



# Coccidioidomycosis

STUDY GROUP

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# PROCEEDINGS OF THE ANNUAL COCCIDIOIDOMYCOSIS STUDY GROUP MEETING

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## Abstract 1: Coccidioidal Meningitis: A Decade of Experience

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A total of 115 patients presenting with coccidioidal meningitis to Kern Medical Center from July 1991 to January 1999 are reviewed. The distribution of new diagnoses reflects the epidemic of coccidioidomycosis that occurred in the earlier part of the decade. Initial treatment was with fluconazole in the majority of patients. A minority received intrathecal amphotericin B. Initial complaints included headache, fever, nausea and vomiting, meningismus, visual abnormality and mental status changes. Demographics showed a preponderance of males and a racial distribution reflective of patients served at Kern Medical Center. A minority of patients had co-existing medical conditions including HIV, diabetes mellitus, and intrauterine pregnancy. Co-existent non meningeal dissemination was found in approximately ten percent of the patients. A minority of patients disseminated while receiving azole therapy. The majority of patients receiving skin testing at diagnosis had a negative result. Abnormalities of cerebrospinal fluid at presentation showed a wide diversity in pleocytosis, glucose and protein. Cerebrospinal fluid eosinophilia had significant diagnostic specificity. The majority of patients had complement fixing antibodies in cerebrospinal fluid and all had complement fixing antibodies in serum. Most mortality occurred in the early months of infection. Approximately fifty percent of patients showed a good response to initial fluconazole therapy. Additional therapy often including intrathecal amphotericin B was required in non responders.

A large number of new coccidioidal meningitis patients have been seen especially in the earlier part of the decade. Fluconazole after nearly a decade of experience appears to be as effective as intrathecal amphotericin B as primary therapy.

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## Abstract 2: Comparative efficacies of fluconazole and terbinafine in a rabbit model of coccidioidal meningitis

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A rabbit model of coccidioidal meningitis was used to compare the therapeutic efficacies of fluconazole (FCZ) and terbinafine (TBF). Hydrocortisone acetate-treated NZW rabbits were infected intracisternally with either  $2.2 \times 10^4$  or  $6.4 \times 10^4$  *Coccidioides immitis* arthroconidia. Oral treatment with PEG-200 B.I.D. (n=8), TBF B.I.D. (n=9; 200 mg/kg/day) or FCZ Q.D. (n=8; 80 mg/kg/day) began on day 5 and continued for 21 days. Mean survival times were 20, 24, and 32 days for PEG, TBF, and FCZ, respectively. All of the FCZ-treated (100%; p=0.003), 56% of the TBF-treated (p=0.4), and 25% of the PEG-treated animals survived the length of the study. Both FCZ and TBF were effective at reducing the incidence of paresis. Only FCZ was effective at reducing most neurological and systemic signs. FCZ treatments resulted in lower CSF protein concentrations and WBC counts, and faster clearing of CSF fungal cultures when compared with PEG-treated controls, but TBF treatments had no effect on these parameters. Neither drug affected CSF glucose levels. Mean serum TBF levels by bioassay were within the range of 3.5 to 6.2 g/ml at 1, 2, and 4 hr post-dose and 0.35 to 7.0 g/ml at 14 hr post-dose. No TBF was detected in CSF. Mean FCZ levels (24 to 25.5 hr post-dose) by bioassay were 16.4 to 19.2 and 13.5 to 19.2 g/ml for serum and CSF, respectively. CFU reduction in the spinal cord and brain was over 100-fold (p=0.0005) in FCZ-treated animals and 2-fold (p=0.2) in TBF treated animals when compared with PEG-treated animals. Histopathologic severity (semi-quantitative scoring system) was significantly attenuated by FCZ treatment (p=0.05) and slightly attenuated by TBF treatment as compared with controls. In conclusion, TBF appeared to have a modest effect on survival, histology, and tissue CFU reduction, however, it was not significant. FCZ was effective at controlling coccidioidal meningitis.

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### Abstract 3: Evaluation of interferon gamma as adjunctive therapy in combination with fluconazole in the treatment of coccidioidal meningitis in a rabbit model

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A rabbit model of coccidioidal meningitis was used to evaluate the therapeutic efficacy of recombinant rabbit IFN alone and in combination with fluconazole (FCZ). Hydrocortisone acetate-treated NZW rabbits were infected intracisternally with either  $4.4 \times 10^4$  or  $6.0 \times 10^4$  *Coccidioides immitis* arthroconidia. Treatments began on day 5 and continued for 21 days. Treatment groups consisted of PEG-200/saline (n=9), PEG-200/IFN (n=8), FCZ/saline (n=8), FCZ/IFN (n=8) or uninfected rabbits receiving PEG/IFN (n=7). PEG and FCZ in PEG (40 mg/kg) were given orally Q.D. Saline and IFN (106 U/kg) were given subcutaneously Q.O.D. Mean survival times were 22.3, 29.4, 32, and 32 days for PEG/saline, PEG/IFN, FCZ/saline, and FCZ/IFN, respectively. All of the FCZ-treated (100%; p=0.02), 38% of the PEG/IFN-treated (p=0.3), and 44% of the PEG/saline-treated animals survived the length of the study. Both FCZ treatments were effective at reducing neurological and systemic signs, lowering CSF protein concentrations and WBC counts, and faster clearing of CSF fungal cultures when compared with PEG-treated controls (p0.04). PEG/IFN treatment delayed the onset of paresis/paralysis. CFU reduction in the spinal cord and brain was over 30-fold (p0.003) in FCZ-treated animals and 2-fold (p0.56) in PEG/IFN-treated animals when compared with PEG/saline-treated animals. Histopathologic severity (semi-quantitative scoring system) was significantly reduced by both FCZ treatments (p0.004), but not by IFN alone. Uninfected IFN-treated animals showed fever spikes, but no adverse clinical signs. In conclusion, IFN appeared to have a modest effect on survival and tissue CFU reduction, however, it was not significant. FCZ was effective at controlling coccidioidal meningitis. At the dosages tested, it is not clear whether IFN-FCZ combination therapy has an advantage over fluconazole alone.

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## Abstract 4: Efficacy of sordarins for the treatment of experimental systemic *Coccidioidomycosis*

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A new class of compounds, sordaricin derivatives (Glaxo Wellcome), which selectively inhibit fungal protein synthesis and have a broad spectrum of activity were tested for efficacy. Systemic coccidioidomycosis was established in female CD-1 mice with 382 arthroconidia of *Coccidioides immitis*, Silveira, given iv. Therapy was begun 4 days postinfection and mice received either GM193663 (663), GM211676 (676), GM237354 (354), fluconazole (fcz) or no Rx. All compounds were given at 20 or 100 mg/kg/day (MKD) in sterile water PO BID for 19 d. Deaths were tallied through 49 days postinfection and quantitation of residual burdens of *C. immitis* in organs done. Serum pharmacokinetics of the compounds were studied in uninfected mice. The MICs of 663, 676 and 354 for *C. immitis* were 1.56, 0.39 and 0.39 g/ml and the MFCs were 6.25, 3.13 and 0.39 g/ml, respectively. Peak serum levels (sampled 1-2 h) after a 50 mg/kg dose were 9.8 g/ml for 663; 13 g/ml for 676; 6.0 g/ml for 354. No accumulation occurred after 19 d dosing; peak levels of 3.2 g/ml for 663, 4.0 g/ml for 676, and < 2.5 g/ml 354 was detectable. We estimate that the t<sub>1/2</sub> for each compound is < 2 hours. In vivo, all drugs had dose-responsive efficacy. All regimens significantly prolonged survival over the control groups (LD<sub>100</sub>); 80-100% of mice given the 100 mg/kg doses of fcz or a GM drug survived. All 100 MKD regimens were equivalent. At 20 MKD, 676 was equivalent to 100 MKD of fcz, indicating that 676 was »5-fold more efficacious. Both 663 and 354 at 100 MKD were equivalent to 100 MKD of fcz. No mice were free of infection. All drugs dose-responsively reduced fungal burden in all three organs. 354 at 100 MKD was superior to all other regimens in the reduction of burden in all organs. The next best regimen, 100 MKD of 676, was more efficacious than 100 MKD of fcz in all organs. 663 showed efficacy, but was superior to 100 MKD of fcz only in the spleen. In conclusion, GM compounds were found to be equivalent or superior to fcz in the treatment of systemic coccidioidomycosis. *C. immitis* was susceptible both in vitro and in vivo. These results are encouraging, indicating that further testing in other models of fungal disease is warranted.

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## Abstract 5: Current Trends in Management of Coccidioidomycosis

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### **INTRODUCTION:**

Coccidioidomycosis is a systemic fungal infection endemic to the southwestern United States and other parts of the western hemisphere. Most primary coccidioidal infections resolve without therapy. There exists no persuasive evidence that any treatment is useful for either shortening the course of the initial illness or preventing more serious problems from arising. There are no definite guidelines.

Kern County Health Department and the Kern County Coccidioidomycosis Study Group thus conducted a series of CME lectures for the local physicians over these four years inviting local experts and various research groups from all over the country. Numerous interviews with experts from both the print and broadcast media were organized. An increased awareness of coccidioidomycosis was cultivated amongst the local population and the physicians. Various diagnostic, controlling, preventive and treatment measures were discussed and debated intensely in an effort to develop a consensus on management of the disease.

### **DESIGN:**

A needs assessment survey of a cohort of local physicians was done to outline; the current trends in management of coccidioidomycosis, factors affecting their decision patterns, their exposure to the various CME programs on the subject, the perceived contribution of these programs and the type of programs they would be more interested in future. A key question was to see if their management of coccidioidomycosis has changed in the past four years. All Kern county physicians were eligible for the survey. As this is an endemic disease specific to this area the survey will be helpful to local physicians only.

### **SUMMARY:**

An increased participation in various CME activities on Coccidioidomycosis, introduction of new anti-fungal agents, research interests and legal concerns has changed the practice patterns of local physicians in managing coccidioidomycosis. There has been a development in consensus of opinion on the types of treatment and their dosages. Most of the physicians agreed that recurrent disease, disseminated disease or meningitis

should be treated and would initiate treatment only after positive cocci titers have returned or there has been a worsening of clinical status. However, there are considerable variations in the perception of demographic, clinical and laboratory parameters as risk factors associated with serious illness. There is also considerable disagreement on the importance of early treatment in reducing chances of dissemination, chronic disease or mortality and the duration of treatment. More studies are needed to clearly define the indications of treatment in primary coccidioidomycosis and the duration of treatment. Most of the physicians, including those who do not treat, do attend the CME programs and would like to see more panel discussions. The focus of the future CME programs should thus be on panel discussions, generating a debate on the indications and duration of treatment. A randomized controlled trial of the efficacy of available treatment in preventing complications and serious illness in primary coccidioidomycosis over a defined period may help outline the indications and duration of treatment. However, due to financial, ethical, Medico-legal and time constraints this may not be possible. There may thus be a need to direct future efforts on basic research and development of a vaccine to prevent this disease instead. Coccidioidomycosis being an endemic disease, developing a vaccine would be a very successful alternative. Until then CME programs should be continued to keep the debate open and to prevent unnecessary utilization of anti-fungal agents.

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## Abstract 6: Cerebrospinal fluid protein vs pleocytosis as a predictor of outcome in coccidioidal meningitis

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**Background:** Disseminated coccidioidomycosis involves the meninges in one half of all cases. Cerebrospinal fluid (CSF) leukocytes and protein have traditionally been used as diagnostic and prognostic indicators of coccidioidal meningitis. Penrose et al observed that higher CSF protein levels were associated with radiographic abnormalities, but observed no apparent association between CSF-WBC and radiographic changes.

**Purpose:** Objective of this study is to analyze whether and to what extent either of these parameters is predictive of symptoms and clinical status.

**Methods:** This is a retrospective study of 80 patient's charts from July 1, 1991 to January 1999. Data was collected at diagnosis, 6,12 and 24 months for signs, symptoms, laboratory and radiographic (brain CT/MRI) findings. Symptoms were scored using the symptom portion of the mycosis study group (MSG) scoring scale. The CSF protein subset was divided into two groups: low protein (<120 mg/dL) and high protein ( $\geq 120$ mg/dL). The CSF leukocyte subset was divided into two groups: low WBC (<100 cells/mL) and high WBC ( $\geq 100$  cells/ mL). Statistical analysis using Chi-square and Pearson correlation were performed.

**Results:** Initial CSF protein level and MSG symptoms score had a statistically significant correlation at 6 and 24 months ( $r=0.50$ ,  $r=0.26$ ). Initial CSF protein level and hydrocephalus had statistically significant relationship ( $P=0.00005$ ). Initial CSF protein and mortality showed a relative linear relationship. No statistical significance was found between CSF-WBC and mortality, hydrocephalus, or the symptom score.

**Conclusion:** Baseline protein level may be a predictor of hydrocephalus, and had a positive correlation with portion of the MSG score. No correlation existed between CSF-WBC and mortality, hydrocephalus, or MSG symptom score. However, we still consider it prudent to use CSF pleocytosis in its current capacity and further study of this relationship is warranted.

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## Abstract 7: Coccidioidal knee synovitis: appraisal of the management since the introduction of the azoles

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**Introduction:** *Coccidioides immitis* is a pathogenic dimorphic fungus, endemic in northwest Mexico, southwestern United States, and portions of Central and South America. In the past decade, the use of azoles has increased as therapy of coccidioidal synovitis of the knee.

**Objective:** To evaluate the role of medical versus medical/surgical (synovectomy) treatment of coccidioidal knee synovitis in the era of azole therapy.

**Design:** Retrospective study

**Methods:** A total of 17 (12 from KMC, 5 from a local private office) patient's medical records from 1988 to January 1999 were reviewed. The diagnostic criteria were based on 1) positive culture of synovial fluid or tissue 2) histologic granulomatous synovitis with evidence of spherules with endospores or histologic granulomatous synovitis with a positive complement fixation (CF) titer 3) clinical symptoms with a positive serum CF titer.

**Results:** All patients presented with pain and swelling of the involved knee and had increased uptake on bone scans. 11/17 (65%) of the cases had surgical intervention in conjunction with medical treatment. 7/11 (64%) had synovectomy, 3/11 (27%) had meniscectomy, and 1/11 (9%) had a total knee replacement. The patients that underwent synovectomy 2/6 (33%) relapsed and 4/6 (67%) did not relapse. Medical therapy alone 4/6 (67%) relapsed and 2/4 (33%) did not relapse. The long term prognosis of patients with and without synovectomy was relatively equal.

**Conclusions:** Knee is a relatively common site of coccidioidal dissemination. Chronic pain and swelling in the knee with or without the history of antecedent trauma should be considered as possible. dissemination. Fluconazole is the mainstay of treatment, but amphotericin B may be used in more severe cases. Relapse with medical therapy is very common. Synovectomy may decrease the frequency of relapse, however it did not effect the ultimate outcome. Repeated and/or long term treatment often lead to stability on

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## Abstract 8: Surgical Pathology of *C. immitis* in Tijuana River Valley

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*Coccidioides immitis* is a thermaldimorphic saprophytic soil dwelling fungus, a known pathogen limited to the Lower Sonoran Desert regions of the Western Hemisphere. Infection is usually acquired by inhalation of ubiquitous airborne arthroconidia. This is to orient you to *C. immitis* pathology as we see it in the Tijuana River Valley, Baja California Norte, Mexico. The forty-third Annual Cocci Study Group meeting takes place in the heart of the Tijuana River Zone, in the valley most notable for containing a high incidence of *C. immitis* infection. Just abutting the meeting facility is the Tijuana River Aqueduct, essentially the Tijuana River as it rolls through the valley. During most of the year, almost as many as 300 days a year, it is a dry riverbed. When the seasonal rains do arrive, this riverbed acts as a flood channel. It drains both the river valley slopes and the high Sonoran type desert plateau just 30 miles east of the valley. Although currently a very populated commercial and residential area, just twenty-five years ago this entire basin was a dry wasteland saturated only when the rains arrived. This Tijuana River Valley along with its basin is quite a unique geographical area. From one vantage point you can see both the Sonoran type desert while looking east and a clear view to the Pacific Ocean while looking west. Within this geographical area the most important aspect for the continual seeding of *C. immitis* arthrospores is the course of the Westerly winds known locally as the Santa Anna's. These Westerly winds, at times reaching considerable speed with devil dust swirling, act to perpetuate the continual source of inhalable *C. immitis* spores. With this singular background, coupled with the increased commercial and human land use, *C. immitis* pathology is as unique here as the spherule, which identifies it. Along with Cocci's presence in this valley, Tuberculosis is attendant in alarming rates. Each hospitalized patient carries a diagnosis of rule out Tuberculosis. Adding to the variety of pathology at times, Cocci and Tuberculosis is diagnosed concomitantly. As Tuberculosis is known as the Great Imitator; *C. immitis* is to be known as the Great Imitator II. For technical reasons serology is not the mainstay of diagnosis in the Tijuana River Valley area. Culture and histopathology are the pillar of diagnosis. As a rule cases are not discovered early in their disease course. When considering pathology of *C. immitis* in Tijuana River Valley, cases of persistent pneumonia and the single solitary lesion do not play a significant role. In contrast, at any time during the year a spectacular case of disseminated *C. immitis* may be found.

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## Abstract 9: Coccidioidomycosis in a Veteran's Affairs clinic: an on-going project

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Coccidioidomycosis is a disease of protean manifestations with variable outcomes. We have recently established a clinic which prospectively follows patients with various forms of coccidioidomycosis with the aim of determining factors associated with outcome. All providers at the Tucson VAMC are encouraged to refer patients with coccidioidomycosis to the clinic and culture and serologic results are reviewed monthly. Patients with positive results are scheduled for clinic. From 9/16/98 to 3/15/99, 34 subjects were entered into the study. The mean age of the patients was 57 years (22 - 83); 32 were male; 32 were non-hispanic; 24 were white, 10 were black; and 28 were non-smokers. Only 7 had an identifiable underlying disease (2 cancer; 2 diabetes, 1 COPD, 2 other [sarcoidosis, scleroderma]). Of the 32 patients where it was known, 19 had pulmonary coccidioidomycosis (6 primary, 2 chronic, 11 nodules) while 13 had disseminated (6 cutaneous, 5 bone & joint, 4 meningitis). The time of coccidioidal diagnosis was 2.8 years (0 - 23); the mean IDCF titer was 2.4 (0 - 8); and of those tested, only 7 of 24 had positive spherulin skin tests. 18 were on no therapy; 12 were on fluconazole, and 2 each on itraconazole and ketoconazole. These early results indicate that patients with coccidioidomycosis in this clinic are older and predominantly white. Smoking and underlying disease were relatively uncommon and pulmonary nodule was the most common manifestation. Continuing results are expected as this study continues.

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## Abstract 10: Epidemiologic studies in coccidioidomycosis in a geriatric population

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Previous state-wide studies in Arizona have suggested an increased incidence of coccidioidomycosis in persons aged  $\geq 65$  years (28.0 per 100,000 population). Clinical observations had been made that the two retirement communities of Sun City and Sun City West, Arizona had high rates of hospitalization for coccidioidomycosis. Reported cases of coccidioidomycosis from the two hospitals serving these two communities increased from 15 cases in 1991 to 108 in 1998. The hospital records of a sample of ninety patients hospitalized with coccidioidomycosis from 1996-1998, were reviewed. Eighty-three patients (92%) fit the case definition of laboratory proven coccidioidomycosis. The age distribution for these patients was: 5(6%)  $\leq 54$  years; 7(8%) 55-64 years and 71 (86%)  $\geq 65$  years. For 1998, the annual incidence rate of coccidioidomycosis in these two communities was at a minimum of 183 per 100,000 population more than 6 times the rate for the State of Arizona. This study supports the observation that coccidioidomycosis is a significant public health problem in this geriatric population.

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## Abstract 11: Diagnosis of Early Coccidioidomycosis at Davis-Monthan AFB

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### **Background:**

Although primary coccidioidomycosis is a common illness in Arizona with an estimation of 20,000 infections per year, less than 2,000 cases a year are reported to the state department of health. Standard medical practice fails to diagnose greater than 90% of illness due to *C. immitis*. Education of providers in the primary care setting should improve awareness and therefore diagnosis of this illness.

### **Method:**

Primary care providers attended an hour-long presentation about primary coccidioidomycosis during the summer of 1998. A case controlled chart review of seropositive and seronegative patients seen at the Davis-Monthan facility during the period of September through December 1998 was then done and the results compared with the previous years. Providers were given a reminder list of some of the presenting signs and symptoms of primary infection of *C. immitis* and the charts reviewed was screened for the following: erythema multiforme or erythema nodosum; eosinophilia; increased ESR; lower respiratory tract symptoms and either multiple visits, abnormal CXR or treatment with empiric antibiotics; two of the following -- fever, arthralgia or fatigue.

### **Results:**

During the time frame of September through December of 1997 there were 94 patients tested serologically for coccidioidomycosis and 5 were serologically positive for the infection. During the same time period in 1998 after the intervention of provider education there were 207 patients tested and 13 cases were identified. The ratio of infections/test went from 1:19 in 1997 to 1:16 in 1998. The following signs and symptoms from the providers reminder list were found to be significant: erythema multiforme ( $p=0.003$ ), fever ( $p=0.04$ ), abnormal CXR ( $p=0.02$ ), increased ESR ( $p=0.002$ ), and eosinophilia ( $p=0.006$ ).

### **Conclusion:**

Education of primary care providers about the signs and symptoms of early infection with Coccidioidomycosis increased the testing and yield of positive cases. In a patient with a consistent clinical history and fever, erythema multiforme, an abnormal chest xray, an elevated ESR, eosinophilia or when considering empiric antibiotic treatment, consideration of serologic testing for Coccidioidomycosis could be productive.

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## Abstract 12: Treatment of coccidioidomycosis and lipid metabolism: is there a link?

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Patients on anti-fungal treatment for coccidioidomycosis infections were noticed to have lipemic sera by the laboratory. This prompted further evaluation of the lipid profiles of patients on fluconazole. Lipoprotein abnormalities have been known to be a part of the acute phase reaction and can be both beneficial as well as harmful to the host. Harmful effects are primarily due to the production of dense LDL particles and increased LDL oxidation which promote atherogenesis. We hypothesized that patients begun on therapy had a greater increase in lipid abnormalities than could be explained by the acute phase reaction alone. Retrospective study of 12 patient's charts followed in Kern Medical Center's Cocci Clinic were examined for serial lipid profiles at baseline, and during follow-up over 1 year. Ethnic profile was as follows: Latino (6), African-American (2), Caucasian (3), and other (1). Two groups were identified. Group 1 had a secondary cause for hyperlipoproteinemia (DM type 1 (1), DM type 2 (4), Hypothyroidism (1)). Group 2 had no identifiable cause for hyperlipoproteinemia. Group 1 was found to have higher baseline triglyceride (TG) levels when compared to Group 2 (means: 527mg/% vs 276mg/%) In both groups, mean TG levels rose while on treatment (1002 mg/% vs 468mg/%). One patient in Group 1, who had DM type 1, developed the complications of DVT and MI during the first year of treatment. Only in Group 1 were lipid lowering treatments used. The predominant abnormality was high TG and low HDL. Group 2 patients will require further evaluation and longer follow up to determine if secondary causes or vascular complications emerge.

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## Abstract 13: Coccidioidal syringomyelia

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Syringomyelia following coccidioidal meningitis has never been reported. We have documented four cases syringomyelia in patients with coccidioidal meningitis. The syrinx developed 1 month to 10 years following the meningitis diagnosis. All four patients suffered arachnoiditis that antedated the diagnosis of syringomyelia. The diagnoses of syringomyelia in these patients were suggested by clinical features and confirmed by neuroimaging in all 4 cases and operative findings in 2 of the cases. The clinical features include a central cord syndrome with dissociated sensory loss and areflexic weakness of the upper extremities, muscle wasting of neck, shoulders, and upper extremities, and can progress to spasticity and weakness of the lower extremities. One patient who developed dysphasia, tongue weakness, and nystagmus was diagnosed with syringobulbia. Syringobulbia is the result of rostral extension of pre-existing syrinx of the upper cervical cord. The patient with syringobulbia died of massive aspiration from an impaired gag reflex attributed to the syrinx involvement of the brain stem. The treatment of coccidioidal syringomyelia is primarily surgical, including posterior fossa decompression (suboccipital craniectomy, upper cervical laminectomy, placement of dural graft), shunting of hydrocephalus, and lysis of adhesion. The three surviving patients have undergone surgical intervention, intrathecal amphotericin B and fluconazole therapy, and have minimal neurologic deficits. The patients highlight the importance of coccidioidal syringomyelia for its potential to cause long term neurological injury and fatal complications.

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**Abstract 14: Soil Isolation and Molecular Identification of *Coccidioides immitis***

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*Coccidioides immitis*, the causative agent of coccidioidomycosis or valley fever, is a fungal pathogen endemic to the semi-arid regions of the Southwestern United States, parts of Mexico, Central America and South America. Isolation of the fungus has been sporadic and retrieval rates vary tremendously from year to year. In the spring of 1997 and 1998, *C. immitis* was isolated from 5 out of 935 soil samples from throughout the San Joaquin Valley. The fungus was isolated using double pour method and identified using species specific primers in a multiplex PCR. The isolates were further typed using microsatellite markers developed for genotyping of clinical specimens for population genetic studies. Two soil samples adjacent to each other yielded identical genotypes, a soil sample obtained one year later at the same location again yielded the same genotype. Another soil sample consistently yielded a single genotype; however, the last soil sample yielded 2 different genotypes, one more predominant than the other. Using discriminant analysis, it was possible to group the isolates by location. Thus, even though a single point source may contain more than one genotype, those genotypes appear to be more similar than genotypes from a different location. Using microsatellite markers in conjunction with discriminant analysis may be useful in identification of geographic clusters of the clusters and may prove useful in epidemiological investigations.

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## Abstract 15: Mapping Canine Coccidioidomycosis: A Pilot Study for Epidemiologic and Environmental Research

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Coccidioidomycosis is a common fungal disease of both dogs and humans in the endemic regions of the southwestern United States. As the dog population in general is less mobile than the human one, we hypothesized that addresses of canine cases may be a good way to locate areas of higher fungal density for both epidemiologic analysis and targeting of soil collection for culture. Addresses of serologically positive dogs were collected over a six month period. Cases were mapped and compared to numbers of licensed dogs to evaluate possible patterns of high density of disease in certain areas of Pima County. This study is limited by the fact that cases were collected from only one laboratory, that some veterinarians did not participate, and that we are unable to distinguish from laboratory records which are new cases and which are rechecks. Nevertheless, the method used here can be expanded and refined to perform an epidemiologic study to evaluate the incidence of coccidioidomycosis in dogs in southern Arizona.

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## Abstract 16: Host Genetic Influences on Severity of Coccidioidomycosis.

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Coccidioidomycosis is usually expressed as a mild flu-like illness in ~40% of infected individuals, progressing to severe pulmonary or disseminated disease in 1-10% of symptomatic cases, depending on race. This report examines host genetic influences on disease severity among class II HLA loci and the ABO blood group. Subjects include African American, Caucasian, and Hispanic individuals with mild or severe disseminated coccidioidomycosis from Kern County, California. Among Hispanics, predisposition to symptomatic disease and severe disseminated disease is associated with blood types A and B, respectively. HLA class II allele DRB1\*1301 allele marks predisposition to severe disseminated disease within each of the three ethnic groups. Reduced risk of severe disease is associated with DRB1\*0301-DQB1\*0201 among Caucasians and Hispanics, and with DRB1\*1501-DQB1\*0602 among African Americans. These data support the hypothesis that host genes, in particular HLA class II and the ABO blood group, play a role in susceptibility to severe coccidioidomycosis.

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**Abstract 17: Cloning of the Echinocandin-binding Subunit of a Glucan Synthase from *Coccidioides immitis* and Its Expression During Mycelial and Spherule Growth**

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Previous studies in our laboratory had shown that cilofungin, an echinocandin antifungal drug, inhibited mycelia but not spherules of *C. immitis*. To begin to understand this anomaly better, we have partially cloned and sequenced a homolog from *C. immitis* of the -1,3-glucan synthase subunit (GS1) which binds and is sensitive to echinocandins and pneumocandins. The ultimate goal of this study is to compare the relative expression of the gene in mycelia and spherules at the level of both transcription (mRNA) and translation (protein). Thus far, the partial DNA sequence (Genbank AF1595) has been deduced to encode for a protein with 87% and 90% overall similarity to the *Aspergillus nidulans* and *Paracoccidioides brasiliensis* FKS genes, respectively. There are 2 small introns in the completed nucleotide sequence, and 16 transmembrane helices in the deduced amino acid sequence, which are expected based on other genes and proteins of the class, respectively. These studies are ongoing.

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## Abstract 18: Identification of an Aspartyl Proteinase from *Coccidioides immitis*

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A crude subcellular vaccine derived from formalin killed *Coccidioides immitis* spherules, designated F27K, and has been shown to protect mice against a lethal intranasal challenge. Con A lectin affinity chromatography was used to separate components of the F27K vaccine. SDS-PAGE of the eluted material yielded multiple discrete bands. One of these bands, 45 kDa, was subjected to N-terminal amino acid sequencing. The derived 18 amino acid sequence showed greatest homology to fungal aspartyl proteinases. Degenerate oligonucleotide primers derived from the 45 kDa N-terminal sequence and from a consensus region of several aspartyl proteinases were prepared and were used to amplify the intervening region of *C. immitis* DNA. The 870 BP amplicon was cloned and the nucleotide sequence determined. The deduced amino acid sequence showed 85.9% and 82.4% homology to aspartyl proteinases from *Aspergillus fumigatus* and *A. niger* respectively. These data support the identification of the protein as an aspartyl proteinase. Studies are underway to clone the entire gene and to express the recombinant protein. This will permit evaluation of this protein as a possible immunogen.

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**Abstract 19: Development and characterization of a new soluble, subcellular vaccine against infection in mice by *Coccidioides immitis***

*C.R. Zimmermann, S.M. Johnson, K.M. Kerekes, N.M. Ampel, L. Christian, R. Williams, D. Pappagianis*

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Previously, we have shown that a soluble vaccine (F-27K)(extracted from formalin-killed, endosporulating whole spherules) when combined with an alum adjuvant, protected mice from lethal infection as well as the whole spherule starting material. We fractionated the F-27K vaccine in an attempt to isolate and identify the protective protein component(s) of this preparation. However, the fractionation methods we used that were expected to work were unable to resolve any individual protective components. We hypothesized that the formaldehyde used to kill the endosporulating spherules was causing an irreversible crosslinking of the contents of the spherules, and, therefore, inhibiting our ability to separate individual components from the F-27K vaccine mixture. We therefore changed our killing and preserving procedure to a thimerosal-killing/thimerosal preserving procedure. A vaccine (T-27K) was prepared from the thimerosal-killed spherules using the same procedure used to produce the F-27K vaccine and used to vaccinate groups of mice. In addition, other groups of mice were vaccinated with thimerosal-killed spherules, formalin-killed spherules, and saline alone. Thirty days following intranasal challenge with 1,500 and 15,000 arthroconidia, we found that the thimerosal-killed spherules (7/7 and 5/7, respectively) and the T-27K vaccine (7/7 and 4/7, respectively) protected mice from lethal infection as well as the formalin-killed spherules (7/7 and 4/7, respectively). All the saline vaccinated mice died at both challenge levels. When lymphocyte proliferation assays were performed using PBMCs from either skin-test positive or negative human donors, we obtained similar proliferation responses from the positive donor PBMCs when using either the F-27K or T-27K vaccine. PBMCs from negative donors did not produce a significant proliferation response due to either vaccine. When the T-27K vaccine was fractionated on SDS-PAGE gels, numerous individual, well-defined protein bands were present compared to the continuous, unresolved smear produced by the F-27K vaccine. We have also used FPLC chromatofocusing and FPLC gel filtration to separate the T-27K vaccine into fractions that show resolved, individual protein bands upon separation by SDS-PAGE. In addition, we have been able to detect chitinase, alkaline phosphatase, chitinase, and protease activity in separate fractions derived from our chromatographic separations. We conclude that we have developed a new approach to the isolation of individual immunogens from the endosporulating spherules of *C. immitis*.

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**Abstract 20: Protection and immunologic surrogate markers associated with protection from a proline-rich antigen (PRA) with either recombinant protein or DNA vaccination**

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Two inbred strains of mice (BALB/c and C57BL/6) were vaccinated with either recombinant expression protein of a *Coccidioides immitis* spherule-derived proline-rich antigen (rPRA) in MPL oil emulsion adjuvant or a DNA vaccine based on the same antigen. Four weeks after vaccination, mice were infected intraperitoneally with arthroconidia. By two weeks, groups of mice receiving saline or plasmids without PRA insert exhibited significant weight loss, and quantitative CFUs in the lungs ranged from 5.9 to 6.4 log<sub>10</sub>. In contrast, groups of mice immunized with either rPRA or DNA vaccine had significantly smaller pulmonary fungal burdens, ranging from 3.0 to 4.5 log<sub>10</sub> fewer CFUs. *In vitro* immunologic markers of lymphocyte proliferation and IFN- release after splenocytes were stimulated with rPRA correlated with protection. Also, plasma concentrations of rPRA-specific total IgG, IgG1 and IgG2a showed increases in vaccinated mice. These studies expand earlier work by demonstrating protection in mice which differ in their H-2 background, using an adjuvant that is potentially applicable to human use, and achieving comparable protections with a DNA-based vaccine. Our *in vitro* results substantiate a Th1 responses as evidenced by IFN- release and increased IgG2a. However, IgG1 was also stimulated, suggesting some Th2 response as well. PRA is a promising vaccine candidate for prevention of coccidioidomycosis and warrants further investigation.