Histoplasmosis in the Eastern and the Western world: Is it endemic in India?

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University of Delhi, Delhi, India
**Histoplasma capsulatum**

- Infectious spores
- Soil enriched by bat or bird excretion
- **Intracellular, dimorphic ascomycete**
- Causes respiratory infection by inhalation.
- Diagnostically important tuberculate macroconidia

Infection may spread from lungs to the RES (liver, spleen, lymph nodes)

*Modified from Talare & Talare, 1999: Foundations in Microbiol, McGraw Hill*
Three varieties

1. *Histoplasma capsulatum* var. *capsulatum* - Worldwide, highly endemic in some regions of USA, Europe and from Asian countries including India, China, Japan

2. *Histoplasma capsulatum* var. *duboissii* - Worldwide, mainly endemic in regions from Central Africa, Madagascar Island

Clinical manifestation

- cause disease in healthy subject, but more severe disease in ICH
- asymptomatic (90%) to disseminated infection (10%).

- Acute pulmonary histoplasmosis
  - Sequelae: mediastinal granuloma, pericarditis, rheumatologic syndromes

- Chronic pulmonary histoplasmosis

- Disseminated histoplasmosis

- Other: mediastinal fibrosis, central nervous system histoplasmosis, broncholithiasis

- Both new infection and reactivation can occur.
Difficulties in Diagnosis (Darling’s Disease)

The diagnosis is often missed or delayed.

- Clinical signs and symptoms are nonspecific and often mimic those of TB.
- Proven: Isolation in culture or microscopy, histology.
- Culture has notable limitations, insensitivity, a need for invasive procedures, delayed growth and expertise.
- Fatal in the absence of timely diagnosis and appropriate antifungal treatment.
- Histology requires expertise: C. glabrata and Leishmania look similar although tissue reaction is different.
Histoplasmosis - the Americas

- Endemic area
- Regions of highest incidence
Incidence and Prevalence

- An estimated population of 40 million is exposed in US.

- Approx. 500,000 new cases of histoplasmosis occur annually in the US, of which 200,000 become clinically ill and 4000 require hospitalization.

- 5% - 20% of opportunistic infections in AIDS patients in Indianapolis (Wheat et al., 1990).

- Death rates observed in AIDS associated histoplasmosis differ between the USA (12-23%) and South America (19-39%) (Couppie et al., 2006)

Perspective Piece

How Many Have Died from Undiagnosed Human Immunodeficiency Virus–Associated Histoplasmosis, A Treatable Disease? Time to Act

Mathieu Nacher,* Antoine Adenis, Christine Aznar, Denis Blanchet, Vincent Vantileke, Magalie Demar, Bernard Carme, and Pierre Couppié
With 1.5 cases per 100 patient-years, histoplasmosis is the most common cause of AIDS defining illness.
Histoplasmosis - Africa

- **Endemic area**
- **Regions of highest incidence**
Europe

- Disease is mainly found in immigrants

- 118 cases reported from 10 countries during 5-year period (1995–1999)

- 8 are autochthonous cases.

- Reactivation upto 50 yrs after the initial infection,

- 20% of HIV infected person develop PDH
Histoplasmosis in Europe

1996-1999 (n=118)

Imported Pulmonary Histoplasmosis in Three French Cavers After a Trip to Cuba

Agathe Sénéchal, MD,† Tristan Ferry, MD, PhD,‡ André Boibieux, MD,‡ Jean-Paul Brion, MD,‡ Olivier I'paulard, MD,§ Christian Chidiac, MD,‡ and Dominique Peyramond, MD,‡†

An Outbreak of Acute Pulmonary Histoplasmosis in Members of a Trekking Trip in Martinique, French West Indies


Histoplasmosis in a group of bat researchers returning from Cuba

K. Erkens1,2, M. Lademann1, K. Tintelnot3, M. Lafrenz1, U. Kaben4, E. C. Reisinger1

Severe Histoplasmosis in Travelers to Nicaragua

Imported Acute Histoplasmosis With Rheumatologic Manifestations in Spanish Travelers

David Alonso, MD,* Jose Muñoz, MD,* Emilio Letang, MD,* Elisa Salvador, MD,* Manuel Cuerva-Estrada, MD,* Maria Jose Boitrago, PhD,* Josep Maria Torres, MD,† and Joaquim Gascon, MD, MPH†

Imported Histoplasmosis in Spain

J. Gascón, J.M. Torres, P. Luburich, J.R. Ayuso, A. Xaubet, and M. Corachán

Pulmonary Histoplasmosis in Three Austrian Travelers After a Journey to Mexico

M. Hoeggl, L. Schwetz, R. Wurm, S. Scherl, H. Olschewski, and R. Kroese

Acute Histoplasmosis in Spelunkers Returning from Mato Grosso, Peru

Paola Nasta, Alessandra Donisi, Anna Cataneo, Alessandro Chiodera, and Salvatore Cesari

Outbreaks among European travellers

Equador

Gautemala
Geographic Distribution of Histoplasmosis in Europe
Potential focus
Northern-Italy
Soil, Animals, Humans

First report in canine histoplasmosis with lesions confined to CNS
Geographic distribution of histoplasmosis in Asia

- Lebanon
- Saudi Arabia
- Iran
- Pakistan
- India
- Bangladesh
- Sri Lanka
- Thailand
- Cambodia
- Malaysia
- Indonesia
- Vietnam
- Philippines
- Japan

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Status in Asia

Original Article
Histoplasmosis in India: Truly Uncommon or Uncommonly Recognised?
Ram Gopalakrishnan, P Senthur Nambi, V Ramasubramanian, K Abdul Ghafor, Ashok Parameswaran

Progressive disseminated histoplasmosis in an immunocompetent patient as an underrecognised imported mycosis in Japan
Goh Ohji, Ken Kikuchi, Katsunori Komorita, Kazuya Imoto, Shigoro Yamamoto, Naoto Hosokawa, Kazuhiro Kamei, Kentaro Itsuta

Histoplasmosis: a new endemic fungal infection in China? Review and analysis of cases
Bo Pan, Min Chen, Weiwei Pan, and Wanqing Liao

Epididymal histoplasmosis diagnosed by isolation of Histoplasma capsulatum from semen

A rare presentation of progressive disseminated histoplasmosis in an immunocompetent patient from a non-endemic region
M.V.S. Subbalaxmi, P. Umabala, Roshni Paul, Naval Chandra, Y.S. Raju, Shivaprapesh M. Rudramurthy

Case report
Disseminated histoplasmosis in an apparently immunocompetent individual from north India: a case report and review
Sushruta Kathuria, Malini Rajinder Kapoor, Sachin Yadav, Avinder Singh, & V Ramesh
Scenario in India

- The first case of histoplasmosis was reported in 1954 by Panja and Sen, Kolkata.
- Well documented in India but endemic areas remain undetermined.
- Occurrence of histoplasmosis constitutes apparently immunocompetent patients in China, Japan and India. (Pan et al., 2013, Kathuria et al.; unpublished)
**Pattern of histoplasmosis in India (1954–2014)**

(Number cases reviewed = 187)

<table>
<thead>
<tr>
<th>Period</th>
<th>No. cases reported</th>
<th>Cases/ year (Mean value)</th>
<th>Increase in prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1954–1993 (40 Yr)</td>
<td>36 (19.2%)</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>1994–2014 (20 yr)</td>
<td>151 (80.7%)</td>
<td>7.5</td>
<td>8.3-fold higher*</td>
</tr>
</tbody>
</table>

(Number cases reviewed = 187)
Diagnostic methods utilized for histoplasmosis (1954-2014) (n=187)

<table>
<thead>
<tr>
<th>Method</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histopathology</td>
<td>167/187 (87%)</td>
<td></td>
</tr>
<tr>
<td>Culture</td>
<td>71/187 (23%)</td>
<td></td>
</tr>
<tr>
<td>Direct PCR based detection</td>
<td>10/187</td>
<td></td>
</tr>
</tbody>
</table>

60% of the cases in apparently immunocompetent
Diagnostic Modalities

1. Direct Microscopy and special stain/Histopathology
   - KOH Mount
   - Staining: Geimsa, Grocotts Methanamine silver nitrate stain, Periodic Acid Schiff

2. Culture and detailed microscopic features:
   - Conversion to yeast form
   - Confirmation by sequencing ITS and LSU regions of isolates

3. PCR-based detection of Histoplasma DNA
   - DNA extraction from clinical specimens
   - Amplification and confirmation of product by sequencing using Histo-specific primers

4. Demonstration of *H. capsulatum* precipitins in patient’s sera
**Isolation of *Histoplasma capsulatum* in the environment of India**

Isolation from a soil sample admixed with bat guano from an abandoned, 350-year-old palatial building in Serampore near Kolkata in West Bengal (*Sanyal and Thamayya, 1975*).

PCR was positive from environmental samples.
Evidence of presence of *H. capsulatum* DNA inferred that

“Histoplasmosis is endemic in India”
**In vitro antifungal susceptibility profile of *H. capsulatum* isolates reported globally**

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Number investigated (Dimorphic form)</th>
<th>AMB</th>
<th>ITC</th>
<th>VRC</th>
<th>POS</th>
<th>ISA</th>
<th>FLU</th>
<th>CAS*</th>
<th>RAV</th>
<th>Refere nce</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>North America</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>St. Louis</td>
<td>1990</td>
<td>8+4§(Y)</td>
<td>0.30-1.04</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.95-1000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indiana</td>
<td>1997</td>
<td>1 (Y)</td>
<td>-</td>
<td>0.004</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.62 (parent); 1.25 (8th wk); 2.5 (12th wk) 20 (16th wk)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Virginia</td>
<td>1998</td>
<td>5 (M)</td>
<td>0.25-0.5</td>
<td>0.06</td>
<td>0.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>16</td>
</tr>
<tr>
<td>Virginia</td>
<td>1998</td>
<td>5 (M)</td>
<td>-</td>
<td>0.04</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CAS: 1.3, AFG: 3.6</td>
<td>17</td>
</tr>
<tr>
<td>Indiana</td>
<td>2000</td>
<td>20 (Y)</td>
<td>0.5-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>Texas</td>
<td>2000</td>
<td>100 (M)</td>
<td>≤0.03-2</td>
<td>≤0.03-0.5</td>
<td>≤0.03-2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CAS:0.5-4; AFG:2.4; MFG: &gt;0.03-0.06</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Indiana</td>
<td>2001</td>
<td>65 (M)</td>
<td>0.019-0.077</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.31-10</td>
<td>-</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Virginia</td>
<td>2003</td>
<td>4-5 (M)</td>
<td>&lt;0.01-0.03</td>
<td>&lt;0.03-0.06</td>
<td>-</td>
<td>1-16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>19</td>
</tr>
<tr>
<td>Mexico</td>
<td>2005</td>
<td>28 (M)</td>
<td>0.06-1</td>
<td>0.06-2</td>
<td>0.03-2</td>
<td>-</td>
<td>-</td>
<td>2-32</td>
<td>-</td>
<td>0.125-2</td>
<td>20</td>
</tr>
<tr>
<td>Indiana</td>
<td>2006</td>
<td>17 (Median MIC) (Y)</td>
<td>-</td>
<td>0.015</td>
<td>0.007</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>0.007</td>
<td>0.007</td>
<td>21</td>
</tr>
<tr>
<td>Mexico</td>
<td>2009</td>
<td>28 (M)</td>
<td>0.06-0.25</td>
<td>0.25-2</td>
<td>0.06-2</td>
<td>0.03-2</td>
<td>0.125-2</td>
<td>4-32</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td><strong>South America</strong></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Brazil</td>
<td>2012</td>
<td>68 (M)</td>
<td>0.007-0.5</td>
<td>0.001-0.031</td>
<td>0.0078-0.5</td>
<td>-</td>
<td>-</td>
<td>3.9-125</td>
<td>0.016-32</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>2012</td>
<td>8 (Y)</td>
<td>0.06-0.5</td>
<td>0.0039-0.03</td>
<td>0.002-0.03</td>
<td>-</td>
<td>-</td>
<td>3.9-7.8</td>
<td>1-4</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td>Asia</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Japan</td>
<td>2010</td>
<td>3 (M)</td>
<td>0.013-0.05</td>
<td>&lt;0.0004</td>
<td>0.006-0.025</td>
<td>-</td>
<td>0.03-0.006</td>
<td>0.55-1.2</td>
<td>-</td>
<td>-</td>
<td>23</td>
</tr>
<tr>
<td>India</td>
<td>2013</td>
<td>21+2§ (M)</td>
<td>0.03-0.25</td>
<td>&lt;0.03-0.125</td>
<td>&lt;0.03-0.25</td>
<td>0.015-0.125</td>
<td>0.015-0.25</td>
<td>2-32</td>
<td>0.015-0.5</td>
<td>Present study</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>21+2§ (Y)</td>
<td>0.03-0.5</td>
<td>0.03-0.25</td>
<td>0.03-0.5</td>
<td>0.03-0.5</td>
<td>0.015-0.125</td>
<td>2-8</td>
<td>0.03-1</td>
<td>-</td>
<td>23</td>
</tr>
</tbody>
</table>

*AMB, amphotericin B; ITC, itraconazole; VRC, voriconazole; POS, posaconazole; ISA, isavuconazole; FLU, fluconazole; CAS, caspofungin; RAV, ravuconazole; MFG, micafungin; AFG, anidulafungin; §denotes number of reference strains of *H. capsulatum* tested.*
Challenges determining the AFST

- No guidelines or recommendations
- Slow grower dimorphic pathogen
- Prolong incubation time for adequate growth
- Differences in growth conditions for both yeast and mold form
- High chances of contamination.
- Takes about 4-8 weeks for conversion from mold to yeast form
- Requires highly sensitive and fastidious medium i.e BHI with 5% sheep blood
- Tedious and time consuming.
AFST of which form is more clinically relevant
In vitro antifungal susceptibility profile of yeast and mold form of *H. capsulatum* (N=23)

<table>
<thead>
<tr>
<th></th>
<th>AMB</th>
<th>ITC</th>
<th>VRC</th>
<th>POS</th>
<th>ISA</th>
<th>CAS</th>
<th>FLU</th>
<th>5FC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H. capsulatum</strong></td>
<td></td>
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<tr>
<td><strong>(mold form)</strong></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td><strong>GM</strong></td>
<td>0.11</td>
<td>0.043</td>
<td>0.102</td>
<td>0.05</td>
<td>0.055</td>
<td>0.094</td>
<td>7.05</td>
<td>64</td>
</tr>
<tr>
<td><strong>MIC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>0.125</td>
<td>0.03</td>
<td>0.125</td>
<td>0.06</td>
<td>0.06</td>
<td>0.125</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td><strong>MIC&lt;sub&gt;90&lt;/sub&gt;</strong></td>
<td>0.25</td>
<td>0.125</td>
<td>0.25</td>
<td>0.125</td>
<td>0.25</td>
<td>0.25</td>
<td>16</td>
<td>&gt;64</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0.03-0.25</td>
<td>&lt;0.03-0.125</td>
<td>&lt;0.03-0.25</td>
<td>0.015-0.125</td>
<td>0.015-0.25</td>
<td>0.015-0.5</td>
<td>2-32</td>
<td>8-64</td>
</tr>
<tr>
<td><strong>H. capsulatum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>(yeast form)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GM</strong></td>
<td>0.18</td>
<td>0.058</td>
<td>0.12</td>
<td>0.20</td>
<td>0.08</td>
<td>0.35</td>
<td>6.1</td>
<td>48.2</td>
</tr>
<tr>
<td><strong>MIC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>0.125</td>
<td>0.06</td>
<td>0.125</td>
<td>0.06</td>
<td>0.06</td>
<td>0.25</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td><strong>MIC&lt;sub&gt;90&lt;/sub&gt;</strong></td>
<td>0.25</td>
<td>0.125</td>
<td>0.25</td>
<td>0.25</td>
<td>0.125</td>
<td>1</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0.03-0.5</td>
<td>0.03-0.25</td>
<td>0.03-1</td>
<td><strong>0.06-2</strong></td>
<td>0.03-0.25</td>
<td><strong>0.06-2</strong></td>
<td>2-16</td>
<td>8-64</td>
</tr>
</tbody>
</table>
generate data on antifungal susceptibility of the two forms and correlated the two growth forms.

Yeast and mold form showed statistically significant concordance in MICs for all the antifungals except POS and CAS.

The cumbersome technique of yeast conversion for susceptibility testing is not mandatory.

Susceptibility of mold form is recommended which is easier and less time consuming.
Mold form

- FLU, 2 μg/ml
- FC, 32 μg/ml
- ITC, <0.03 μg/ml
- VRC, 0.06 μg/ml
- ISA, 0.03 μg/ml
- AMB, 0.06 μg/ml
- CAS, 0.5 μg/ml
- POS, <0.03 μg/ml

Yeast form

- FLU, 4 μg/ml
- FC, 8 μg/ml
- ITC, <0.03 μg/ml
- VRC, <0.03 μg/ml
- AMB, 0.125 μg/ml
- ISA, 0.03 μg/ml
- POS, 0.03 μg/ml
- CAS, 0.25 μg/ml
First study for the phylogeny of this pathogen was reported by Kasuga et al., in 1999.

DNA sequence variation or polymorphism in four protein-coding genes (H-anti gene, Ole, Tub1, Arf) were analysed.

1. North American-1,
2. North American-2,
3. Latin American Group A,
4. Latin American Group B,
5. Australian,
6. Netherlands,
7. Eurasian
✓ Phylogenetic analysis of the concatenated sequences of all the 4 genes showed genetic diversity among the Indian strains.

✓ The Indian isolates revealed marked heterogeneity.

✓ Further analysis of multiple genes with large number of global isolates may reveal significant data on distribution of unique genotypes prevalent in India.
Future Perspectives

✓ Warrants screening of larger number of environmental samples from varied regions.

✓ AFST recommended usage of mycelial form to generate substantial data.

✓ Marked heterogeneity using MLST and further studies are warranted to explore the population structure of this pathogen.

✓ Survey for the occurrence of histoplasmosis in patients with TB like Syndrome and patients who were anti-tubercular therapy resistant and had AFB negative sputa.
Take Home Message

Patient with respiratory disorder and/or chest x-ray mimicking TB or misdiagnosed as TB and Anti-tubercular treatment resistant and AFB stain negative

“Don’t miss histoplasmosis!”
Multiple Strategies to combat histoplasmosis

- Increase diagnostic facilities and strengthening infrastructure.

- Development of new, rapid, affordable diagnostic assays such as molecular techniques should be used to improve the diagnosis, particularly for the detection of disease in an early stage and to improve the specificity of the diagnosis.

- Improvements in expertise to diagnose cases of histoplasmosis by filling the knowledge gap between clinicians, pathologists and microbiologists.

- Access to the most effective antifungal drugs to reduce mortality.

- Improved estimates of disease burden i.e. knowledge of local epidemiologies.

- Restricted niches: so geographical history (both past and recent) crucial important.
To quote Ainsworth 1966*

“The available literature reflects the distribution of mycologists or mycologically oriented investigators more accurately than it does the distribution of mycoses and their relative importance in a given area”.

Histoplasma capsulatum: More Widespread than Previously Thought

Spinello Antinori*

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* Address correspondence to Spinello Antinori, Department of Biomedical and Clinical Sciences, “Luigi Sacco,” University of Milano, Italy. E-mail: spinello.antinori@unimi.it
Think about Fungus
Thank you