Serological evidence of increased *Coccidioides immitis* infections in western Canada in 1996

Errol Prasad DChM, Patricia Diediw RT, Donna Fernandes RT, Lorreen Hodge ART, Katherine Ower BSc, Robert Rennie PhD

E Prasad, P Diediw, D Fernandes, L Hodge, K Ower, R Rennie. Serological evidence of increased *Coccidioides immitis* infections in western Canada in 1996. Can J Infect Dis 1998;9(6):377-381.

OBJECTIVE: To investigate the epidemiology of *Coccidioides immitis* infection in persons returning to western Canada from *C immitis* endemic zones in southwestern United States.
DESIGN: Review of *C immitis* serology requests from 1996.
METHODS: Data were based on review of enzyme immunoassay and immunodiffusion results from 1993 to 1996 inclusive. Detailed information on clinical presentation, treatment and outcome of disease process was obtained through questionnaires and interviews with physicians who submitted *Coccidioides* serology requests in 1996.
RESULTS: Positive serology for *C immitis* increased from 4.7% to 5.2% (between 1993 and 1995 inclusive) to 10.7% in 1996. Enzyme immunoassay for immunoglobulin G and/or immunoglobulin M or immunodiffusion was positive in 25 patients in 1996. The mean age of these patients was 62 years, and the predominant clinical presentation was pulmonary infiltrate with fever. All patients with positive serology were known to have travelled to central or southwestern Arizona or southern California.
CONCLUSIONS: Travel to a defined coccidioidomycosis endemic zone presents a risk for the older traveller. Serology for *C immitis* supported the clinical, histological and microbiological diagnoses in patients who had travelled to this defined endemic zone.

Key Words: *Coccidioides, Epidemiology, Serology, Western Canadians*

Preuve sérologique d'un accroissement des infections à *Coccidioides imitis* dans l'Ouest du Canada en 1996

OBJECTIF : Se pencher sur l'épidémiologie de l'infection à *Coccidioides imitis* chez des personnes retournant dans l'Ouest du Canada après un séjour dans des zones du Sud-Ouest américain où prévaut l'endémie à *C. imitis*.
MODÈLE : Revue des demandes d'analyses sérologiques pour le dépistage de *C. imitis* en 1996.
MÉTHODES : Les données ont été basées sur une revue des résultats d'analyse par immunodosage enzymatique et immunodiffusion entre 1993 et 1996 inclusivement. Les renseignements détaillés sur le tableau clinique, le traitement et l'issue de la maladie ont été obtenus au moyen de questionnaires et d'entrevues auprès des médecins qui avaient demandé des tests de dépistage de *C. imitis* en 1996.

voir page suivante

National Centre for Mycology, Microbiology and Public Health Laboratory, University of Alberta Hospital, Edmonton, Alberta
Correspondence and reprints: Dr R Rennie, 2B3.08 Walter C Mackenzie Health Sciences Centre, 8440-112 Street, Edmonton, Alberta T6G 2J2. Telephone 403-492-4461, fax 403-492-3864, email rpr@bugs.uah.ulberta.ca
Received for publication January 9, 1998. Accepted March 31, 1998

RÉSULTATS : La sérologie positive à l'endroit de *C. imitis* a augmenté de 4,7 % et 5,2 % (entre 1993 et 1995 inclusivement) à 10,7 % en 1996. L'immunodosage enzymatique pour l'immunoglobuline G et/ou l'immunoglobuline M ou les tests d'immunodiffusion se sont révélés positifs chez 25 patients en 1996. L'âge moyen de ces patients était de 62 ans et le tableau clinique prédominant est un infiltrat pulmonaire avec fièvre. Tous les patients séropositifs avaient séjourné dans le centre ou le Sud-Ouest de l'Arizona ou le Sud de la Californie.

CONCLUSION : Les déplacements vers une zone où la coccidiomycose est endémique représentent un facteur de risque pour le voyageur âgé. La sérologie de *C. imitis* a confirmé les diagnostics cliniques, histologiques et microbiologiques chez les patients qui s'étaient rendus dans cette zone endémique connue.

Coccidioidomycosis is caused by the dimorphic fungus *Coccidioides immitis*. Infection is often asymptomatic but may result in acute or chronic disease. Approximately 40% of persons exposed to this fungus develop mild to acute pulmonary disease; less than 1% develop serious disseminated infection.

The fungus is restricted to soil in the southwestern United States, west Texas and in selected areas of Central and South America (1). Coccidioidomycosis in these endemic areas is usually sporadic, but outbreaks or increased incidence of infection have been noted following windstorms, earthquakes and unusually heavy rainfalls, all resulting in widespread landslides (2,3).

Since 1990, marked increases in recorded active cases of infection have been reported throughout the southwestern United States but particularly in Arizona (144% increase) and parts of California (4-6). Reports from *Morbidity Mortality Weekly Report* have also highlighted cases that have occurred in areas where *C immitis* is not endemic (7).

The National Centre for Mycology (NCM), Edmonton, Alberta, operates in conjunction with the Laboratory Centre for Disease Control, Ottawa, Ontario. The NCM receives samples of sera for serological analysis of fungal infections from all provinces in Canada with the exception of Quebec. Our observations from 1993 through 1995 indicated only sporadic serological evidence of exposure to *C immitis* in travellers returning from the United States. Almost all requests for *C immitis* serology are for patients who reside in western Canada, and an earlier report on coccidioidomycosis in Canada indicated that 94% of *C immitis* cases were identified from western Canada (8). In 1996, we noticed a 100% increase in positive serology for *C immitis* in samples submitted to NCM. In the present study, we present clinical evidence to support serological evaluations of persons from western Canada who had travelled to the southwestern United States and returned with suspected *C immitis* infection.

METHODS

Enzyme immunoassay: The Premier *Coccidioides* enzyme immunoassay (EIA) (Meridian Diagnostics, Inc, Ohio) was used as the screening test. This is a qualitative assay for the detection of immunoglobulin (Ig) M and IgG antibodies directed against the purified tube-precipitating and complement fixing antigens of *C immitis* in serum and cerebrospinal fluid. The test has a microwell configuration, and the procedure followed was that recommended by the manufacturer in the product insert.

Immunodiffusion test: Immunodiffusion test (ID) (Immuno-Mycologics, Inc, Oklahoma) was used as a supplementary test for all specimens giving positive and/or indeterminate results with EIA to establish definitive diagnostic criteria in the laboratory. The test was performed in accordance with the manufacturer's recommendations.

Clinical data: Clinical information was acquired by sending a questionnaire to and requesting relevant diagnostic findings and symptomatology from the physicians of all patients with positive serology results. Information requested included patient demographics, clinical presentation and diagnosis, travel history, mycology culture results if available and performed in a laboratory other than NCM, types of specimens collected, biopsy and/or histological evidence of disease, use of any antifungals, and patient outcomes. Travel histories were available for all of these patients. Patients with negative serology but whose laboratory requests indicated that the patients had travelled to a *Coccidioides* endemic area also had clinical information collected using the same format as that for patients with positive serology. In cases with negative serology where there was no travel indicated on the requisition or when the travel indicated was to nonendemic areas such as Idaho or the southeastern United States, no additional clinical information was collected.

RESULTS

Seropositive cases: Between 250 and 320 samples are received annually for *C immitis* serology at NCM. Statistics from 1993 through 1995 showed that 4.7% to 5.2% of samples each year were positive. The seropositive rate increased to 10.7% in 1996 (a 100% increase from that of the previous three years) without a significant change in the number of samples submitted. In 1996, a total of 25 patients had positive serology for *C immitis*. Serology results on 30 samples from these patients are summarized in Table 1. IgM was positive in 19 patients; IgG was positive in 23 patients, negative in one and indeterminate in one. IgG and IgM were both positive in 18 patients. One patient was positive for IgM only (patient 15); another had IgM only in the first sample, but a follow-up sample collected 15 days later was positive for IgM as well as IgG (patient 2a/b). Sixteen patients had positive ID results. One patient, who had positive IgM and IgG by EIA and a negative ID initially, had positive ID in a follow-up specimen collected two weeks later (patient 8a/b). Six patients had positive IgG and IgM by EIA but had negative ID, five had positive IgG and ID but negative IgM, one was positive for IgG only (patient 12) and one had an indeterminate IgG with a negative IgM and ID (patient 4). ID is a highly specific test. The positive EIA IgG and ID but negative EIA IgM results in five patients indicated a definitive exposure to *C immitis* in the past and correlated with travel history to an

TABLE 1
Demographics and serology results on 25 *Coccidioides immitis* seropositive patients

				Enzyme immunoassay		
Patient	**Age (years)**	**Sex**	**Collection date of specimen**	**Immunoglobulin M**	**Immunoglobulin G**	**Immunodiffusion**
1	66	M	16/12/95	+	+	+
2a	68	M	08/02/96	+	–	+
2b	68	M	23/02/96	+	+	+
3	65	F	21/02/96	–	+	+
4	68	F	29/03/96	–	Indeterminate	–
5	60	F	02/04/96	+	+	+
6a	69	M	02/05/96	+	+	+
6b	69	M	26/06/96	–	+	+
7a	61	F	06/05/96	+	+	+
7b	61	F	22/05/96	+	+	Weak+
8a	70	M	07/05/96	+	+	–
8b	70	M	21/05/96	+	+	+
9	76	M	14/05/96	+	+	+
10	70	M	15/05/96	+	+	–
11	58	F	16/05/96	+	+	+
12	64	M	16/05/96	–	+	–
13	81	M	17/05/96	+	+	+
14	60	M	23/05/96	–	+	+
15	38	F	28/05/96	+	–	–
16	44	M	28/05/96	–	+	+
17	57	F	10/06/96	+	+	+
18a	67	F	17/06/96	+	+	+
18b	67	F	13/08/96	+	+	+
19	63	F	19/06/96	+	+	–
20	45	F	06/07/96	+	+	–
21	67	M	17/07/96	–	+	+
22	52	M	29/07/96	+	+	–
23	44	F	11/09/96	+	+	+
24	64	M	05/12/96	+	+	–
25	64	F	12/12/96	+	+	–

'a' and 'b' indicate sequential samples from the same patient. F Female; M Male

endemic area. Of the patients who had positive serology results, five had positive *C immitis* cultures, four were negative by culture and the remaining 16 had no cultures performed.

Clinical histories were provided for all 25 patients, 13 males and 12 females with ages ranging from 38 to 81 years (mean 62 years). All patients either travelled to or spent winter months in central or southwestern Arizona, or southern California. The predominant clinical presentations reported were pulmonary infiltration or consolidation (14 patients), fever (12 patients), cough (eight patients), chest pain (five patients), pleural effusion (two patients) and pneumonia (two patients). Two patients had skin eruptions. Biopsy from one patient was positive for *C immitis* on histology and culture. Ten patients were treated with antifungal agents, of whom six were reported as having a favorable outcome, four continued therapy and two had excisional biopsies. All but one of the remaining patients had radiographic evidence suggestive of *C immitis* infection and were being followed clinically. One patient (patient 12) had no chest x-ray evidence of *C immitis* infection and was thought to have had a previous exposure. This patient was positive for IgG only by EIA. The following case presentations were typical of this group of patients.

Case report 1: A 68-year-old male from western Canada who travels each winter to southern Arizona (patient 2) returned home in 1996 with a dry cough, fever, general malaise and weight loss. As reported by the attending physician, a chest x-ray revealed a mass in the left lower lobe of the lung. The differential diagnosis included malignancy and infection. A lung biopsy was collected for histology, and serum was submitted to the NCM for serology. Culture was not performed. The attending physician reported the lung biopsies positive for coccidioidomycosis. Serology was positive for IgM and IgG by EIA and positive for F antibody by ID. The patient was treated with oral fluconazole 400 mg daily for nine months. Clinical improvement was observed, although a chest x-ray at the end of treatment did not show complete resolution of the lung lesion.

Case report 2: Four days after returning from a two- to three-week vacation in Arizona, a 57-year-old female (patient 17) developed headache, malaise and anorexia. This was followed several days later by fever, chills, a nonproductive cough and

TABLE 2
Demographics and clinical features of 31 *Coccidioides immitis* seronegative patients

Demographics	
Female	19
Male	12
Age (years)	
Mean	60
Range	(25-87)
Clinical features	
Fever	9
Cough	20
Malaise	11
Chest pain	6
Skin eruption	3
Other	14
Abnormal chest x-ray	15
Antifungal therapy	2
Diagnosis	
Pneumonia-bacterial	6
Viral respiratory infection	4
Sarcoidosis	2
Asthma, sinusitis	2
Carcinoma, pulmonary	3*
Rheumatoid disease	1
Congestive heart failure	1
Myocardial infarction	1†
Routine medical	1†
Unknown-coccidioidomycosis (unlikely)	9
Unknown-coccidioidomycosis (probable)	1†

**One of three patients had a solitary nodule on chest x-ray; †Each of these patients had a solitary nodule on chest x-ray*

left lateral pleuritic chest pain. A chest x-ray of the patient revealed a left upper lobe pneumonia, and she was treated with clarithromycin. A repeat chest x-ray performed three weeks later showed a small nodule still present in the left upper lobe. A subsequent computed tomography scan revealed an irregular mass-like lesion in the left upper lobe adjacent to the mediastinum that remained apparent on repeated computed tomography scans. The patient was not treated with antifungal agents and has remained clinically well. *Coccidioides* serology collected at the time of her initial presentation was positive for IgM and IgG by EIA, and positive for F antibody by ID.

Case report 3: A 44-year-old male who made multiple trips to the San Joaquin Valley in California (patient 16) developed a severe flu-like illness that persisted for approximately two months and resolved spontaneously. He subsequently developed erythema multiforme-like skin eruptions on his nose, face and hands. Chest x-ray revealed a single nodule in the right middle lobe. Skin biopsy was positive for *C immitis* by culture and histology. He was treated with ketoconazole for three months with clinical evidence of improvement but was not available for follow-up. *Coccidioides* serology performed before therapy was positive for IgG by EIA and positive for F antibody by ID.

Seronegative cases: There were 31 patients with negative serology for *C immitis* for whom the authors received a documented travel history to a defined *Coccidioides*-endemic area. For the remaining seronegative cases, travel histories or clinical details were not provided with the test request. These cases were not examined further.

Demographic and clinical details for the 31 patients with known compatible travel history are given in Table 2. The mean age (60 years) was approximately the same as the seropositive patients. The predominant clinical features differed in this group of patients. Cough and general malaise were more common. Only two patients were treated with antifungal agents. Diagnoses in this collection of patients were different. Incidental solitary pulmonary nodules were observed on chest x-rays of four patients who demonstrated no evidence of pulmonary disease. Only one patient had a diagnosis for which coccidioidomycosis was considered probable, but only chest x-ray evidence of a lung nodule was supportive of the diagnosis.

DISCUSSION

The increase in 1996 of the number of patients from western Canada with positive serology for *C immitis* is a sequela to natural environmental events that have occurred in southwestern United States in the previous few years. Windstorms, landslides, earthquakes and possibly fires in this part of the country provide a mechanism for disturbing the alkaline soil that naturally harbours arthroconidia of *C immitis*. Travellers to this arid endemic zone are, therefore, at greater risk of exposure to the fungus.

The three cases presented in detail illustrate several important features of *C immitis* infection that we observed in the entire group with positive serology. Patients with suspected coccidioidomycosis are older than 40 years (Table 1). They may have repeated exposure by regular annual travel to the endemic zone, but shorter exposure may also result in acute infection. Resolution of symptoms may occur without specific therapy, but prolonged antifungal therapy may be required for clinical resolution. Residual evidence of exposure is often observed on chest x-ray long after clinical resolution of symptoms.

Serological investigations for coccidioidomycosis are justified in persons who present with a suspected pulmonary infection and a concomitant history of travel to a *C immitis*-endemic area. Clinical histories and other diagnostic findings are also important to rule out other causes of pulmonary diseases such as those identified in Table 2. Nonspecific entities such as fever, cough and general malaise are insufficient to confirm a diagnosis of *Coccidioides* infection in a traveller to an endemic area. In the sample from the seronegative group that was investigated by the same criteria as the seropositive group, only 15 of 31 patients had a chest x-ray, which may be more useful to establish a diagnosis. In four patients, radiographic evidence of solitary 'walled-off' lesions suggestive of *Coccidioides* exposure without serological correlation is of in-

terest. We believe that the initial exposure to *C immitis* may invoke an asymptomatic IgM response, but once the developed spherule has been 'walled-off' by the host's inflammatory response, there is no further presentation of antigenic epitopes to elicit an antibody response. It is possible that *C immitis* exposure in these patients may have occurred in the distant past in the absence of other conditions that predisposed the patients to overt disease.

Of the seropositive patients, 40% received antifungal therapy. Only two (6%) of the group with negative serology that we investigated received antifungal therapy once the results of the serology were known. A recent report suggested that relapse of infection may be more frequent in patients with higher complement-fixing antibody titres to *C immitis* (9). It is possible in the patients included in this study that negative serology implied a lower degree of concern by physicians about the need to treat, particularly when the antifungal treatment course for coccidioidomycosis is long.

Our observations support a role for serology by EIA for IgM and IgG with ID for F antibody as an adjunct for the diagnosis of coccidioidomycosis in patients who have travelled to an endemic area. The EIA used in this study has been reported by others as a sensitive and specific test, particularly for IgM antibodies (10). The ID for IgG to F antigen is considered to have similar sensitivity to complement fixation, but different antibodies are detected. Patients with an initial negative ID result may demonstrate seroconversion to a positive ID if a follow-up specimen is submitted after an interval of three to four weeks (patient 8, Table 1). Previous reports have suggested that complement fixation, which is considered to be less sensitive than ID for IgG, may have useful prognostic value. High complement fixing titres reflect increased risk for extrapulmonary disease (11). In this study, complement fixation testing was performed on paired sera from one patient only. The complement fixation test results were negative, ID was positive and the patient's infection did not spread beyond the respiratory tract.

CONCLUSIONS

Our results indicate that patients with *C immitis* positive serology increased by 100% in 1996 over the previous three years. Our laboratory methodology has remained the same throughout this period. The patients were attended by many physicians in many different locales, and there is no reason to suspect that physician practices changed during the period of study. If this were the case, we might have expected a significant increase in the number of requests for *Coccidioides* serology. Our data do not tell us if the patients in 1996 stayed longer in the endemic area, but many travellers go every year to the southwestern United States. It is possible that regular exposure over several years increases the likelihood of acquiring this fungus. However, our results support data from the United States that the true incidence of coccidioidomycosis has increased over the last few years. In 1996, we identified at least 25 new cases of *C immitis* infection; in one year, almost one-fifth the number reported in Canada over the previous 35 to 40 years (8).

The Premier *Coccidioides* EIA screening test provided laboratory evidence supporting the diagnosis of *C immitis* infection in this nonendemic population. Physicians should consider the diagnosis of coccidioidomycosis in older patients who have travelled to defined endemic zones in the southwestern United States and who present with bronchopulmonary disease. Early recognition of IgM antibodies to *C immitis* by EIA will support the clinical diagnosis; however, negative serology by itself does not exclude a clinical diagnosis of coccidioidomycosis. Additional investigations including chest x-ray and skin testing can provide helpful evidence for other disease processes.

ACKNOWLEDGEMENTS: The authors acknowledge the assistance of all the attending physicians who supplied clinical information, R Gill of British Columbia Centre for Disease Control, Dr S Shafran for his helpful review of the manuscript and A Koutzun for the preparation of the manuscript.

REFERENCES

1. Pappagianis D. Epidemiology of coccidioidomycosis.In: McGinnis M, ed. Current topics in medical mycology, vol 2. New York: Springer-Verlag, 1988:199-238.
2. Pappagianis D, Einstein H. Tempest from Tehachapi takes toll on *Coccidioides* conveyed aloft and afar. West J Med 1978;129:527-30.
3. Coccidioidomycosis following the Northridge Earthquake – California, 1994. Morb Mortal Wkly Rep 1994;43:194-5.
4. Coccidioidomycosis – Arizona, 1990-1995. Morb Mortal Wkly Rep 1996;45:1069-73.
5. Update: coccidioidomycosis – California, 1991-1993. Morb Mortal Wkly Rep 1994;43:421-3.
6. Pappagianis D. Marked increase in cases of coccidioidomycosis in California: 1991, 1992, and 1993. Clin Infect Dis 1994;19(Suppl 1):S14-8.
7. Coccidioidomycosis – United States, 1991-1992. Morb Mortal Wkly Rep 1993;42:21-4.
8. Sekhon AS, Isaac-Renton J, Dixon JM, Stein L, Sims HV. Review of human and animal cases of coccidioidomycosis diagnosed in Canada. Mycopathologia 1991;113:1-10.
9. Oldfield EC 3rd, Bone WD, Martin CR, Gray GC, Olson P, Schillaci RF. Prediction of relapse after treatment of coccidioidomycosis. Clin Infect Dis 1997;25:1205-10.
10. Einstein HE, Johnson RH. Coccidioidomycosis: new aspects of epidemiology and therapy. Clin Infect Dis 1993;16:349-56.
11. Pappagianis D, Zimmer BL. Serology of coccidioidomycosis. Clin Microbiol Rev 1990;3:247-68.