACT 20: Diagnosis of Coccidioidomycosis Using the MVista™ *Coccidioides* Antigen Enzyme Immunoassay (EIA)

M. Durkin, P. Connolly, L.J. Wheat

Diagnosis of Coccidioidomycosis Using the MVista™ *Coccidioides* Antigen Enzyme Immunoassay (EIA)

Michelle Durkin, Patricia Connolly, L. Joseph Wheat

Background. We previously reported detection of a cross-reactive antigen in patients with coccidioidomycosis in the MVista™ Histoplasma antigen EIA (Kuberski, CID 2007). Subsequently we have developed a specific MVista™ *Coccidioides* spp. antigen EIA.

Methods. Polyclonal antibodies to *Coccidioides* spp. were produced in rabbits and used to detect *Coccidioides* antigen. Urine from patients with histoplasmosis or coccidioidomycosis were tested in the MVista™ *Coccidioides* and Histoplasma antigen EIA.

Results. Urine from 9 of 10 patients with coccidioidomycosis were positive compared to 2 of 5 patients with histoplasmosis. The coccidioidomycosis specimens gave much higher results in the *Coccidioides* EIA (4/10 > 10 units) than in the Histoplasma EIA (1/10 > 10 units). The two cross reactive histoplasmosis specimens gave much lower results in the *Coccidioides* EIA (2.0 units, 1.2 units) than in the Histoplasma EIA (42.8 units, 27.7 units).

Conclusions. The new MVista™ *Coccidioides* antigen EIA shows promise for use as an aide in the rapid and specific diagnosis of coccidioidomycosis.

ABSTRACT 21: Ultrasonography in the Evaluation of Canine Coccidioidomycosis

A.P. Davidson, T.W. Baker

Ultrasonography in the Evaluation of Canine Coccidioidomycosis

AP Davidson, TW Baker
School of Veterinary Medicine, University of California, Davis

The clinical evaluation of dogs suspected to have coccidioidomycosis typically includes both clinical pathologic and radiological studies. Radiography of the thorax and, if osseous dissemination is suspected, the appropriate skeletal structures occurs most commonly. The usefulness of abdominal ultrasonography in the evaluation of dogs with confirmed coccidioidomycosis was evaluated.

Medical records of dogs seen at the University of California Veterinary Medical Teaching Hospital between January 1989 and January 2008 were searched; 141 cases of confirmed coccidioidomycosis were found and records reviewed. Abdominal ultrasound was performed in 49 (35%). Abdominal ultrasound was performed with dogs in ventral recumbency; sedation or anesthesia was used only when invasive or painful procedures such as aspirates or biopsies were performed.

Thirty seven (75%) dogs were referred from known coccidioidomycosis endemic regions of California. Eight (15%) were referred from non-endemic regions. Three were referred from Arizona (<1%) and 1 from Texas (<1%).

The dogs were placed into size categories of small (< 4.5kg), medium (4.5-18kg), large (18-45kg) and giant (>45kg). In agreement with previous studies, large dogs (25 cases, 51%) were most commonly affected, followed by medium (14 cases, 28%), small (9 cases, 18%) and giant (1 case, <1%).

Ultrasound requests indicated the clinical signs these dogs exhibited and the tentative diagnosis in most cases. The most common clinical signs described were cough (11 cases 24%), fever (11 cases, 22%), lameness (8 cases, 16%) and pain (8 cases, 16%). A complete list of clinical signs follows (table 1). The most common tentative clinical diagnoses were coccidioidomycosis (10 cases, 20%), undefined pneumonia (8 cases, 16%), polyarthritis (3 cases, 6%), and myelopathy (3 cases, 6%). A complete list of tentative diagnoses follows (table 2).

A normal abdominal study was obtained in 13 cases (26%). Thirty six cases (74%) had abnormalities detected by abdominal ultrasound. Hepatomegaly (10 cases, 20%), lymphadenomegaly (10 cases, 20%), splenomegaly (6 cases, 12%), echogenic renal cortices (4 cases, 8%), a hypoechoic liver (3 cases, 6%), a cystic prostate (3 cases, 6%) and adrenomegaly (3 cases, 6%) were the most common abnormalities found. A complete list of the ultrasonographic findings follows (table 3).

In addition to abdominal scans, ultrasound guided diagnostics were performed including cystocentesis, splenic, hepatic, prostatic and pulmonary mass aspirates, as well as hepatic and osseous biopsies.

ABSTRACT 21 *(continued)*: Ultrasonography in the Evaluation of Canine Coccidioidomycosis

A.P. Davidson, T.W. Baker

Cytologic evaluation of ultrasound guided fine needle aspirates identified lymphatic reactivity, pyogranulomatous inflammation, extramedullary hematopoiesis and myelopoiesis, histiocytic inflammation and hepatic degeneration. One aspirate was inconclusive.

Histopathology of ultrasound guided hepatic biopsies identified neutrophilic hepatitis with spherules; of osseous biopsies pyogranulomatous osteomyelitis with spherules.

Abnormalities found to be associated specifically with disseminated disease included hepatomegaly, splenomegaly, lymphadenomegaly, hyperechoic renal cortices, a cystic prostate, adrenomegaly and pulmonary consolidation.

Altogether, abdominal ultrasound contributed to the confirmation of disseminated disease in 27 of 49 cases (55%), and is concluded to be a useful tool in the evaluation of canine coccidioidomycosis. Additionally, ultrasound permits cytological evaluation of some pulmonary coccidioidal lesions.

Table 1. Clinical signs of dogs presented for abdominal ultrasound (n=49)

	#
Hematuria	1
Lethargy	8
Anorexia	7
Exercise intolerance	4
Cough	12
Pain	8
Hemospermia	1
Fever	11
Lame	8
Arrhythmia	2
Seizure	2
Weight loss	3
Vomiting	3

Table 2. Tentative diagnosis of dogs presented for abdominal ultrasound (n=49)

	#
Cocci	10
Meningitis	1
Polyarthritis	3
Pneumonia	8
Pericarditis	1
Enteritis	1
Lymphoma	2
Pregnancy	1
Prostatitis	2
Neoplasia	2
Seizure disorder	1
Pancreatitis	1
Myelopathy	3
Osteomyelitis	1
Septic shock	1
Liver failure	1

ABSTRACT 21 *(continued)*: Ultrasonography in the Evaluation of Canine Coccidioidomycosis

A.P. Davidson, T.W. Baker

Table 3: Ultrasound findings of dogs with coccidioidomycosis n=49

	#		#
Hepatomegaly	10	Gastric wall thickening	1
Splenomegaly	6	Pancreatitis	1
Cystic prostate	3	Splenic mass	1
Consolidation lung	1	Renal mass	1
Normal	13	Porto systemic shunt	1
Renal pyelectasia	1	Ascites	2
Pregnancy	1	Hypoechoic liver	3
Lymphadenomegaly	10	Cystic ovary (ies)	1
Hepatic vein engorgement	2	Hyperechoic liver	1
Micro hepatica	1	Echogenic renal cortex (ices)	4
Adrenomegaly	3	Peritonitis	1
Pulmonary mass	2	Splenic mottle	2
Bone lesion(s)	2	Small adrenal(s)	2

ABSTRACT 22: A Prospective Cohort Analysis of the Decision to Treat or Not Treat Primary Coccidioidal Pneumonia

N.M. Ampel, A. Giblin, J. Mourani, J.N. Galgiani

An Observational Study on the Outcome of Primary Coccidioidal Pneumonia

Neil M. Ampel, M.D., Andrea Giblin, D.O., John P. Mourani, M.D., Suzette Chavez, B.S., and John N. Galgiani, M.D.
University of Arizona Valley Fever Center for Excellence and the Southern Arizona Veterans Affairs Health Care System, Tucson, AZ

Background. There is no published experience to assess the value of initiating oral antifungal therapy to treat primary pulmonary coccidioidomycosis.

Methods. We analyzed prospectively collected data from subjects with primary pulmonary coccidioidomycosis followed in a single clinic devoted to the management of coccidioidomycosis located within the coccidioidal endemic region. Primary pulmonary coccidioidomycosis was defined as a patient with a documented focal alveolar infiltrate on chest radiograph with respiratory symptoms of less than 3 months with either a positive coccidioidal serologic test or a positive coccidioidal culture.

Results. One hundred five subjects with primary pulmonary coccidioidomycosis were identified from January 2001 through December 2005. Of these, 54 were prescribed antifungal therapy while 51 were not. There were no statistically significant differences between the two groups with regard to age, race, ethnicity, sex, or the presence or type of underlying diseases (for all, $P > 0.100$). Patients were significantly more likely to be prescribed antifungal therapy if they had an elevated total clinical score ($P = 0.001$), a higher symptom score ($P = 0.049$), or a positive sputum culture for *Coccidioides* ($P = 0.048$). Prospective follow-up based on return to the coccidioidomycosis clinic occurred in 43 subjects. Follow-up was for a median of 286 days (35 to 1124). There was no difference in rate of clinical improvement between those treated and not treated ($P = 0.899$). All 16 patients who were not treated were improved after a median of 217 days and none developed complications. Seven subjects remained on antifungal therapy at the end of follow-up and none had evidence of recurrent coccidioidomycosis. However, two of 20 patients who were treated but whose therapy was subsequently stopped during follow-up developed disseminated disease. A retrospective follow-up of the electronic medical record of the total cohort of 105 patients identified six other patients with complications, all among the group initially treated but whose therapy was subsequently discontinued. Five of these six cases were relapses of pulmonary disease while one was a possible extrathoracic dissemination.

Conclusions. Approximately half of patients with primary pulmonary coccidioidomycosis were prescribed antifungal therapy and this was done on the basis of symptoms. Complications were seen only among patients in the group prescribed therapy after treatment was discontinued.

ABSTRACT 23: Continuous Intrathecal Amphotericin B (AB) for Relapsed *Coccidioides(C) immitis* Meningitis (M)

C. Berry, E. Hassid, D.A. Stevens, D. Pappagianis, E. Hepps, K. Sahrakar

Continuous Intrathecal Amphotericin B (AB) for Relapsed *Coccidioides(C) immitis* Meningitis (M)

C. Berry[1], E. Hassid[2], D.A. Stevens[3], D. Pappagianis[4], E. Hepps[1], K. Sahrakar[1]
[1]TPMG, South Sacramento; [2]Sutter Davis, Davis; [3]Sta. Clara Valley Med Ctr., San Jose; [4]Univ Calif Davis, Davis, CA

A 19-year-old African American man with CM was treated with fluconazole (FZ) 800 mg daily. Worsening hydrocephalus, hemiparesis, cognitive impairment and seizure-like activity with cerebral lesions occurred after 1 month (8/00); dexamethasone was added (8/24-9/23/00) and a ventriculo-peritoneal shunt (VPS) placed. Noncompliance with FZ for > 1 mo. resulted in headache and vomiting 9/01. C was cultured from ventricular CSF during new VPS placement, which was complicated by peritoneal adhesions. Vomiting persisted and FZ resumed. AmBisome 4350 g was given IV between 10/9-11/12/01. Vomiting, cognitive and gait abnormalities persisted, and CSF total protein and CF titers increased. On 11/16, a catheter was placed in the basilar cisterns and connected subcutaneously to a Medtronics programmable pump for continuous intrathecal (IT) infusion of 1mg/ml AB, at initial rate 0.05 mg/day, from an 18 ml reservoir. When the residual reservoir solution was replaced 11/29 the AB level was 0.2 mg/ml with inhibitory & killing levels (I&KL) vs. patient's isolate 1:256. The infusion was increased to 0.25 mg/day. By 12/26, he had received 12.6 mg AB and the 27 day old residual reservoir AB solution was assayed at 0.06mg/ml with I&KL 1:32.

Table. Lumbar CSF Results

Date	WBC/ mm3	Eosino-phils, %	Protein, mg/ml	Glucose, mg/ml	CF Titer	I&KL	AB, mcg/ml
10/8/01	135	40	1024	22	1:128	–	–
11/9	220	37	1746	24	1:512	1:16	0.076
12/26	52	44	435	16	1:32	<1:4	<0.06
2/21/02	20	56	78	24	1:8	–	–
6/7/06	9	0	–	51	1:2	–	–

There was no evidence of AB toxicity during continuous IT infusion + high dose FZ . Device malfunction was detected 2/21/02 and he was continued on FZ. He remains well and attends college 4 years later.

Continuous IT infusion provided biologically active AB without neurological toxicity. The 18 ml reservoir provided continuous therapy for a month without reloading, lessening the risk of infection. A rapid escalation in dose in this case of fulminant CNS fungal infection was achieved by adjusting the rate of IT infusion.

ABSTRACT 24: Impregnation of a Ventriculoperitoneal Shunt with Amphotericin B Deoxycholate Used in Meningitis Due to Coccidioidomycosis

T. Kuberski, V. Ianas

Impregnation of a Ventriculoperitoneal Shunt with Amphotericin B Deoxycholate Used for Meningitis Due to Coccidioidomycosis

Timothy Kuberski, MD[1], Voichita Ianas, MD[1], Annette Fothergill, MBA[2]
[1]Phoenix, AZ; [2]University of Texas Health Science, San Antonio, TX

Ventriculoperitoneal shunts (VPS) can be life saving in patients with hydrocephalus secondary to meningitis caused by coccidioidomycosis. A complication of VPS is obstruction due to *Coccidioides*. Impregnation of VPS with antibiotics is known to reduce bacterial infections. However, little is known about prevention of fungal infection of the VPS using antifungals.

We describe a 31-year-old Hispanic man with a VPS for hydrocephalus due to coccidioidomycosis. He presented with headache, nausea and vomiting. The patient had stopped taking fluconazole about a year previously. He was found to have recurrence of hydrocephalus secondary to *Coccidioides* obstruction of his VPS. It was elected to replace the VPS.

BioGlide® VPS (Medtronic) are designed to absorb therapeutic agents which subsequently elute from the shunt over time. We immersed the shunt in a solution of amphotericin B deoxycholate. The amphotericin impregnated shunt was used to replace the nonfunctional VPS. The patient improved and was alive and well at 5 months follow up.

As proof of principle we performed in vitro study on the elution of amphotericin B from the BioGlide VPS. The result of this study suggested that amphotericin B can be absorbed by the BioGlide shunt and eluted over time. The study supports the conclusion that a BioGlide shunt may be a valuable therapeutic approach in patients with hydrocephalus due to coccidioidomycosis and need a VPS or a replacement of VPS.

POSTER ABSTRACT 1: Agent-based Modeling of Physical Factors That May Control the Growth of *Coccidioides* in Soils

M.D. Gettings, F.S. Fisher

Agent-Based Modeling of Physical Factors That May Control the Growth of *Coccidioides*

Mark Gettings[1] and Frederick Fisher[2]
[1]U.S. Geological Survey, Tucson, AZ.; [2]University of Arizona, Tucson, AZ.

A model of the wind-borne spread of spores and spore survival in soils of *Coccidioides*, the fungus that causes valley fever, has been completed using agent-based modeling software. The model assumes that for a new site to become established, four factors must be simultaneously satisfied: 1) there must be transport of spores from a source site to sites with *Coccidioides*-favorable soil geology, texture, topographic aspect, and lack of biomass competition; 2) there must be sufficient moisture; 3) temperature of the soil must be *Coccidioides*-favorable; and 4) temperature and moisture must remain in *Coccidioides*-favorable ranges long enough for the fungus to grow to depths at which spores will survive subsequent heat, aridity, and ultraviolet radiation. Modeling with only these four factors appears adequate to explain observed *Coccidioides* distributions. Rain probability and amount, annual and diurnal temperature variation, and wind direction and intensity were computed from weather records at Tucson, Arizona from 1894 to 2001. *Coccidioides*-favorable ground was defined using a fractal tree algorithm in accordance with observations that sites that are likely to host large populations are often adjacent to drainage channels. Model runs produced five conclusions: 1) if any of the four factors is not isotropic, parts of the favorable areas will never become colonized no matter how long the model runs; 2) the spread of sites is extremely sensitive to soil moisture duration and wind and temperature after a rain control the time before a site becomes too dry; 3) the distribution of wind and rainstorm directions is a strong control on the spread of colonization; 4) soil temperature was the least sensitive control in the model, although it does control dormancy of a site; and 5) the model results range from complete colonization of all favorable sites to no new sites in three years of model simulation. Most model runs were carried out to 23 years.

Subsequent modeling by R. Jamalamadaka and M. Gettings using a non-binary favorable ground model and temperature and moisture models based on observed soil data profiles have produced scenarios with generally slower rates of colonization that are in agreement with what is known of actual site formation rates. These models were run with simulation times of 50 and 100 years, and rates of new site formation were slower than with the simplified model.

POSTER ABSTRACT 2: A Fluorescent Microsphere Immunoassay for Detection of Antibodies to *Coccidioides* Species

S. J. Wong, T. Victor, D. Pappagianis, V. Chaturvedi

A Fluorescent Microsphere Immunoassay for Detection of Antibodies to *Coccidioides* Species

Susan J. Wong[1,2], Tanya Victor[1], Demosthenes Pappagianis[3], Vishnu Chaturvedi[1,2*]*
[1]Wadsworth Center, New York State Department of Health; [2]Department of Biomedical Sciences, State University of New York, Albany, New York 12201; [3]Coccidioidomycosis Serology Laboratory, Department of Medical Microbiology and Immunology, School of Medicine, University of California, Davis, CA 95616

Background. *Coccidioides* immitis and C. posadasii are respiratory and systemic fungal pathogens endemic in the Southwest. Classical complement fixation (CF) and immunodiffusion (ID) tests are the mainstay of laboratory diagnosis. There is need for more rapid, sensitive and specific assays as this disease is being increasingly reported from both within and outside of the endemic zone.

Methods. The patient samples were procured from ARUP Laboratories (Salt Lake City, UT) and UC, Davis (75 *Coccidioides* positive & 20 negative sera, 10 Histoplasma positive, 8 Aspergillus positive and 2 Blastomyces positive sera). The fungal antigens (Meridian Biosciences, Cincinnati, OH) were covalently linked to the surface of polystyrene microspheres following 4-(4, 6dimethoxy [1,3,5] triazin-2-yl)-4-methyl morpholinium modifications. The patient sera were diluted 1:100, incubated with microspheres, and bound human antibodies were detected with fluorescently labeled anti-human immunoglobulins in a Luminex 100 instrument to test sensitivity and specificity of the microsphere immunoassay (MIA).

Results. The results showed 76% overall agreement between MIA and CF. Additionally, 7 CF negative sera were positive by MIA while 7 CF positive sera tested negative in MIA. Serial dilutions of representative positive samples showed that MIA was approximately 100-fold more sensitive than CF. Multiplex analysis with Apergillus, Blastomyces and Histoplasma antigens beads also permitted specific identification of *Coccidioides* positive samples.

Conclusions. The suspension phase MIA for coccidioidomycosis offers good specificity, broad dynamic range and multiplex capacity.

POSTER ABSTRACT 3: The Utility of *Coccidioides* PCR in Clinical Specimen

D. Vucicevic, J.E. Blair, M.J. Binnicker, A.E. McCullough, S. Kusne, H.R. Vikram, N.L. Wengenack

The Utility of *Coccidioides* PCR in the Clinical Setting

D. Vucicevic, J.E. Blair, M.J. Binnicker, A.E. McCullough, S. Kusne, H.R. Vikram, N.L. Wengenack

Background. Recently, a PCR test to detect *Coccidioides* DNA in clinical specimens was developed at our institution. In the assay validation phase of the test development, the PCR test demonstrated 100% sensitivity and 98.4% specificity when compared to culture-based detection of *Coccidioides* sp. in respiratory specimens.

Aim. To compare the PCR test with other diagnostic methods and to set the parameters for a larger study that will eventually determine the sensitivity and specificity of *Coccidioides* PCR in the clinical setting.

Methods. A retrospective chart review was performed for all patients who have undergone *Coccidioides* PCR testing on clinical specimens since the implementation of the assay into routine testing. Clinical, microbiological, serological, radiographic, treatment and follow-up information regarding the course of coccidioidomycosis, if any, were all abstracted.

Results. 88 PCRs were performed on 86 respiratory and 2 CSF specimens from 78 patients. There were 4 positive and 84 negative PCR results. All 88 specimens had a fungal culture performed, and *Coccidioides* was grown from 5 of these specimens. 2 patients had negative PCR result with a concurrent positive culture from the same specimen. In one patient, the PCR assay was positive and the culture was negative. Compared with culture, the PCR sensitivity was 3/5 60%, positive predictive value 3/4 75%, specificity 82/83 99%, and negative predictive value 82/84 98%. There were 6 patients with a negative PCR and a negative culture who had either confirmed, highly probable or probable coccidioidomycosis diagnosis.

Conclusion. When compared with fungal culture, *Coccidioides* PCR appears to accurately identify a negative result in the clinical setting. The sensitivity of the PCR test in the clinical setting is lower than the sensitivity reported during the assay validation. Further investigation with larger number of *Coccidioides*-positive specimens is needed to better define the sensitivity in this setting.

POSTER ABSTRACT 4: Surgical Consideration in Pulmonary Coccidioidomycosis: 10 Year Experience and Review of the Literature

W.J. Halabi, D.E. Jaroszewski, J.E.Blair, R. Wong, J. Parish, P.A. DeValeria, L. Lanza, V. Trastek, F.A. Arabia

Surgical Considerations in Pulmonary Coccidioidomycosis: 10 Year Experience

W.J. Halabi, D.E. Jaroszewski, J.E. Blair, J. Parish, P.A. Devaleria, L.A. Lanza, V.F. Trastek, F.A. Arabia
Mayo Clinic Hospital, Phoenix AZ

Background. Coccidioidomycosis is a fungal disease that mainly affects the lungs. 5-10% of infections result in lung nodules and cavities. While coccidioidal nodules represent a diagnostic challenge due to their resemblance to malignancy and hence require surgical biopsy, cavitary disease can have a more complicated course and require therapeutic surgery. There have been not studies in the literature examining the surgical considerations of this disease for more than 28 years, when diagnostic measures and medical treatment were different.

Methods. A retrospective chart review of 1136 patients with pulmonary coccidioidomycosis who presented to Mayo Clinic Arizona over 10 years was conducted to identify patients with nodular, cavitary or infiltrative pulmonary coccidioidomycosis and review the characteristics of the 73 patients who had surgery.

Results. 6.4% of patients with pulmonary coccidioidomycosis had surgery over a 10 year period. Surgery done for patients with nodular disease included VATS (35%) and diagnostic wedge biopsy (85%) to achieve diagnosis. The complication rate was 14%, primarily consisting of small air leaks that closed spontaneously. Medical treatment was given in 22 % of cases. There were no recurrences.

Surgery done for cavitary coccidioidomycosis was done for therapeutic reasons in 95% of pts, with thoracotomy and lobectomy in 68% of the cases. Extensive adhesions were seen intraoperatively in 68% of cases. The complications rate was 26%, primarily due to bronchopleural fistula. Medical treatment after surgery was given in 95% of cases and was prolonged. There were no recurrences in this group after a mean follow up of 30 months.

Conclusions. Surgery still has a role to play in the management of pulmonary coccidioidomycosis in both diagnosis and treatment. It has an acceptable complication rate and should be done with minimal invasiveness and extent possible to limit the incidence of post-operative complications and preserve good lung function. It also offers the advantage of minimizing recurrences. Indications for medical treatment as an adjunct to surgery should be more clearly defined.

Poster Abstracts Presented But Not Submitted

POSTER ABSTRACT 5: Evaluation of Environmental Samples Collected in Utah, Arizona and California for the Presence of *Coccidioides*
S.M. Johnson, F.S. Fisher, C.N. Miller, D. Pappagianis

POSTER ABSTRACT 6: Community-Based Epidemiological Study of Valley Fever in Tucson, Arizona
J.A. Tabor, M.K. O'Rourke, M.D. Lebowitz

POSTER ABSTRACT 7: Environmental Search for *Coccidioides* spp. in Soil Samples of Endemic Areas from Baja California, Mexico
R.C. Baptista Rosas

POSTER ABSTRACT 8: Comparison of Two Coccidioidins in an Endemic Region in Mexico
H.A. Avila, A. Calleros, V.E. Castañeda, E. Chávez, C. Escobar, N. Gallegos, J. González, J.A. Limón, J.E. Paniagua, D.M. Rosales, I.A. Zepeda, R. Laniado-Laborín

POSTER ABSTRACT 9: *In silico* Genome Analysis
D.M. Engelthaler, et al.

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
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	

• Publishing courtesy of Mayo Clinic Arizona •