

Proceedings of the Annual Coccidioidomycosis Study Group Meeting

Guest Editor: John N. Galgiani, MD
Production Editor: Robert J. Brauer Jr.
Meeting Number 46
University of California, Davis, California

April 6, 2002

I: Epidemiology

Chairperson: Peter Kelly

1. Pappagianis D, VanKeresix K, et al. Resurgent coccidioidomycosis in California – emphasis also on Tulare County and prison inmates.
2. Komatsu K. Coccidioidomycosis in Arizona, 2001.
3. Fisher FS, Bultman MW, Pappagianis D, Orbach MJ, Chatigny JM, Barton C, Haddad M, Panebaker D. The ecology of the Swelter Shelter *C. immitis* site, Dinosaur National Monument, Utah.
4. Crum N, Lamb C, Utz G, Amundson D, Wallace M. Coccidioidomycosis outbreak in Navy Seals during training in an endemic area – Coalinga, California.
5. Clark T, Mark K, Hahn, S, Asgari-jirhandeh N, Lindsley M, Johnson E, Morgan M, Evans B, Nicoll A, Werner B, Hajjeh R. Coccidioidomycosis associated with the World Championship of Model Aeroplane Flying – Kern County, California, October 2001.
6. Hajjeh R. Update on recent coccidioidal outbreaks: new sites and new at-risk population.

Session II: Annual presentations of interesting coccidioidomycosis cases and vaccine activity

Chairperson: Paul Williams

7. G. Rutherford, R.F. Hector and Valley Fever Vaccine Project Investigators. Update on the Valley Fever vaccine project.
8. Lerche N, Christe K, Hector R, Pappagianis D. Experimental coccidioidomycosis in FKS vaccinated and unvaccinated *Cynomolgus* Macaques (*Macaca fascicularis*).
9. Kirkland T. The genome of *C. immitis* – what good is it?
10. Laniado-Laborin R, Castaneda-Godoy R, Torres P. Coexisting tuberculosis and coccidioidomycosis.
11. Morrill GB, Brauer RJ, Galgiani JN. Development of a customized database to document activities of the Valley Fever Center for Excellence.

Tour of the Veterinary Teaching Facilities
Business Meeting

Chairperson: Hillel Levine

Session III: Immunology

Chairperson: David A Stevens

12. Ampel NM, Hector RF, and Pappagianis D. Coccidioidin skin testing response and *in vitro* cellular immune assessment using T27K in a cohort of healthy immune donors.
13. Daniels JI, Wilson W, DeSantis T, Shinn JH, Andersen GL, Layton DL, Johnson SM, Pappagianis D. Development of a specific and sensitive quantitative taqman-PCR assay for detecting *Coccidioides immitis* in environmental media-application to air samples.
14. Zucker K, Kamberi P, Leib SL, Sobel RA, Leppert D, Clemons KV, Stevens DA, Williams PL. Temporal analysis of pro-inflammatory cytokines and chemokines within the brain basilar artery of *C. immitis* infected rabbits.
15. Richards JO, Lake DF, Ampel NM. Reversal of *C. immitis*-specific anergy by dendritic cells from patients with disseminated coccidioidomycosis.

Session IV: Vaccines and Clinical Topics

Chairperson: Tony Catanzaro

16. Soogoor M, Johnson R, Louie J, Helenius M. Analysis of coccidioidal synovitis with and without adjacent osteomyelitis to determine the nature and extent of disease as served in the population at Kern Medical Center and Kern Faculty Medical Group.
17. Blair JE, Smilack JD. Coccidioidomycosis in patients with hematological malignancies.
18. Bowers JM, Ampel NM. Assessment of fatigue and cytokine response in coccidioidomycosis.
19. Kuberski T. Coccidioidomycosis as a cause of sarcoid in Arizona.
20. Tangella KV, Klein RR, Stephen G, Sobonya RE, Schiffman R. *Coccidioides immitis* in bronchoalveolar lavages: Comparison of cytologic and microbiologic techniques.

1. Resurgent coccidioidomycosis (coccy) in California – emphasis also on Tulare County and prison inmates

Pappagianis D, Van Kekerix V.

University of California, Davis

Following the epidemic years of 1991 through 1995, the number of new cases of coccy reported in California declined somewhat in the late 1990's and through the year 2000, although never dropping to the pre-epidemic levels. However, in 2001, there was a sharp rise in cases.

Year		Year	
1989	4.3×10^2	1995	11.2×10^2
1990	4.4×10^2	1996	9.4×10^2
1991	12.0×10^2	1997	6.9×10^2
1992	45.4×10^2	1998	7.2×10^2
1993	41.1×10^2	1999	9.4×10^2
1994	27.1×10^2	2000	8.4×10^2
		2001	14.3×10^2

The surge in 2001 was particularly noted in Kern and Tulare Counties, also California as a whole.

Year	CSDHS	Calif.*	Kern Cnty.**		Total	TulareCnty.*	Out of State*
1999	940	748	504	=	1252	102	425
2000	840	656	406	=	1062	82	430
2001	1430	827	995	=	1822	128	445

CSDHS = Calif. Dept. of Health Services.

*U.C. Davis Coccy Serology Laboratory.

**Data kindly provided by Kirt Emery, M.P.H.

Occurrence of coccidioidomycosis among inmates of prisons of the endemic area has been noted before (e.g., C.E. Smith and others reported on the disease in prisoners of war during World War II). Dr. Smilovitz has noted the disease in inmates of California prisons. However, our attention was focused on this problem by Drs. Jerry Wheadon and Joel Pullen in an (August-October 2000) occurrence of coccy among some 23 inmates at a California Youth Authority, infections acquired while they were fire-fighting near McKittrick in Kern County. One of the 23 developed disseminated coccy. Interestingly, B. Hoar, M. Stars and B. Werner of CSDHS noted that 30% of these young men were asymptomatic but seropositive for coccidioidomycosis. The association of cases with various prison sites in 2000–2001* is indicated in the following table:

Facility	Number of cases
Avenal	36**
Corcoran	14
Chuckawalla	1
Ironwood	1
Vacaville	8
CMC-San Luis Obispo	16
Miscellaneous	10
CA Youth Authority	<u>23</u>
Total	109

*In a few instances onset of disease may have been earlier than 2000.

**One case in a prison employee.

This increase in cases of coccy, emphasizes the markedly increased medical and economic burden on the general population and on correctional institutions.

3. The ecology of the Swelter Shelter *C. immitis* site, Dinosaur National Monument, Utah

Fisher FS, Bultman MW, Pappagianis D, Orbach, MJ, Chatigny JM, Barton C, Haddad M, Panebaker D

An outbreak of coccidioidomycosis occurred at the Swelter Shelter archeological site during June–September 2001. This outbreak is unusual in that the site is located at least 200 miles north of the recognized *C. immitis* endemic zone in southern Utah. A total of 15 people were exposed to dust created from construction of a stone retaining wall and steps; from the sifting of approximately one cubic yard of soil for archeological artifacts; and from the collection of soil samples for the isolation of *C. immitis*. Three of the fifteen people exposed to the dust wore dust protective equipment and were not infected. Only one individual who was exposed to the dust and did not wear a protective facemask escaped infection.

The site is within the Upper Sonoran Life zone at an elevation of 4825 feet and is characterized by cold winters and hot summers. Annual precipitation ranges from 8 to 11 inches. Vegetation near the site is sparse and consists

of scattered clumps of greasewood (*Sarcobatus vermiculatus*), big sagebrush (*Artemisia tridentata*), rabbitbrush (*Chrysothamnus* spp.), Indian rice grass (*Oryzopsis hymenoides*), and other perennial bunch grasses and cacti.

Swelter Shelter is formed by a rock overhang in the Upper Cretaceous Frontier sandstone member of the Mancos Shale Formation. The Frontier member is a resistant light-gray to light-brown, thin-bedded, calcareous, locally crossbedded sandstone. The space beneath the overhang is approximately 15 feet wide, 8 feet deep, and 8 feet high at the opening. The ceiling slopes steeply towards the rear of the shelter allowing rainwater to run down the ceiling and back wall. The opening of the shelter faces south and receives full sunlight from dawn to dusk throughout the year. Terrace deposits of the Green River (possibly several 1000 years old) make up the soils surrounding Swelter Shelter. These soils are a fine-grained sandy loam containing approximately 10% clay, 10% silt, and 80% fine sand. Within, and in the immediate vicinity, of the shelter the soils also contain material derived from the weathering of the Frontier sandstone (mostly fine quartz grains). These soils have a low water holding capacity, an organic matter content of less than 5%, a pH of 8.5 to 11.0, and salinity of 8 to 16 mmhos/cm (EC).

Our working hypothesis is that; Swelter Shelter provides an isolated microclimate favorable to the growth of *C. immitis*; that *C. immitis* was introduced to the soils in the shelter by native Indian cultures between 7000 and a few hundred years ago; that *C. immitis* was introduced via fomites or by the carcasses of infected domestic dogs associated with the native cultures or possibly by carcasses of infected wildlife (e.g. coyotes, wolves, bats); and that other such microclimate/Indian sites may be present in the area. We believe that it is unlikely that *C. immitis* was introduced to the soils of the area via airborne transport.

4. Coccidioidomycosis outbreak among U.S. Navy Seals training in an endemic area – Coalinga, California

Crum N, Lamb C, Utz, G, Amundson, D, Wallace M

Naval Medical Center San Diego, San Diego, California

Introduction: Coccidioidomycosis is an endemic mycosis of the southwestern U.S. and acquisition is by inhalation of arthroconidia during soil disruption. Risks for disease development include activities such as construction or archeological digs as well as natural events such as earthquakes or dust storms. We describe an outbreak of *C. immitis* infection among Navy Seals undergoing military training exercises in Coalinga, California in September–October of 2001.

Methods: Case definition was established as a Navy Seal training in Coalinga in September–October 2001 who had a positive *C. immitis* enzyme immunoassay for IgM or a positive CF titer (≥ 2). Personal interviews and physical examinations were conducted in 22 Navy Seals. Laboratory data including *C. immitis* enzyme immunoassay and complement fixation titers, and a chest radiogram were performed. An epidemiologic investigation was conducted to determine the extent of the outbreak and potential risk factors for infection.

Results: Of the 22 men, all were immunocompetent males and 95% were Caucasian with a median age of 29 years (range 20–40). Ten (45%) of the 22 men developed acute coccidioidomycosis by definition with titers between <2 to 16. Approximately 8 weeks elapsed from onset of symptoms to recognition of the outbreak. This delay was due to missed diagnosis by local clinicians. Cases reported symptoms of fevers (90%), night sweats (70%), chills (60%), weight loss (60%), and arthralgias (40%) which were statistically more common in cases than controls. Eighty percent of cases also described cough. The onset of symptoms in the majority of cases began 2–3 weeks after arrival in Coalinga. Symptom duration was 2–63 days, and three Navy Seals missed workdays due to their illness. Fifty percent of cases had chest radiographs abnormalities of consolidation, adenopathy and/or cavity. No patient has developed disseminated disease to date and there were no deaths.

Conclusions: This outbreak of coccidioidomycosis among 10 of 22 Navy Seals training in Coalinga is the highest attack rate (45%) ever described in a military unit. All cases developed symptomatic disease and 50% had abnormal chest radiographs. The missed diagnosis of cases initially resulted in a delay in the recognition of the outbreak, emphasizing that clinicians should consider the diagnosis of coccidioidomycosis in persons who recently traveled to an endemic area. This outbreak emphasizes the importance of ‘Valley fever’ in U.S. military personnel.

5. Coccidioidomycosis associated with the World Championship of Model Airplane Free Flight – Lost Hills, California, 2001

Clark TA, Hahne S, Johnson E, Mark K, Werner SB, Lindsley M, Evans B, Hajjeh R

Background: Coccidioidomycosis, a fungal infection caused by inhalation of *Coccidioides immitis* spores, can cause severe disease in older and immunocompromised persons. *C. immitis* is endemic to the southwestern United States. Although local outbreaks have occurred following dust exposures, travel-related international outbreaks have rarely been reported. In early December 2001, a 72 year-old male in England and a 55 year-old male in Finland were hospitalized with coccidioidal pneumonia, confirmed by bronchoalveolar lavage fluid culture. Both attended the World Championship of Model Airplane Free Flight, October 8–13 in Lost Hills, California, in which 32 nations participated. We initiated an investigation to alert participants about coccidioidomycosis and define the magnitude of this outbreak.

Methods: A case was defined as positive coccidioidomycosis serology by complement fixation or immunodiffusion in a championship participant. Case finding was conducted through public health authorities and airplane modeling clubs. We conducted a cohort study among participants, administering a standardized questionnaire and collecting serum for anti-coccidioidal antibody testing.

Results: Of 337 participants, 175 (52%) from 13 countries completed questionnaires, 134 (40%) had serology performed, and 95 (28%) completed both. The median age was 54 years (range 12–78); 83% were male; 122 (70%) reported prior travel to an endemic area; 98 (56%) reported large amounts of dust exposure, 84 (48%) reported previous knowledge of coccidioidomycosis, and 16 (9%) wore masks. Thirteen (10%) participants met the case definition. Among case patients with completed questionnaires (n = 7), 57% reported any symptoms; rash, fatigue (57% each), fever, shortness of breath, and night sweats (43% each) were most common.

Conclusions: This outbreak highlights the need to be aware of coccidioidomycosis as a travel-related illness. Travelers to endemic areas, especially those at risk for severe disease, should be advised about coccidioidomycosis prior to travel.

Key words: Valley Fever, coccidioidomycosis, travel, *Coccidioides immitis*

6. Update on recent coccidioidal outbreaks: new sites and new at-risk population

Hajjeh R

Over the last five years, the Mycotic Diseases Branch at CDC has investigated at least 4 outbreaks of coccidioidomycosis, 3 of them had been among travelers to endemic areas (Mexico (2), Kern County (1)) and one involved a newly identified endemic area in northern Utah. In recent years, Mexico has become the country most frequently visited by Americans and several outbreaks of the disease have been reported among returning travelers. In 1996, 21 (17%) cases of coccidioidomycosis occurred among a group of 126 church group members from Washington State who had recently returned from a 6-day stay at an orphanage near Tecate, Mexico. Members of the group had assisted with construction projects requiring excavation, but the trip organizers were unaware of the potential for *C. immitis* infection. More recently, church group members from Pennsylvania traveled to Hermosillo, Mexico, to assist with a church construction project. Within 2 weeks of returning home, 27 travelers developed a flu-like illness, and 8 (30%) had serologically confirmed disease. Visitors from other countries have also developed coccidioidomycosis following trips to the southwestern United States. In October 2001, over 300 individuals from 30 countries participated in the World Championship of Model Airplane Flying in Lost Hills, Kern County, California. One week after returning home, a participant from the United Kingdom developed influenza-like symptoms and was later diagnosed with coccidioidomycosis, and many others were later serologically confirmed to have acute coccidioidomycosis. These three outbreaks illustrate the importance of informing travelers about regions where *C. immitis* is endemic, and the risk of developing coccidioidomycosis and its clinical manifestations.

Recent coccidioidomycosis outbreaks have shared many common features: high attack rates of infection, an increased incidence of some clinical manifestations (such as rash among young adults), an association with massive dust exposures, as well as the usual delay in diagnosis, and the lack of awareness about the disease among travelers to endemic areas. In addition, public health professionals face many difficulties investigating these outbreaks: with the travel-related ones, case finding is a challenge, especially when travelers return to their respective states or countries; environmental studies are quite difficult, especially when it comes to quantifying exposures and isolation of *C. immitis* from the environment. Health care providers – especially those in non-endemic areas – need to be familiar with coccidioidomycosis, its diagnosis, and the need to obtain a detailed travel history. In addition, they need to be aware of the IDSA guidelines for the management of coccidioidomycosis. Prevention of exposure to sources of *C. immitis* in endemic areas is difficult. Nevertheless, travelers should be advised to decrease their risk by limiting their exposure to outdoor dust. Wearing well-fitted masks, or using outdoor vehicles with enclosed air-conditioned cabs can provide some protection. Groups undertaking construction work or other dust-generating activities should be informed about dust-control measures which include wetting soils before disturbing the earth.

7. Update on the Valley Fever Vaccine Project

Rutherford G, Hector RF and Valley Fever Vaccine Project investigators

The Valley Fever Vaccine Project is in the 3rd year of a 5-year effort to identify, evaluate and develop a suitable vaccine for the prevention or amelioration of coccidioidomycosis. Through the efforts of the five academic-based laboratories, a series of recombinant antigens have been identified and testing using murine models and associated immunologic assays. At present, four antigens are considered candidates for the final vaccine; each has been shown to increase survival and decrease fungal burden in a variety of mouse models. In addition, the Project is sponsoring efforts to establish a primate model of coccidioidomycosis, and is also conducting incidence/prevalence studies in naturally-acquired disease in domestic dogs. Both of these species may be utilized for the evaluation of a vaccine prior to human clinical trials, tentatively scheduled to begin in 2004. The Project is also conducting a Phase 1 study of the skin test antigen coccidioidin in human volunteers as a prelude to anticipated incidence/prevalence studies in target populations in California and Arizona.

8. Experimental coccidioidomycosis in FKS vaccinated and unvaccinated Cynomolgus Macaques (*Macaca fascicularis*)

Lerche N., Christe K, Hector RF, Pappagianis D

In efforts to establish an appropriate model of coccidioidomycosis in primates for the evaluation of experimental vaccines, groups of female cynomolgus monkeys were vaccinated with the formalin killed spherule (FKS) preparation or saline control $\times 3$ and then infected intratracheally 30 days after the final immunization with 250 or 2500 arthroconidia of strain Silveira. Animals were monitored for deaths and various clinical and immunological parameters at regular intervals for a six-month period. All animals showed good evidence of pulmonary disease, based on radiographic findings. Weight loss, signs and symptoms, serologic assays (including a CD69 + assay) and skin test results were variable, with no correlation to experimental group discernible. Results from an IFN- γ assay showed control animals to have statistically significant increases compared to vaccinated animals ($p = 0.017$). However, 5/10 vaccinated animals succumbed or were euthanized, compared to 0/10 control animals ($p = 0.033$), suggesting that under the experimental conditions employed, FKS did not confer protection to vaccinated animals.

9. The genome of *C. immitis* – what good is it?

Kirkland T

10. Coexisting Tuberculosis and Coccidioidomycosis

Laniado-Laborin R, Castañeda-Godoy R, Torres-San Miguel P

Introduction: Tuberculosis (TB) and Coccidioidomycosis share epidemiological, clinical, radiographic, and even histopathological features, which can make the correct diagnosis extremely difficult in cases where both diseases coexist. We present two cases with coexistent TB and Coccidioidomycosis.

Clinical cases:

Case 1: A 17 year-old malnourished female is diagnosed with pulmonary tuberculosis, and started on first line antituberculosis drugs; two months later she is readmitted with multiple wart-like skin lesions in the face and neck regions, and osteolytic lesions in fingers, humerus, femur and toes. Aspiration of the elbow joint revealed *Coccidioides immitis* spherules. She was started on amphotericin-B, and the bone abscesses were surgically drained. HIV test was non-reactive. She completed a short-course regimen for TB, and one year on itraconazole, and she is currently asymptomatic.

Case 2: A 42 year-old male, intravenous drug user is admitted with typical signs and symptoms of pulmonary tuberculosis; the routine examination of sputum however, revealed both acid-fast bacilli and spherules of *C. immitis*. HIV was non-reactive. Cultures later grew both *Mycobacterium tuberculosis* and *C. immitis*. He was started on first-line antituberculosis drugs and itraconazole, but was lost to follow-up.

Conclusions: In endemic areas for both diseases, the pertinent studies for diagnosing these two ailments should be performed in every patient with compatible clinical features. Unfortunately in Mexico, sputum is examined routinely only for TB. The diagnosis of one of them, does not exclude the possible existence of the other. When coexistence is proven, treatment for both diseases should be administered simultaneously in an orthodox fashion. Liver function should be carefully monitored due to the additive toxicity of both regimens.

11. Customized database to document activities of the Valley Fever Center for excellence

Morrill GB, Brauer Jr. RJ, Galgiani JN

*Pfizer, Inc., Southern Arizona Veterans Affairs Health Care System (SAVAHCS),
and the University of Arizona, Tucson, AZ*

The Valley Fever Center for Excellence (VFCE) was founded in 1996. It is jointly sponsored by the University of Arizona and the Southern Arizona Veteran Affairs Healthcare System. The Missions of the Center are to develop public awareness and provide education about valley fever, to promote high quality patient care for patients with valley fever, and to pursue and encourage research into all aspects of coccidioides immitis and the diseases that it causes. One of the activities that support this mission is the staffing of a hotline to answer questions from healthcare professionals and the public regarding valley fever. In order to facilitate information entry, retrieval, and analysis, a menu-driven Access database was developed. This database records information about the call, the caller, the patient, treatments, outcomes, and advice, information and referrals generating from the VFCE. Standard reports that can be printed include a Call Subject Report, Contact Type Report, Contact Disposition Report, Contact Summary Report, Origin of Contacts Report, and a Treatment Report. These reports are used for administrative review, to serve as legal documentation of the activities of the VFCE, and to serve as a potential source of epidemiological data for future data analyses.

12. Coccidioidin skin-testing and in vitro cellular immune response using T27K in a cohort of healthy immune donors

Ampel NM, Hector R, Hale B, Pappagianis D

From the Valley Fever Vaccine Project, SAVAHCS, the University of Arizona, the Naval Medical Center, San Diego, and the University of California at Davis

No FDA approved coccidioidal skin-test reagent is currently available. This study represents an initial attempt to examine the current utility of coccidioidin as a skin-test reagent. A prior study had demonstrated reactivity to thimerosal, a preservative contained in coccidioidin. In this study, coccidioidin was diluted in phenol and thimerosal was tested alone. For each donor, six skin tests were placed including 1:2000, 1:500, 1:100, and 1:25 coccidioidin in phenol; phenol alone; and phenol with 1:250,000 thimerosal (v/v). In addition, the relationship between skin-test reactivity to coccidioidin and in vitro lymphocyte responses to the coccidioidal antigen T27K was examined. Twenty-eight subjects with evidence of prior coccidioidal cellular immunity and no evidence of active coccidioidomycosis were studied. No significant adverse reactions to skin-testing were encountered. Twenty-seven subjects developed induration at least one of the coccidioidin test sites. All reactions occurred by 48 hrs after testing. Comparison of the sum of the diameters of induration in response to coccidioidin to a historical control indicated that the current coccidioidin was dilute; an approximately 1:66 dilution of the current material appeared to be equivalent to standard 1:100 coccidioidin. In addition, a total of 7 individuals demonstrated significant induration at the site of thimerosal injection. In vitro test of coccidioidal cellular immunity included measurement of expression of CD69 on CD3 lymphocytes and production of interferon-gamma (IFN- γ) in supernatant plasma of incubated whole blood using 22 subjects at one site. There was a close association IFN- γ production and CD69 expression (Spearman rho = 0.6872, p = 0.0004). The association between the sum of induration after 48 hr to 1:25 coccidioidin and in vitro IFN- γ production was also significant (Spearman rho = 0.4842, p = 0.0224) but not to in vitro CD69 expression (Spearman rho = 0.3271, p = 0.1373). These data indicate that coccidioidin can have a future role in assessing coccidioidal cellular immunity. However, a fraction of subjects can respond to thimerosal, so this preservative should be avoided in future preparations. There was an association between the degree of skin-test reactivity and magnitude of in vitro response.

13. Development of a specific and sensitive quantitative Taqman-PCR assay for detecting *Coccidioides immitis* in environmental media-application to air samples

Daniels JJ¹, Wilson W¹, DeSantis T¹, Shinn JH¹, Andersen GL¹, Layton DL¹, Johnson SM², and Pappagianis D²

¹University of California, Lawrence Livermore National Laboratory, Livermore, CA;

²University of California, Davis, School of Medicine, Davis, CA

[ABSTRACT for 46th Annual CSG, held April 6, 2002, at UC Davis. This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48].

Researchers in the Health and Ecological Assessment Division and the Biology and Biotechnology Research Program at the Lawrence Livermore National Laboratory, and in the Department of Medical Microbiology and Immunology at the School of Medicine of the University of California at Davis, collaborated on a study designed to develop and apply a specific, sensitive, and quantitative Taqman-PCR assay and an advanced high-volume environmental air collection system to demonstrate the feasibility of using these devices to collect and safely detect spores of *Coccidioides immitis*, the fungal organism responsible for Valley Fever (i.e., coccidioidomycosis), in environmental air samples from regions where it is endemic. We confirmed that spores of *C. immitis* (i.e., arthroconidia) are steam killed at 95 °C and such spores can then be handled and prepared safely so their extracted

DNA can be detected by the Taqman-PCR assay. Moreover, we determined the Taqman-PCR assay to be specific, sensitive, and quantitative based on testing for *C. immitis* DNA in the presence of other DNAs, and using different quantities of *C. immitis* DNA to establish a calibration curve. Finally, results from applying the Taqman-PCR assay to air samples collected using an advanced high-volume air sampling system [which includes an 8 × 10 in, track etched (1-μm pore size), polyester filter] positioned on either relatively undisturbed ranch land or on a cultivated agricultural field in Kern County, CA, strongly suggest that it is feasible to collect and detect spores of *C. immitis* in environmental air samples from different locations in a region where *C. immitis* is endemic. We conclude from our study that unique tools are now available to study the occurrence and ecology of *C. immitis* in the environment and such work could yield new methods to reduce or eliminate the microorganism responsible for incidents of Valley Fever.

14. Temporal analysis of Pro-Inflammatory Cytokines and Chemokines within the Brain Basilar Artery of *C. immitis* infected rabbits

Zucker KE¹, Kamberi P^{2,3}, Leib, SL⁴, Sobel RA^{2,5}, Lepert D⁴, Clemons KV^{2,3},
Stevens DA^{2,3}, and Williams PL⁶

¹Children's Hosp. Central CA, Madera, CA; ²CA Inst. Med. Res., Sta. Clara Vly. Med. Ctr., San Jose, CA;

³Stanford Univ, Stanford, CA; ⁴Inst. Infect. Dis., Univ. Bern, Switzerland;

⁵Veterans Affairs Med Ctr, Palo Alto, CA; ⁶Kaweah Delta Hosp., Visalia, CA

Stroke complications associated with CM, due to transmural V, result in significant morbidity and mortality. The immunological/inflammatory processes responsible are poorly understood. We sought to determine whether a number of immunological mediating molecules (IMMS) including a number of cytokines, chemokines, iNOS, matrix metalloproteinase 9 (MMP-9) might contribute to V by evaluating their temporal expression in the brain basilar artery (BA) of male NZW rabbits. BAs were removed at necropsy, snap frozen and mRNA extracted for RT-PCR amplification of specific IMMS mRNAs. Five infected and 3 uninfected rabbits were euthanized on days 4, 9, 14, and 20 post infection. All infected rabbits had neurological abnormalities and severe V in the BA on days 9 through 20. Statistically significant up-regulation of the following IMMS mRNAs were found.

TABLE

IMMS	+ = P # 0.05/day(d)			
	d4	d9	d14	20
IL-6	+	+	+	+
TGF-β	+			
iNOS, IFN-γ		+	+	+
IL-2				+
IL-1, IL-10			+	
TNF-α, CCR-1, MMP-9		+		

In conclusion, numerous IMMS are up-regulated in the BA during experimental CM. Assessment of individual contribution of each mediator to V as well as relative importance in promoting immunological competency during coccidioidal CNS infection requires further studies.

15. Reversal of *C. immitis*-specific anergy by dendritic cells from patients with disseminated coccidioidomycosis

Richards J, Lake D, Ampel N

Coccidioides immitis is a pathogenic, dimorphic fungus found in the Southwestern United States and is the causative agent of coccidioidomycosis. Extrathoracic dissemination of coccidioidomycosis is associated with a lack of cellular immunity. Dendritic cells (DCs) have been shown to initiate and modulate cellular immune responses. To determine if DCs could modulate or initiate the immune response in this disease, monocyte-derived DCs were generated from coccidioidal antigen non-responsive patients with disseminated coccidioidomycosis and healthy, non-immune individuals. DCs generated from both groups demonstrated phenotypes characteristic of DCs and stimulated strong allogeneic mixed lymphocyte reactions. DCs from patients and healthy, non-immune individuals pulsed with the coccidioidal antigen preparation T27K induced lymphocyte proliferation. Mature DCs were much more efficient than immature DCs in these stimulations. Furthermore, restimulation of T27K-primed PBMC with antigen-pulsed DCs generated a *C. immitis*-specific cellular immune response in PBMC from patients with disseminated coccidioidomycosis as well as healthy, non-immune individuals. These results show that (i) DCs have the capacity to stimulate specific cellular immune responses from patients with disseminated coccidioidomycosis who are non-responsive to coccidioidal antigen and healthy, non-immune individuals *in vitro*; (ii) DC can be employed to screen coccidioidal antigens as candidates for human vaccine development; and (iii) DC therapy may be useful in the treatment of disseminated coccidioidomycosis.

16. Coccidioidal Synovitis: A review of 52 cases

Soogoor M, Johnson R, Louie J, Helenius M, Cha T, Einstein, H

University of California at Los Angeles, Kern Medical Center, Bakersfield, CA

Objective: To evaluate the clinical, laboratory, radiological features and treatment of coccidioidal synovitis.

Method: Retrospective observational study of 52 patients diagnosed between December 1990 and December 2000. Inclusion criteria was: compatible history, physical examination and one or more of the following: (1) Complement fixation titre of 1:4, (2) *C. immitis* culture from the joint, (3) Histopathology from the joint demonstrating endospore-forming spherules. Exclusion criteria: HIV, non-synovial joints and isolated osteomyelitis.

Results: Coccidioidal synovitis has predilection for males: mean age of the patients was 34.9 years. The predominant ethnic group affected was latino group. 28 patients had prior diagnosis of pulmonary coccidioidomycosis before dissemination. 24 patients presented as disseminated disease with synovitis. 76% patients had monoarticular involvement. Knee, ankle and wrist were the most common. Pain and swelling of joint was the most common presentation. Osteomyelitis was the commonest X-ray finding. >76% had CF titre of 1:32 Joint pathology showed *C. immitis* in 35 patients. 36 patients had cocci skin test and 55% were negative. All patients received medical treatment; two thirds received concomitant surgical treatment. 56% patients had one surgical intervention. 49 patients received Fluconazole, 3 patients received Amphotericin. Duration of treatment in 41 patients was more than a year. 49% patients showed no disability and 8% had major disability. 33% patients had relapse.

Conclusion: Coccidioidal synovitis can present concomitantly with pulmonary infection or as disseminated disease and affects the larger joints. Pain and swelling heralds the onset of synovitis. Therapy is very protracted. Fluconazole was the mainstay of medical treatment.

17. Coccidioidomycosis in patients with hematological malignancies

Blair JE, Smilack JD, Caples S

Mayo Clinic Scottsdale

Background: Coccidioidomycosis (cocci) is generally a self limited disease, but has increased risk of extrapulmonary infection and mortality in some immunocompromised hosts. The clinical characteristics of coccidioidomycosis in patients with hematological malignancies has not been described for several decades.

Methods: A retrospective chart review of all cases of coccidioidomycosis in persons with hematological malignancies at Mayo Clinic Scottsdale, January 1987 through December 2001.

Results: 56 patients were identified, 42 with adequate information to include in study. The ages ranged 36–84 years, mean 66.8 years. Thirty (71%) were male, and 95% were Caucasian. Predominant cancer types included chronic lymphocytic leukemia (CLL) and non Hodgkin's lymphoma (NHL). The chemotherapy regimens varied widely among all patients; corticosteroids were administered in 25 (59%). The majority of infections were new infections, not reactivation of prior disease. Bronchoalveolar lavage and serology were the primary means of diagnosis; however, serology was positive in only 58% of those for whom the test was performed. Nearly all cases (90%) involved one or both lungs, and additional extrapulmonary spread was also common (33%). Overall crude mortality was 29%. Patients with CLL (n = 14) had primarily pulmonary (without extrapulmonary) infection (86%), positive serology in 29%, and had a crude mortality of 35%. Patients with NHL (n = 14) had higher incidence of extrapulmonary infection (43%), positive serology in 50%, and an overall crude mortality of 35%. Three patients had chronic myelogenous leukemia, and all died with coccidioidal infection (p = 0.03). All of the 5 patients with multiple myeloma had been treated with steroids, yet none had extrapulmonary disease. Serology was positive in 80%, and 80% survived. On the whole, there were no significant risk factors for extrapulmonary coccidioidomycosis in this group already at risk for disseminated disease. Risk factors for death due to coccidioidal infection included the presence of chronic myelogenous leukemia (p = 0.007) and steroid treatment (p = 0.03).

Conclusion: Coccidioidal infection in patients with hematological malignancies is a serious infection. Extrapulmonary infection is common, and overall crude mortality is high. There may be differences in the clinical presentation among the various types of malignancies, but further study is needed to clarify these issues.

18. Fatigue severity in patients with coccidioidomycosis

Bowers JM¹, Ampel NM²

¹*Department of Nutritional Sciences, University of Arizona, Tucson, AZ;* ²*Southern Arizona Veterans Affairs Healthcare System (SAVAHCS), and Department of Medicine, University of Arizona, Tucson, AZ*

Fatigue is a major debilitating symptom of coccidioidomycosis. The incidence of Patients' complaints of fatigue was documented as early as the 1940's, but has not yet been quantified in previous research. We examined the severity of fatigue in coccidioidomycosis patients by using the Fatigue Severity Scale (FSS). The FSS consists of 9 statements. Subjects were instructed to choose a number from 1 to 7 that indicated their level of agreement with each of the nine statements. The nine scores were averaged, yielding the FSS score. The FSS scores were related to nutrition factors, infection duration and severity, and demographics. The FSS scores for coccidioidomycosis patients were compared to FSS scores from age-matched healthy controls using the student's t-test. Forty-eight coccidioidomycosis patients and twenty controls completed the FSS questionnaire. The patients surveyed included any new or existing patient seen in the SAVAHCS Coccidioidomycosis Clinic who gave their consent. No patients were excluded. The mean FSS score for the coccidioidomycosis patients was 4.796 (\pm 1.91), with a broad range of 1.22–7.0. The mean FSS for the controls was 2.12 (\pm 0.71). FSS scores for coccidioidomycosis patients were significantly higher than scores for control subjects (p < 0.0001). Using criteria from FSS validation studies and

applying that criteria to this population of coccidioidomycosis patients, 64.6% of patients were classified with severe fatigue. Coccidioidomycosis patients scored significantly higher than control subjects on six out of nine questions. The range in FSS scores among patients with severe fatigue was 4.78–7.0, with a mean of 6.096 + 0.66. When comparing patients with severe fatigue to those without severe fatigue, there were no differences in age, duration of infection, coccidioidomycosis serology, clinical coccidioidomycosis score, serum albumin, serum prealbumin, %usual body weight, %ideal body weight or body mass index. No significant correlations were found between FSS scores and markers of infection severity (clinical coccidioidomycosis score and serology titer) and duration. Significant negative correlations were found between FSS scores and body mass index (-0.364 , $p < 0.05$) and FSS scores and %ideal body weight (-0.411 , $p < 0.01$). This indicates that as weight status declines, symptoms of fatigue worsen. There were insignificant relationships found between FSS scores and serum albumin, serum prealbumin and %usual body weight. Follow-up FSS scores are being collected in an attempt to track the course and severity of fatigue in coccidioidomycosis.

19. Coccidioidomycosis as a cause of Sarcoidosis in Arizona

Kuberski T

Phoenix, Arizona

Because of their clinical similarities, patients with sarcoidosis can be misdiagnosed as coccidioidomycosis and vice versa. The clinical observation was made that many patients who had sarcoidosis diagnosed in Arizona ultimately had evidence of coccidioidomycosis. The question being addressed is whether *Coccidioides immitis* infection is primary or secondary in sarcoidosis. Four patients with sarcoidosis diagnosed in Arizona were followed prospectively. All four patients eventually demonstrated infection with *C. immitis* at variable times (years) after the diagnosis and treatment of their sarcoid. The hypothesis proposed is that these sarcoid patients have a relatively unique immune response to *C. immitis* manifest by an intense cell mediated response resulting in the formation of non-caseating granulomas, lack of an antibody response and no obvious organisms present. Treatment of sarcoid patients with steroids would tend to impart their immune response and eventually allow a *C. immitis* infection to manifest itself. These clinical observations would support the possibility that *C. immitis* is the cause of the sarcoidosis in Arizona. To prove this hypothesis the presence of *C. immitis* DNA should be demonstrated in tissues at the time of the sarcoid diagnosis when no organisms are evident and also demonstrating these patients have a unique cell mediated immune response.

20. *Coccidioides immitis* in bronchoalveolar lavages: comparison of cytologic and microbiologic techniques

Tangella KV¹, Sobonya RE¹, Schiffman R²

¹Department of Pathology, University of Arizona, Tucson, Arizona, United States;

²Southern Arizona VA Health Care System, Tucson, Arizona, United States

Background: Coccidioidomycosis, more commonly known as Valley fever, is caused by the fungus *Coccidioides immitis*. It is a thermally dimorphic fungus that is prevalent in the alkaline soils of the Southwest region of the United States confined to the Lower Sonoran Life Zone. Tucson is considered a “hot spot” for valley fever. We studied and analyzed the cases of coccidioidomycosis identified in the Cytology and the Clinical Microbiology sections of the Department of Pathology and Laboratory Sciences during the past 15 years.

Design: We reviewed all BAL cytological examinations that were positive for coccidioidomycosis from 1986 to Feb. 2001. We categorized all these cases of *C. immitis* according to year, age and sex. We investigated the correlation between diagnosis of coccidioidomycosis through bronchoalveolar lavage (BAL) cytology versus BAL culture. *C. immitis* can readily be identified through Pap stain, though cultures are considered gold standard. Since

at our laboratory all the BALs undergo a cytological examination with a pap stain, we examined the sensitivity and specificity of this method. At least two slide preparations were made for each case.

Results: There were 6 BALs that were positive through cytological examination. Age of these patients ranged from 25 years to 81 years. All six cases had fungal cultures done, which grew out *C. immitis*. Since 1990, there were 42 BAL cultures positive for *C. immitis*. The sensitivity of cytological examination of BAL for diagnosis of *C. immitis* in culture positive patients in our institution is 14.3%. Also, the specificity of BAL cytological examination for diagnosis of *C. immitis* is 100%. All the 36 cultures positive, cytology negative cases remained negative after re-review.

Conclusion: Our results show that the sensitivity of BAL cytological examination to diagnose valley fever is 14.3%. We find this to be surprisingly low. We recommend that although cytology is an important diagnostic tool for rapid diagnosis, it is not very sensitive and hence, it should be utilized in conjunction with culture. The possibilities of utilizing molecular technique like PCR using *C. immitis* specific primers on BAL specimens directly should be explored. A PCR technique may be more specific and sensitive and could aid in more rapid diagnosis than traditional culture and microscopic examination methods.

Annual Presentations of Interesting Coccidioidomycosis Cases

Winner of the Annual Award

Cocci Endocarditis

La Via WV

We recently evaluated a 10-year old male who was previously treated for Disseminated Coccidioidomycosis (DC), with amphotericin (AmB) for 1 month followed by fluconazole (FLC) PO. He returned 6 months later with respiratory symptoms, fevers and anorexia that started about 1 month following self-discontinuation of FLC treatment. *C. immitis* complement fixation (CF) titers were 1:2048. At presentation he had severe orthopnea as well as hepatosplenomegaly and dilation of superficial abdominal and chest veins. During hospitalization arrhythmias developed; an echocardiogram revealed a 20 × 14 mm, pedunculated, mobile vegetation in the right atrium. This mass was surgically excised and pathology revealed *C. immitis* organisms in amorphous vegetation material and was culture positive within 24 hours. CSF CF titers and fungal cultures were negative. His PMH was significant for Juvenile Rheumatoid Arthritis treated with systemic and intra-articular steroids as well as methotrexate in the past. He was treated with 5 months of AmBisome (5 mg/kg/day) followed by PO itraconazole (300 mg bid) and continues to improve clinically. He developed numerous complications during his therapy including vitritis, multiple punctate cerebral lesions and molluscum-like skin rash. These have subsequently improved. This is the first case of pediatric infective endocarditis due to *C. immitis* in the literature.

“Runner Ups”

A Breast Implant Infected with Coccidioides immitis

bf Blair JE, Schnur P

Mayo Clinic Scottsdale

A 55 year old female was diagnosed with breast cancer in 1984. She underwent a modified radical mastectomy, followed by adjuvant chemotherapy, and had no evidence of recurrence. Eight years later, she had biopsy proven pulmonary coccidioidomycosis that symptomatically resolved without specific therapy; follow up chest x-rays showed decreasing nodule size. In 1996, she began breast reconstruction, initially with contralateral simple mastectomy and bilateral tissue expanders. Over the subsequent 6 months, the expanders were periodically injected with saline. At the 2nd stage of surgical reconstruction, silicon implants were placed, although turbid fluid was encountered around the right tissue expander. The breast fluid ultimately grew *Coccidioides immitis*. She was treated with fluconazole for 6 months. Her serology was initially positive with complement fixation of 1:2, positive

immunodiffusion, and positive enzyme immunoassay (EIA). Following treatment, cocci serology was only positive for EIA IgG, which persisted for 6 years without further evidence of active infection.

Longest Case of Cocci Ever!

Johnson R, Einstein H

Notes not available