An Outbreak of Histoplasmosis among Healthy Young Japanese Women after Traveling to Southeast Asia

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Abstract

Histoplasmosis, caused by *Histoplasma capsulatum*, is an endemic mycosis in many countries of the world except for Japan. Outbreaks of histoplasmosis among Japanese people are very rare and are mainly imported by travelers. We report an outbreak of histoplasmosis among healthy Japanese people who traveled to a resort area in Southeast Asia. Three young Japanese women traveled to Langkawi island, Malaysia and stayed on the island for five days without visiting caves, a known reservoir of *H. capsulatum*. All three individuals developed flu-like symptoms with multiple nodule shadows on chest X rays or chest CT scans at around ten days after their return to Japan. Serum samples obtained from the three subjects were positive for anti-*Histoplasma* antibody and specific PCR for *H. capsulatum* on lung biopsy specimens and the serum from one patient was positive. The clinical course of all three patients improved without the use of anti-fungal agents and no recurrence has been confirmed. Clinical attendants should consider histoplasmosis when they see patients with flu-like symptoms with abnormal chest X-rays after visiting *H. capsulatum* endemic areas, especially Southeast Asia.

Key words: histoplasmosis, outbreak, serological test, PCR

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Introduction

Histoplasmosis is one of the endemic mycoses in North America (Ohio and Mississippi river valleys), Central and South America, Africa, and Asia (1). In Japan, histoplasmosis is considered an imported mycosis because most patients had recently traveled to endemic areas (including cave exploration) and very few had been suspected of domestic infection (2). Although several outbreaks of histoplasmosis have been reported all over the world, most infections have been reported in North and South America (3-7) and few reports describing outbreaks of histoplasmosis among Japanese people (7, 8). Here, we report an outbreak of histoplasmosis in three Japanese women who had visited a resort area in Southeast Asia for which serum and *Histoplasma*-specific PCR tests of lung tissue and blood samples facilitated making a confirmatory diagnosis.

Case Report

A 27-year-old Japanese woman traveled to Langkawi Island, Malaysia, with two other Japanese friends in November 2007. During her stay, she spent most of her time in a...
Figure 1. Time course of chest CT scan images of the patient. A) Multiple small nodules were confirmed in both lung fields during the first visit to the hospital (December 2007). B) The number of nodular shadows decreased and had almost disappeared without specific treatment (January 2008). C) Multiple small nodules were evident in both lungs concurrent with the emergence of back pain (February 2008).

five-star class resort hotel surrounded by sea and jungle. The woman took all meals and foods in the hotel, did not walk in the jungle and never visited caves. After a five-day stay on the island, she returned to Japan. Ten days after her return, she felt general fatigue with flu-like symptoms (fever of 38°C, headache, and muscle pain) and saw a local doctor. A conventional influenza antigen detection test was performed but was confirmed negative. Symptoms were treated as for the common cold for a duration of 4 days due to a moderate fever. Thereafter, all symptoms subsided.

Twenty days later, the individual developed back pain with dyspnea, and visited a nearby hospital the following day. The woman’s condition was almost fully recovered and no significant findings on physical examination were confirmed. Several examinations were performed, and a chest CT scan revealed multiple nodules in bilateral lungs (Fig. 1A). Blood tests, biochemical examination, and serum examination results were as follows: white blood cell count 7,370 cells/μL (47% neutrophils, 31% lymphocytes, 5% monocytes, 5% eosinophils); hemoglobin 13.4 g/dL; C-reactive protein 0.48 mg/dL; all liver function and renal function parameters were within the normal range; urine occult blood (+/-); and WBC (+). Microbiological tests including bacterial and mycological culture of sputum showed the presence of only indigenous bacteria and tests for (1→3)-beta-D-glucan, Aspergillus galactomannan antigen, Candida mannan, and Cryptococcal antigen were all negative. At this time, although some infectious diseases including fungal infections were suspected, no specific treatments were given due to the good condition of the patient, and the clinical course was observed. During the course observation period, no remarkable change in condition was observed and a follow-up chest CT scan revealed improvement of the shadows (Fig. 1B). Approximately two weeks later, the individual felt the return of mild back pain, however, chest X-rays did not show any significant changes. Back pain disappeared within several days and another follow-up chest CT scan was performed again three weeks after the onset of the recent back pain. CT images revealed the return of multiple nodules in bilateral lungs were (Fig. 1C) and, therefore, a lung biopsy under video-assisted thoracoscopy (VATS) was proposed to make a definite diagnosis.

VATS was performed in April 2008 during which a lung biopsy specimen and serum samples were obtained and immediately transferred to National Institute of Infectious Diseases (NIID) in Tokyo, where microbiological and histopathological examinations were performed. Broad bacterial, mycobacterial and mycological examinations of the lung specimen for aerobic and anaerobic bacteria, Mycobacterium spp and common fungi were carried out and showed no microorganism growth. Likewise, PCR for Burkholderia spp. and M. tuberculosis were also negative. Histopathological examination of the lung tissue revealed multiple small, white nodules (Fig. 2A). In addition, granulomas with caseous necrosis-like tubercles were observed by microscopic examination, and the small size (2-4 μm) and round morphology were confirmed by gomori methenamine silver (GMS) (Fig. 2B) and fluorescent (Fungiflora Y) staining.

Aliquots of two serum samples collected from the patient in January and February 2008 were subjected to serological tests to identify fungal diseases. Both patient samples were positive for anti-Histoplasma antibody by ELISA (Histoplasma DxsSelect™, Focus Diagnostics, Cypress, CA, USA) and, therefore, histoplasmosis was suspected for the patient.

Prior to PCR for the diagnosis of H. capsulatum, we investigated the specificity of the PCR against other known fungal and bacterial pathogens. As shown in Fig. 3, this assay is specific for H. capsulatum. For the PCR analysis of patient samples, DNA extraction from a portion of the lung specimen was performed using the DNeasy Blood & Tissue Kits (Qiagen, Germany) according to the manufacturer’s instructions. Concurrently, another aliquot of serum was mixed with an equal volume of lysis buffer (9), followed by proteinase K and Westase (Takara, Ohtsu, Japan) treatment
Figure 2. Histopathological examinations of a lung biopsy specimen. Multiple small, white nodules were observed in lung tissues (A), and GMS staining showed multiple small, rounded morphological yeast-like features (B).

Figure 3. Agarose gel showing the specificity of the PCR assay (single round PCR: expected amplicon size is 318 bp). DNA was extracted from several fungal species to test the specificity of the PCR. Lane 1, molecular size marker (100 bp ladder); 2, Aspergillus fumigatus; 3, A. niger; 4, A. flavus; 5, Candida albicans; 6, C. glabrata; 7, C. parapsilosis; 8, Cryptococcus neoformans; 9, Trichosporon asahii; 10, Penicillium spp.; 11, Nocardia brasiliensis and; 12, Histoplasma capsulatum.

Figure 4. Agarose gel of products from nested PCR targeting the Histoplasma capsulatum M antigen gene in patient samples. Lanes 1 and 10, molecular size marker (100 bp ladder); 2, specimen of lung biopsy DNA; 3, serum sample DNA of the patient; 4, serum sample DNA of another patient (29 years of age, anti-Histoplasma antibody positive); 5, serum sample DNA of an unrelated non-infectious disease case (anti-Histoplasma antibody negative); 6, serum sample DNA of another patient (28 years of age, anti-Histoplasma antibody positive); 7, serum sample DNA of healthy volunteer (anti-Histoplasma antibody negative); 8, positive control; 9, negative control.

and used for PCR. Finally, 5 μL of each sample was used for subsequent PCR. Nested PCR was carried out using our modified sets of primers which amplify a segment of M antigen gene of H. capsulatum, as previously reported by de Matos Guedes et al (10). After the second round PCR, 10 μL of each reaction was subjected to agarose gel electrophoresis stained with ethidium bromide. PCR amplicons were confirmed for both the lung specimen and serum samples for the patient (Fig. 4). Furthermore, direct sequencing of positive PCR amplicons was performed for both DNA strands and revealed 99.3% identity with the H. capsulatum M antigen gene sequence using the BLAST algorithm. Finally, a confirmed diagnosis of acute pulmonary histoplasmosis was made for the patient.

Similarly, two other women (28 and 29 years old) that had traveled with the first patient to Langkawi Island also developed fever, muscle pain, general fatigue or mild diarrhea at around ten days after their return. These two women were admitted to different hospitals in December 2008 where chest X-rays and CT scans revealed that they both had multiple small nodules on bilateral lungs (Fig. 5). Clinical examinations for diagnosis, including bronchofiberscopic examination, were carried out for each patient but a confirmed diagnosis could not be made. However, one patient showed granuloma formation in the biopsy specimen collected by transbronchial lung biopsy. Several sequential serum samples were collected from both women and frozen for later examination. In May 2008, the serum samples were analyzed at NIID for the presence of anti-Histoplasma antibody. The findings confirmed that both patients were positive for antibody to Histoplasma as determined by ELISA.
and the diagnosis of suspected histoplasmosis was made. However, the BALF specimen from one patient showed a negative fungal culture and negative Histoplasma-specific PCR result, thus a definitive diagnosis could not be obtained.

For all three cases, the individuals were carefully observed during their clinical course and their symptoms and signs gradually disappeared without the need for chemotherapy.

**Discussion**

In Japan, the number of histoplasmosis patients has increased dramatically since the mid-1980s, with a total of 63 cases reported as of March 2009 (11). This number is predicted to increase over the next decade as travel abroad is becoming increasingly popular with Japanese people. In this report, all three women had visited Langkawi Island together and all developed flu-like symptoms at approximately the same time after their return to Japan. As all three women reside in different regions of Japan separated from each other by more than two hundred kilometers, it is likely that these women were infected by *H. capsulatum* during their stay on Langkawi Island. The source or reservoir of *H. capsulatum* in the island is unknown but none of the patients visited high-risk areas such as caves. Usually, immunocompetent hosts with *H. capsulatum* infection are asymptomatic or develop mild symptoms (1); however, all three of the young women described in this study showed flu-like symptoms with multiple nodular shadows in the lungs. We speculate that these women had inhaled a high burden of *H. capsulatum* and/or were possibly exposed to a highly virulent strain of *H. capsulatum*. Further studies including environmental surveys are expected.

Clinical laboratory methods for *Histoplasma* detection are likely to become important due to the projected increase in the number of patients. Typically, histoplasmosis is diagnosed using fungal culture, antigen detection methods, serological tests, or histopathological examination (12). Culture of *H. capsulatum* in the clinical laboratory is difficult as it is classified as a biosafety level-3 (BSL-3) pathogen, and it takes approximately four weeks for *H. capsulatum* to grow in vitro. Additionally, the sensitivity of fungal culture is reported as relatively low (12), especially in the case of acute pulmonary histoplasmosis. In this report, *H. capsulatum* isolates could not be recovered for all three cases but specimen storage conditions may have influenced these results.

Serological testing for histoplasmosis cases is considered a highly sensitive assay but is reduced for acute histoplasmosis or disseminated disease. In contrast, the antigen detection assay for *H. capsulatum* has been reported as useful in clinical settings with a 75% sensitivity of detection for acute pulmonary histoplasmosis cases but it can cross-react with other pathogens such as coccidiomycosis, paracoccidiomycosis, and blastomycosis (12). In this report, although we could not confirm infection by *H. capsulatum* using the antigen assay, all three cases were positive for antibody and these results helped make the diagnoses. Generally, antibody assays can be used to detect previously infected individuals with no signs of active disease. However, histoplasmosis is not endemic in Japan and the majority of the Japanese population has never been to regions with endemic *H. capsulatum* and, therefore, would be negative for antibody to the organism. To determine if this is indeed the case in Japan, a comprehensive epidemiological study is needed.

Diagnostic methods, such as PCR, are powerful tools for the diagnosis of some infectious diseases but the usefulness of genetic detection methods against fungal diseases is still controversial (12). In this report, we used PCR for the diagnosis of histoplasmosis and confirmed that this assay is specific for *H. capsulatum* (Fig. 3) and could detect at least 1 fg of genomic DNA (data not shown). Therefore, this technique was considered to be another useful method for the diagnosis of histoplasmosis. The PCR assay described here could detect one out of three cases using a lung specimen and serum samples and this may reflect the clinical status of diseases such as disseminated histoplasmosis.

It has been reported that the symptoms of acute pulmonary histoplasmosis are fever, chills, malaise, headache, weakness, coughing, dyspnea, or chest pain with associated chest radiograph findings described as patchy pneumonia, enlarged mediastinal lymph nodes, bilateral diffuse reticulonodular infiltrates, or diffuse pulmonary infiltrates (1, 13, 14). Flu-like symptoms such as fever, headache, or muscle pain were seen in all patients, in addition to multiple small nodules on bilateral lungs observed on chest images. Therefore, the symptoms and signs in this report were consistent with acute pulmonary histoplasmosis. All three patients in this study recovered from the disease without specific chemotherapy for histoplasmosis and the immune status of the hosts was likely the major contributing factor for the complete control and recovery. Chest CT

![Figure 5. A chest CT scan image of a 29-year-old woman who also developed flu-like symptoms after returning to Japan (December 2007). The image also revealed multiple small nodules in both lung fields.](image-url)
scans for one of the patients showed an interesting dynamic state where most of the nodules were absent at one time but reappeared approximately one month later. Although the reason for this is unclear, we speculate that this phenomenon was due to the relationship between the pathogen and the host immune status. Furthermore, although one patient was suspected of disseminated histoplasmosis, all three cases could be considered as acute pulmonary histoplasmosis.

Generally, therapeutic guidelines for histoplasmosis state that acute self-limited syndromes and acute pulmonary histoplasmosis with mild-to-moderate illness are not indications for treatment and these cases should be closely observed (15). However, it is also recommended that treatment with itraconazole for 6-12 weeks should be considered for acute pulmonary histoplasmosis patients who have shown no clinical improvement after one month of observation. For moderate or severe acute pulmonary histoplasmosis cases, treatment with amphotericin B plus methylprednisolone is recommended. Treatment with an anti-fungal agent such as amphotericin B or itraconazole is suggested for treatment of all patients with chronic pulmonary histoplasmosis and disseminated histoplasmosis (15). Considering these therapeutic guidelines, chemotherapy might have been required for the 27-year-old patient because CT images had revealed the return of multiple nodules in bilateral lungs and Histoplasma DNA had been detected in her serum by PCR. Since all three patients had no underlying diseases or immunosuppressive factors, and they also showed a good clinical course, close observation was considered appropriate care. Thus far, these patients have never had recurrence of histoplasmosis, even without the use of anti-fungal agents. However, careful continued observations will be necessary for all cases in this report.

To date, the number of histoplasmosis infections in Japan is still relatively small compared with endemic countries such as the United States. As the population is increasingly mobile and able to reach anywhere in the world within 24 hours, it is important that there is awareness of the diagnosis and care of histoplasmosis patients who have traveled abroad, especially to endemic areas.

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