

HISTOPLASMIN SURVEY IN HIV-POSITIVE PATIENTS: RESULTS FROM AN ENDEMIC AREA IN NORTHEASTERN BRAZIL

Fabricia Salvador BEZERRA(1), Rosely Maria ZANCOPÉ-OLIVEIRA(2), Raimunda Sâmia Nogueira BRILHANTE(3), Bodo WANKE(2), Rosa Maria Salani MOTA(1), Ana Paula Gomes RAMOS(4), Alberto Novaes RAMOS Jr.(1), Mônica Cardoso FAÇANHA(1) & Terezinha do Menino Jesus Silva LEITÃO(1)

SUMMARY

Background. Disseminated histoplasmosis is common in AIDS patients with advanced immunosuppression in Ceará, Northeastern Brazil. The goal of this study was to determine the prevalence of *Histoplasma* infection in patients with HIV/AIDS living in Fortaleza, the capital of Ceará. **Methods.** Intradermal tests with histoplasmin (mycelial phase) were performed in 161 HIV patients with CD4 \geq 350 cells/mm³. Evidence of recent illness was evaluated with immunodiffusion (ID) tests in 76 of these individuals. **Results.** A total of 11.8% of patients reacted to histoplasmin and 2.63% had ID test positive to *Histoplasma*. The presence of mango trees (*Mangifera indica*) in the patient neighborhood (OR = 2.870; 95% CI = 1.081-7.617; p = 0.040) and past activity involving soil (OR = 2.834; 95% CI = 1.045-7.687; p = 0.045) or visits to a farm (OR = 3.869; 95% CI = 1.189-12.591; p = 0.033) were significantly associated with *Histoplasma* infection. **Conclusions.** Patients with HIV living in Fortaleza have an expressive prevalence of infection with *Histoplasma*.

KEYWORDS: Histoplasmosis; Histoplasmin; Epidemiology; AIDS; HIV.

INTRODUCTION

Histoplasmosis is an infection caused by *Histoplasma capsulatum* that affects humans and several other animal species^{18,20}. The organism grows profusely in soil that is rich in the droppings from birds and bats¹⁸. Old abandoned buildings and caves often contain high concentrations of *H. capsulatum*¹⁸. Demolition of buildings, movement of soil, cleaning of bridge structures, and spelunking have been implicated as the point source for dispersal of the organism¹.

Prior to the AIDS epidemic, histoplasmosis was most frequently reported in outbreak events^{20,23}, and the disseminated form was found primarily in patients with lymphoma or other cancers and sporadically in renal transplant patients¹³. However, since the 1980s, the disseminated form has been observed mainly in HIV-infected patients with advanced immunosuppression^{13,16}. Several Brazilian states have reported cases of histoplasmosis associated with HIV infection^{2,26}, however, the state of Ceará in Northeastern Brazil has had the highest incidence of AIDS/histoplasmosis association in recent years^{7,24} and one of the highest in the world⁶. In a retrospective study that was conducted from 1995 to 2004 in Fortaleza, the capital of Ceará, 164 patients were diagnosed with concurrent HIV infection and disseminated histoplasmosis⁸.

Histoplasmin skin-test surveys carried out in the general Brazilian

population in different regions have reported a positivity of 4.4-63.1% in the Midwest and 6.3-89.0% in the South¹⁵. Previously, another survey of several cities throughout the country identified a prevalence ranging from 2.60% to 61.50% in populations living in Northeast Brazil¹². Studies from Ceará performed several decades ago indicated a high prevalence (23.6% to 61.5%) of infection in individuals from rural areas^{9,11}.

Based on the paucity of studies concerning the prevalence of histoplasma infection in Ceará, mainly in urban areas, and the high prevalence of disseminated histoplasmosis in HIV patients from this state, studies to address the epidemiology of this fungal infection in the immunosuppressed population in this area are urgently needed.

METHODS

A cross-sectional study was performed at the Center of Medical Specialties José de Alencar (*Centro de Especialidades Médicas José de Alencar* - CEMJA), a public outpatient clinic in Fortaleza (estimated population in 2012: 2.5 million inhabitants), the capital of Ceará State. CEMJA began to offer HIV outpatient assistance in 2006 and has received most of the newly diagnosed patients from the area since then (communication from the local health department). This study enrolled HIV-positive patients (enzyme-linked immunosorbent assay positive, confirmed with an indirect immunofluorescence test) of both sexes

(1) Department of Community Health, School of Medicine, Federal University of Ceará. Fortaleza, Ceará, Brazil.

(2) Mycology Department. Instituto de Pesquisa Evandro Chagas. Fundação Oswaldo Cruz, Fiocruz. Rio de Janeiro, Brazil.

(3) Medical Mycology Research Center. Federal University of Ceará. Fortaleza, Ceará, Brazil.

(4) Center of Medical Specialties José de Alencar. Municipal Health Department. Fortaleza, Ceará, Brazil.

Correspondence to: Dra. Terezinha M. J. Silva Leitão, Departamento de Saúde Comunitária, R. Prof. Costa Mendes 1608, 5° andar, Rodolfo Teófilo, 60430-140 Fortaleza, Ceará, Brasil.
E-mail: tsilva@ufc.br

over 18 years old, residents in Fortaleza, and included patients with a CD4 count ≥ 350 cells/mm³ regardless of their use of highly active antiretroviral therapy (HAART). Patients with a previous history of histoplasmosis and those who did not return for skin-test reading were excluded.

After providing written informed consent, the patients completed a questionnaire concerning socio-economic and demographic data and known risk factors associated with *Histoplasma* infection, such as recent (up to a year before) or past (more than a year ago) activities involving soil (gardening, civil construction or agriculture) or visits to farms or caves, or the presence of birds, bats, or mango trees (*Mangifera indica*) in the home or neighborhood. Clinical and laboratory data, such as time of AIDS and HIV diagnosis, CD4 cell count, and highly active antiretroviral therapy (HAART) use, were obtained from patient records.

Initially, 5 mL of blood was collected and stored at -20 °C for subsequent analysis. Skin tests were subsequently performed by intradermally injecting 0.1 mL of histoplasmin antigen (HMIN)⁹, in a 1:1000 dilution¹², into the inside of the left forearm of each selected individual⁷. HMIN is a metabolic antigen from the filamentous phase of *H. capsulatum* produced in-house, isolated from sputum of a case of chronic pulmonary histoplasmosis, (IPEC3356), in Rio de Janeiro provided by the Mycology Laboratory at FIOCRUZ - Rio de Janeiro. Tests that produced induration ≥ 5 mm in transverse diameter after 48 or 72 hours were considered to be histoplasmin positive⁹. The intradermal tests and readings were performed by the same investigator using the same instrument (gauge).

All patients who had a positive reaction to the intradermal test were analyzed with a double immunodiffusion test; the negative histoplasmin sera selected for testing were taken from patients with the highest CD4 counts. To control for possible cross-reactivity between *H. capsulatum* and *Coccidioides immitis* antibodies, 36 sera were randomly selected and tested with commercial antibodies and antigens for *H. capsulatum* IDCF and anti-IDCF, (Immy Immunodiagnostics, OK, USA) and *C. immitis* (IDCF and anti-IDCF, Immy Immunodiagnostics, OK, USA). The double radial immunodiffusion assay was performed as described by SIDRIM & OLIVEIRA²⁵.

Statistical analyses were performed with STATA version 8 (Stata Corporation, College Station, USA) and adopted a statistical significance level of 5%. This study was approved by the Ethics in Research Committee of the São José Hospital for Infectious Diseases in Ceará under protocol 018/2008.

RESULTS

A total of 344 patients were invited to participate in the study, and 161 met the inclusion criteria; the remaining 183 had CD4 < 350 cells/mm³ (169) or were excluded because they did not return for skin-test readings (14). The studied population averaged 35.1 years of age (29-38 years), and the majority of the patients were men (76.4%). As demonstrated in Table 1, approximately 61.4% were born in Fortaleza, and 85.1% lived in houses.

Nineteen patients (11.8%) were positive (≥ 5 mm) for the skin test. All of these patients were men, with a mean age of 36 years. Twelve

Table 1
Socio-economic and demographic characteristics of the study population, CEMJA. Fortaleza, Ceará, Brazil, 2008-2009

Variables	N (%)	Variables	N (%)
Age (years)	35.10 (mean)	Monthly family Income	2.03 (mean ¹)
18-28 years	48 (29.81)	Unknown	8 (4.96)
29-38 years	59 (36.65)	0 to 3 MI ²	132 (81.99)
49-58 years	9 (5.59)	4 to 6 MI ²	14 (8.70)
59 years or older	4 (2.48)	7 MI ² or more	7 (4.35)
Sex		Dwelling type	
Male	123 (76.40)	House	137 (85.10)
Female	38(23.60)	Apartment	24 (14.90)
Education		Place of Birth	
Illiterate	2 (1.24)	Fortaleza	99 (61.49)
9 years	60 (37.27)	Country side	48 (29.81)
12 years	71 (44.10)	Other States	14 (8.70)
More than 12 years	28 (17.39)		

Source: CEMJA, 2008-2009. ¹: Average family income; ²: 2008 Minimum Income: US\$ 226.54

(63.1%) had an average monthly income of US\$ 244.11 and had completed approximately 10.5 years of school. Most lived in downtown Fortaleza (three cases) and in two areas in the periphery of the city (two patients each). Seventeen of these patients (89.4%) were born in Ceará State. The distribution of the skin test results according to patient CD4 values is shown in Table 2. Although all participants have CD4 counts ≥ 350 cell/mm³, 53.41% of the studied patients have already met the Brazilian Ministry of Health criteria of AIDS.

Table 2
Distribution of patients according to skin tests and CD4 counts results. CEMJA. Fortaleza, Ceará, Brazil, 2008-2009

CD4+	N/(%)	Positive TEST
		N
350-500	70(43.5)	11
501-750	62(38.5)	5
751 or more	29(18.0)	3
TOTAL	161(100.00)	19

Source: CEMJA, 2008-2009.

There was no significant difference in the average time since HIV diagnosis between patients with positive (40.4 months) and negative (28.0 months) histoplasmin tests, as well as in the average time since AIDS diagnosis (36.8 months versus 40.7 months for positive and negative results respectively). The mean CD4 counts were also not different

Table 3
Risk factors versus histoplasmin intradermal test (HT) results in HIV positive patients. CEMJA. Fortaleza, Ceará, Brazil, 2008-2009

Variables	Current*				Past**			
	HT + N=19 %	HT- N=142 %	p	OR [CI]	HT+ N=19 %	HT- N=142 %	p	OR[CI]
Presence of Birds***								
Housing	42.10	26.05	0.143	2.063 [0.662-6.108]	36.84	19.01	0.073	2.484 [0.749-7.588]
Neighborhood	63.15	46.47	0.171	1.974 [0.667-6.257]	10.52	15.49	0.568	0.641 [0.067-3.033]
Presence of bats								
Housing	5.26	6.33	1.000	0.821 [0.98-6.866]	5.26	7.04	1.000	0.733
Neighborhood	10.52	7.74	0.653	1.401 [0.286-6.864]	5.26	2.81	0.471	1.917 [0.203-18.106]
Presence of mango three (<i>Mangifera indica</i>)								
Housing	15.78	17.60	1.000	0.878 [0.238-3.241]	0	6.33	-	-
Neighborhood	57.89	32.39	0.040	2.870 [1.081-7.617]	5.26	6.33	1.000	0.821 [0.098-6.866]
Visit to farm	15.78	7.04	0.185	2.475 [0.616-9.943]	26.31	8.45	0.033	3.869 [1.189-12.591]
Visit to cave	10.52	2.81	0.149	4.059 [0.691-23.842]	5.26	11.97	0.698	0.408 [0.51-3.258]
Activity with soil****	10.52	9.85	1.000	1.076 [0.225-5.147]	42.10	20.42	0.045	2.834 [1.045-7.687]

Source: CEMJA, 2008-2009. *Up to one year; **More than one year; ***Pigeons and chickens; ****Gardening, civil constructions or agricultures.

between the positive (590.4 cell/mm³) and negative (577.5 cell/mm³) groups. Nine (47.3%) of the histoplasmin-positive participants used highly active antiretroviral therapy. There was no significant difference ($p = 1.0$) in patients reactive (52.63%) and no reactive (53.52%) to HMIN considering the AIDS diagnosis.

The presence of mango trees (*M. indica*) in the current neighborhood ($p = 0.040$), activity involving soil in the past ($p = 0.045$), and having visited a farm in the past ($p = 0.033$) were significantly associated with HMIN reactivity (Table 3). The cleaning of the attic lining of the home or a chicken house in the past or present was not significantly correlated with histoplasmin status.

Of the sera tested with the double immunodiffusion tests (76), only two showed M-line precipitation, and these sera were obtained from histoplasmin-positive patients (2.63%). No cross-reaction was observed in the 36 sera tested with the commercial *C. immitis* antigen and antibody.

DISCUSSION

The vast majority of immunosuppressed patients with disseminated histoplasmosis have AIDS¹⁶. The reactivation of a latent focus of infection is considered to be a possible mechanism of disease manifestation in patients from endemic areas²¹. Therefore, studies on the prevalence of histoplasmosis infection in specific areas are important to understand the local epidemiology and risk factors associated with the acquisition of *Histoplasma*.

Most infected patients in this series were men; consistent with the results of a histoplasmin prevalence study (95.1%) performed by McKINSEY *et al.* (1997) in AIDS patients in the United States²¹.

Considering the paucity of studies on HIV positive patients, comparisons will be performed mostly with histoplasmin intradermal tests performed on the general population.

A survey performed in three cities in Paraíba State (Northeastern Brazil) found histoplasmin reactivity in 18.5% to 31.5% of 1,957 tests applied in participants from two to > 60 years old⁵. Another serological survey by FAVA & FAVA NETTO (1998) carried out in two localities of Bahia (Northeast Brazil) and 29 Southeast areas of the country revealed histoplasmin reactivity in 13% to 19.6% and 3.0% to 93.2%, respectively¹²; the highest positivity was recorded in the city of Angra dos Reis on the coast of Rio de Janeiro State. In the southwestern Amazon, a study conducted in three native communities (individuals aged one to > 40 years old) observed positive histoplasmin test rates that varied from 5.8% to 80.5%⁴. Another study in native populations from the reservation of Xacriabá in the state of Minas Gerais in Southeast Brazil found a reactivity of 3.9% in 180 participants¹⁹.

Studies performed in Ceará by DIOGENES *et al.* in the early 1990s⁹ found a high prevalence of histoplasmin positivity in Serra do Pereiro in the south of the state (61.5%). Similarly, FAÇANHA *et al.* (1991) found a 38.93% reactivity in residents from a mountainous area in the municipality of Palmácia¹¹. In 1983, another survey, this time with 138 patients from a rural area admitted to the Walter Cantídio University Hospital, showed a histoplasmin positivity rate of 23.6%³.

The prevalence (11.8%) observed in the current series was lower than that observed in a study²¹ conducted on AIDS patients residing in histoplasmosis-endemic areas of the United States (16.7% in a total of 274 patients). This difference, however, was not large, suggesting that Ceará is an area with significant *Histoplasma* transmission.

The AIDS epidemic in Brazil affects mainly young adults²², which explains the lower average age observed in this study. In the study by PONTES *et al.*, the average age of the 134 hospitalized patients with HD and AIDS in a referral hospital for infectious diseases was 35.8 years.

Based on information from the Ministry of Health of Brazil, only 7.7% of AIDS patients have 12 or more years of education²². The current study found that 61.49% of patients had 12 or more years of education. This result may be explained by one of the inclusion criteria, which selected cases with higher CD4 counts. Higher CD4 counts are associated with patients with better HAART adherence and access to health care facilities, which in turn is associated with better education¹⁰. HAART use and time since AIDS diagnosis were not associated with intradermal test response; a similar result was found in a previous study²¹.

Most of the patients enrolled in the current study lived in houses, which could have facilitated fungal infection due to the increased prevalence of tree cultivation (fruit trees or landscaping) or soil activities (cleaning and maintenance). In a retrospective series of 30 patients with deep systemic mycosis living in southern Brazil (2005-2010) analyzed by FREY *et al.*, 26.3% of patients had reportedly performed some type of activity involving soil (farmer or gardener)¹⁴. The importance of *M. indica* trees reported in the current study was most likely due to its status as a bat habitat; these animals enjoy its fruit and are attracted by their presence. Risk variables such as living or working on a farm or direct interactions with birds were not mentioned by any of the patients, possibly because the individuals were from an urban area.

More than 50% of the samples studied already had the AIDS diagnosis. The low prevalence of *Histoplasma* infection found in this study compared with other surveys in Brazil might be explained by the low immune system response of the HIV patients; it is possible that although good levels of CD4 cells were observed in this study, the CD4 cells may not be functionally competent.

Only two patients were positive (prevalence 2.6%) for ID in the current study, and they also reacted to the intradermal histoplasmin test. It is not possible to determine whether or not the antibodies detected at the time of blood collection, resulted from previous illness with *H. capsulatum*. The patients in the current survey had their medical records investigated, and there were no reports (complaints, symptoms or signs) suggestive of histoplasmosis.

KUBERSKI *et al.*¹⁷ reported that patients with *Coccidioides* may have a false-positive reaction to HMIN ID tests due to cross-reactivity, especially in areas where both fungi are endemic. SIDRIM & OLIVEIRA²⁵ reported similar results for patients with paracoccidioidomycosis. Because almost 30% of the patients in this study were born in the countryside, and Ceará State is an endemic area for coccidioidomycoses, it was necessary to evaluate the patient sera for possible cross-reactivity to *Coccidioides*; however, none of the sera tested were positive for coccidioidin.

It can be concluded that patients with HIV/AIDS living in Fortaleza have an expressive prevalence of infection with *Histoplasma*. This study also identified several risk factors associated with *Histoplasma* infection, which will be useful in the implementation of preventive measures for patients who are susceptible to severe forms of this disease.

RESUMO

Inquérito com histoplasmina em pacientes HIV positivos: resultados de uma área endêmica no nordeste do Brasil

Introdução: Histoplasmoze disseminada ocorre com grande frequência em pacientes com aids e imunossupressão avançada no Ceará, Brasil. O objetivo deste artigo é determinar a prevalência da infecção por *Histoplasma* em pacientes com HIV/aids residentes em Fortaleza capital. **Métodos.** Testes intradérmicos com histoplasmina (fase micelial), foram realizados em 161 pacientes com CD4 \geq 350 células/mm³. Doença recente foi estudada por imunodifusão em 76 desses indivíduos. **Resultados.** Reagiram à histoplasmina, 11,8% dos pacientes e à imunodifusão para *Histoplasma*: 2,63%. A presença da árvore mangueira (*Mangifera indica*) na vizinhança (OR = 2,870; IC 95% = 1,081-7,617; $p = 0,040$), atividade com o solo no passado (OR = 2,834; IC 95% = 1,045-7,687, $p = 0,045$) e visitar sítio no passado (OR = 3,869; IC 95% = 1,189-12,591; $p = 0,033$); foram significativamente associados com positividade para o teste. **Conclusões.** Pacientes com HIV que vivem em Fortaleza apresentam uma prevalência expressiva de infecção por *Histoplasma*.

ACKNOWLEDGMENTS

We thank the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES*, the *Centro de Especialidades Médicas José de Alencar (CEMJA)*, and the local data collection agents, particularly João Bezerra da Silva, for their support in laboratory research; the *Centro Especializado em Micologia Médica (CEMM)*, particularly PhD Júlio C. Sidrim, PhD Raquel Fontenelle and PhD Ana Karoline Freire, for their support in laboratory research; and PhD Roberto da Justa P. Neto for his help with the logistics of this study.

CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Cano M, Hajjeh RA. The epidemiology of histoplasmosis: a review. *Semin Respir Infect.* 2001;16:109-18.
2. Chang MR, Taira CL, Paniago AMM, Taira DL, Cunha RV, Wanke B. Study of 30 cases of histoplasmosis observed in the Mato Grosso do Sul State, Brazil. *Rev Inst Med Trop São Paulo.* 2007;49:37-9.
3. Coêlho ICB, Gadelha JB, Câmara LMC. Estudo sobre a paracoccidioidomicose no Estado do Ceará: análise preliminar da paracoccidioidomicose-infecção, utilizando reações intradérmicas com paracoccidioidina e histoplasmina. *Rev Soc Bras Med Trop.* 1986;19(Suppl):97-8.
4. Coimbra Júnior CE, Wanke B, Santos RV, do Valle AC, Costa RL, Zancopé-Oliveira RM. Paracoccidioidin and histoplasmin sensitivity in Tupí-Mondé Amerindian populations from Brazilian Amazonia. *Ann Trop Med Parasitol.* 1994;88:197-207.
5. Costa W, Wanke B, Barros MAD. Paracoccidioidomicose e histoplasmoze capsulata: inquérito intradérmico em três municípios do Estado da Paraíba. *Ciênc Cult Saúde.* 1989;11:181-8.
6. Couppié P, Aznar C, Carme B, Nacher M. American histoplasmosis in developing countries with a special focus on patients with HIV: diagnosis, treatment, and prognosis. *Curr Opin Infect Dis.* 2006;19:443.

7. Daher EF, Silva GB Jr, Barros FAS, Takeda CFV, Mota RM, Ferreira MT, *et al*. Clinical and laboratory features of disseminated histoplasmosis in HIV patients from Brazil. *Trop Med Int Health*. 2007;12:1108-15.
8. De Francesco Daher E, De Sousa Barros FA, Da Silva Júnior GB, Takeda CFV, Mota R, Ferreira MT, *et al*. Risk factors for death in acquired immunodeficiency syndrome-associated disseminated histoplasmosis. *Amer J Trop Med Hyg*. 2006;74:600-3.
9. Diógenes MJ, Gonçalves HM, Mapurunga AC, Alencar KF, Andrade FB, Nogueira-Queiroz JA. Reações à histoplasmina e paracoccidiodina na Serra de Pereiro (estado do Ceará-Brasil). *Rev Inst Med Trop São Paulo*. 1990;32:116-20.
10. Eidam CL, Lopes AS, Guimarães MDC, Oliveira OV. Estilo de vida de pacientes infectados pelo vírus da imunodeficiência humana (HIV) e sua associação com a contagem de linfócitos T CD4+. *Rev Bras Cineantropom Desempenho Hum*. 2006;8(3).
11. Façanha MC, Café VS, Wanke B, Souza AQ, Bornay F, Coelho Filho JM. Estudo soropidemiológico de paracoccidiodomicose em Palmácia, Ceará. *Rev Soc Bras Med Trop*. 1991;24(Supl 2):28.
12. Fava SDC, Fava Netto C. Epidemiologic surveys of histoplasmin and paracoccidiodin sensitivity in Brazil. *Rev Inst Med Trop Sao Paulo*. 1998;40:155-64.
13. Ferreira MS, Borges AS. Histoplasmosis. *Rev Soc Bras Med Trop*. 2009;42:192-8.
14. Frey MN, Bonamigo RR, Ioppi AEE, Prado GP. Estudo sobre as características clínicas, epidemiológicas, histopatológicas e micológicas de pacientes com micoses profundas em um Serviço de Dermatologia de Porto Alegre, RS. *Rev AMRIGS*. 2011;55:123-9.
15. Guimarães AJ, Nosanchuk JD, Zancopé-Oliveira RM. Diagnosis of histoplasmosis. *Braz J Microbiol*. 2006;37:1-13.
16. Kauffman CA. Diagnosis of histoplasmosis in immunosuppressed patients. *Curr Opin Infect Dis*. 2008;21(4):421-5.
17. Kuberski T, Myers R, Wheat LJ, Durkin M, Connolly P, Kubak BM, *et al*. Diagnosis of coccidioidomycosis by antigen detection using cross-reaction with a Histoplasma antigen. *Clin Infect Dis*. 2007;44:50-4.
18. Lacaz CS, Porto E, Martins JEC, Heins-Vaccari EM, Melo NT. Histoplasmosse clássica. In: Lacaz CS, Porto E, Martins JEC, Heins-Vaccari EM, Melo NT, editores. *Tratado de micologia médica* Lacaz. 9. ed. São Paulo: Sarvier Editora de Livros Médicos; 2002. p. 594 -617.
19. Martinez R, Vitali LH, Henriques JHS, Machado AA, Albernaz A, Lima AA. Inquérito soropidemiológico para infecções por fungos causadores de micoses sistêmicas na Reserva Indígena Xacriabá, Estado de Minas Gerais. *Rev Soc Bras Med Trop*. 2002;35:347-50.
20. Martins EML, Marchiori E, Damato SD, Pozes AS, Silva ACG, Dalston M. Histoplasmosse pulmonar aguda: relato de uma microepidemia. *Radiol Bras*. 2003;36:147-51.
21. McKinsey DS, Spiegel RA, Hutwagner L, Stanford J, Driks MR, Brewer J, *et al*. Prospective study of histoplasmosis in patients infected with human immunodeficiency virus: incidence, risk factors, and pathophysiology. *Clin Infect Dis*. 1997;24:1195-203.
22. Ministério da Saúde. Brasil. Secretária de Vigilância em Saúde. Programa Nacional de DST e AIDS. Brasília: Ministério da Saúde; 2012. *Bol Epidemiol AIDS/DST*. 2012;VIII(1). [Cited 2011, Oct 13]. Available from: <http://www.aids.gov.br/publicacao/2011/boletim-epidemiologico-aids-e-dst-2011>
23. Oliveira FM, Unis G, Severo LC. Microepidemia de histoplasmosse em Blumenau, Santa Catarina. *J Bras Pneumol*. 2006;32:375-8.
24. Pontes LB, Leitão TMJS, Lima GG, Gerhard ES, Fernandes TA. Características clínico-evolutivas de 134 pacientes com histoplasmosse disseminada associada a SIDA no Estado do Ceará. *Rev Soc Bras Med Trop*. 2010;43:27-31.
25. Sidrim JJC, Oliveira FGM. Micoses profundas. In: Sidrim JJC, Moreira JLB. *Fundamentos clínicos e laboratoriais da micologia médica*. Rio de Janeiro: Guanabara Koogan; 1999. p.152-69.
26. Unis G, Oliveira FM, Severo LC. Histoplasmosse disseminada no Rio Grande do Sul. *Rev Soc Bras Med Trop*. 2004;37:463-8.

Received: 26 July 2012

Accepted: 11 December 2012