HISTOPLASMOSIS - LABORATORY DIAGNOSIS IN VIETNAM

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Introduction - histoplasmosis

- Caused by *Histoplasma capsulatum*
  - High mortality rate in the case of systemic infection
  - 250,000 individuals/year in the US
  - Clinical manifestations occur in less than 5% of the population
- The most common fungal infections
  - A systemic disease - **the reticulo-endothelial system**
  - Manifesting itself in the bone marrow, lungs, liver, and the spleen
    - in children: primary sign
    - in adults: pulmonary disease
- Histoplasmosis is an **occupational disease**

Introduction - histoplasmosis

- Endemic all over the world
  - High risk for immunocompromised host

    (Not safe for healthy individuals)
- Dimorphic fungus
  - mycelial phase (natural environment)
  - yeast phase (inside host)
- Three *Histoplasma* spp. (*capsulatum, duboisii, farciminosum*)
- Grow best in soils contaminated with bird manure or bat droppings

  (High risk for construction worker or cave explorer: Cave fever)

Introduction – Clinical aspects

- Respiratory tract infection
  - Dissemination to lymph node after pneumonia
  - Asymptomatic or mild flu-like respiratory illness
- Histopathologic image similar to tuberculosis

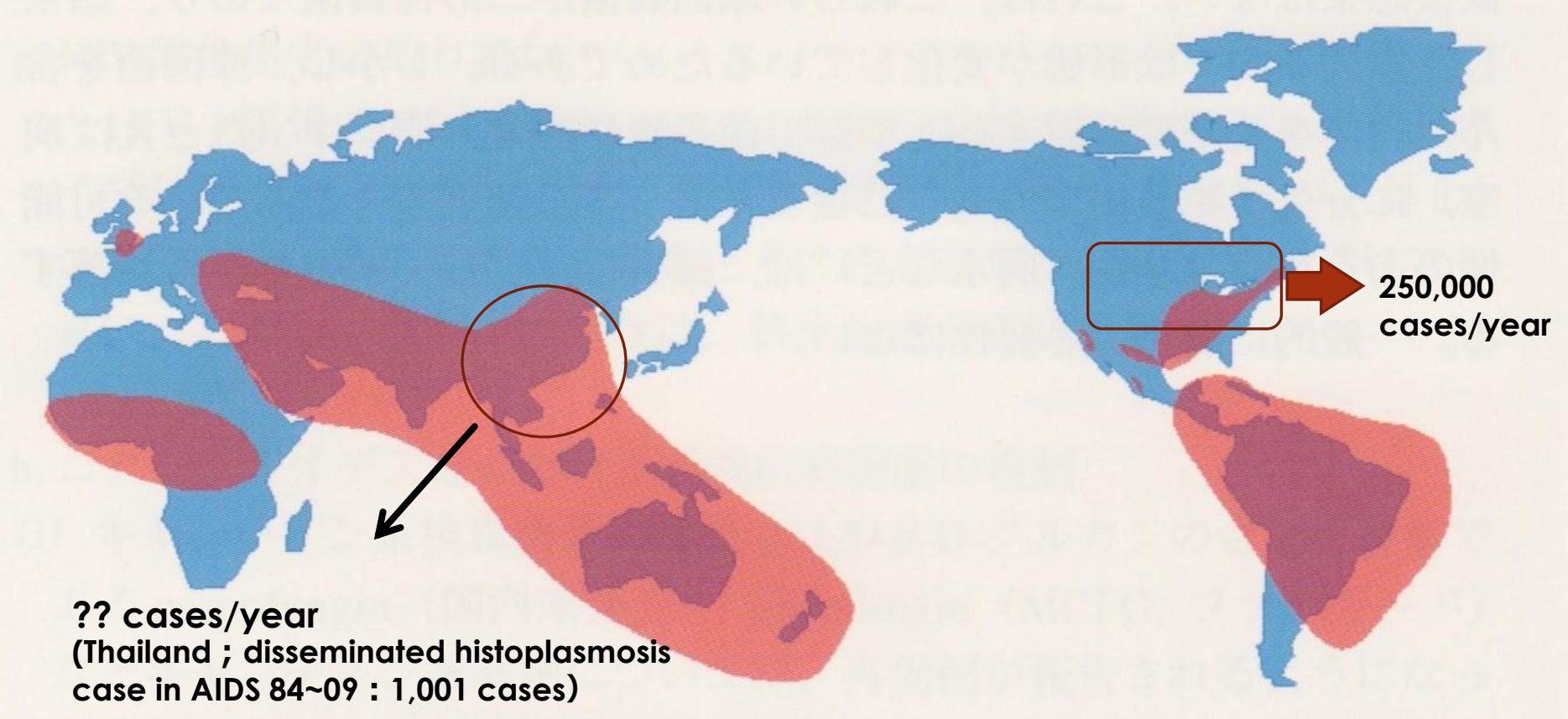
  (95% of infected people show asymptomatic)

  Acute pulmonary histoplasmosis

  Chronic pulmonary histoplasmosis

  Disseminated histoplasmosis (cell-mediated immune deficiency)
- High risk group
  - Hematologic malignancy, Steroid therapy
  - HIV infection (mortality = around 50%)

Introduction - histoplasmosis



Mississippi valley and Ohio valley areas in USA, Central America, Southeast Asia, Australia, Central Africa, and Europe were reported as endemic areas of histoplasmosis
(figure from "Guidelines for diagnosis and treatment of imported mycosis 2006", published in Japan)

Introduction – transmission route

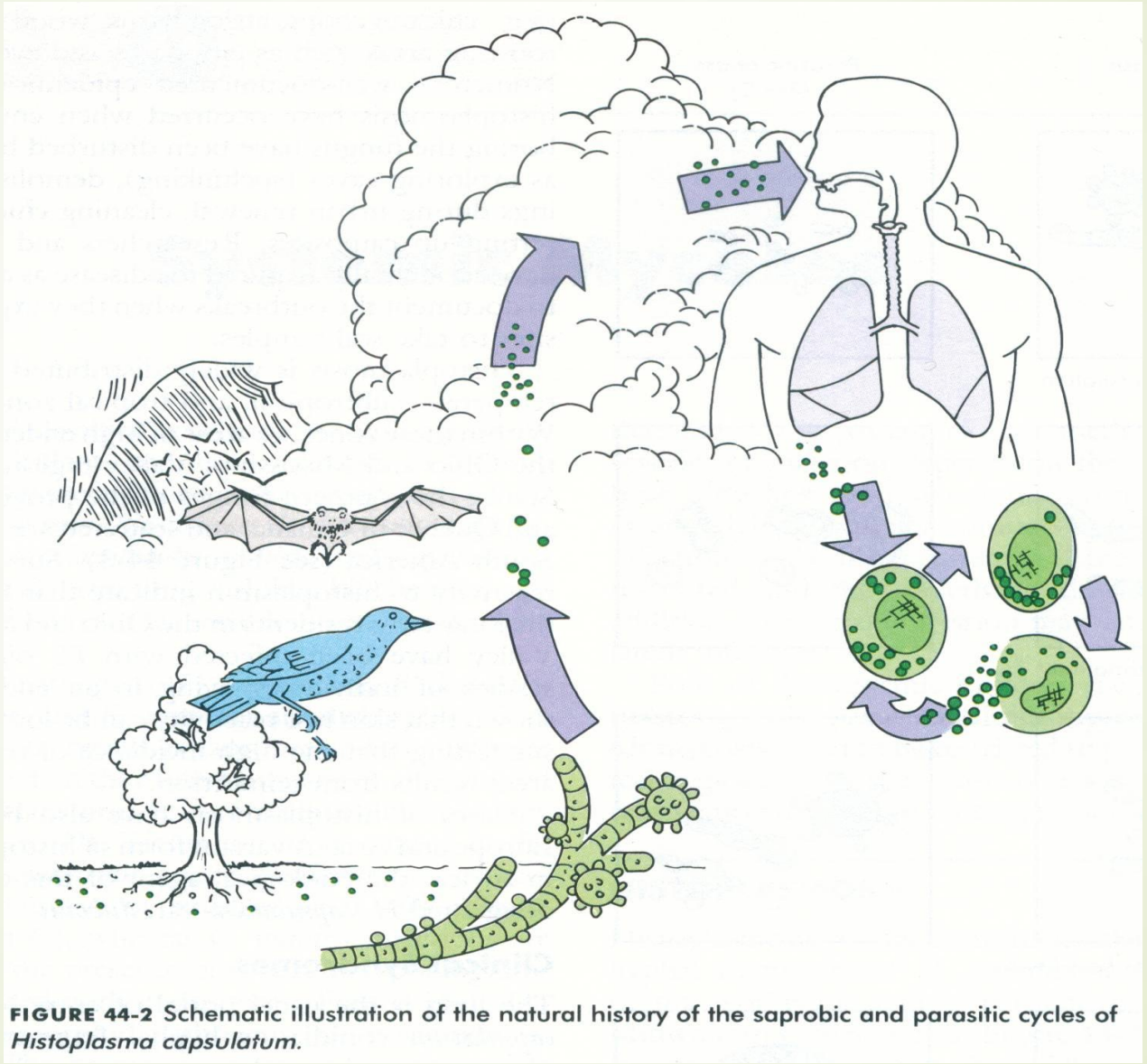
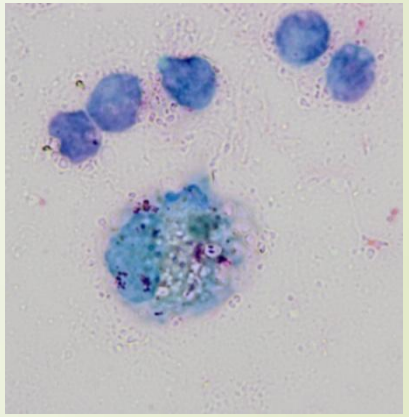


FIGURE 44-2 Schematic illustration of the natural history of the saprobic and parasitic cycles of *Histoplasma capsulatum.*

Introduction – diagnosis

- **IMPORTANT: Check travel history to endemic area**
- Mycological test（yeast cells phagocyted by monocyte or macrophage:
- similar to *Penicillium marneffei*）
- Culture test（false negative, and takes 4-6 weeks）Histopathological examination
- Detection of *H. capsulatum* antibody
- Detection of *H. capsulatum* antigen
- Molecular methods: PCR, MLST



ELISA for anti-histoplasma antibody

1：healthy volunteer
2, 3：histoplasmosis patient
4：blank
5：positive control
6：negative control
7：calibration control

Introduction – reports from Asian countries

- Malaysia:

  A survey of histoplasmin skin test sensitivity showed a prevalence of 10.5% (Randhawa, 1970)

- Thailand:

  between 1966 and 1968: 7–14% in the northern region and 15–36% in the Southeast and Southern regions (Norkaew et al., 2013)

- India:

  histoplasmin sensitivity has been reported to be relatively high in Calcutta and Delhi (4.7–12.3%) with most cases occurring in north- eastern India in West Bengal and Assam states (Randhawa, 1970)

- China:

  A 2013 review found that from 1990 to 2011, 300 cases were reported in China of which 75% occurred along the Yangtze river (Pan et al., 2013)

Introduction - Vietnam

Y Học TP. Hồ Chí Minh * Tập 12 * Phụ bản Số 1 * 2008 Nghiên cứu Y học

NHÂN HAI TRƯỜNG HỢP NHIỄM NẤM *HISTOPLASMA* TẠI BỆNH VIỆN CHỢ RẪY NĂM 2006- 2007

*Nguyễn Thị Tố Như**

TÓM TẮT

Có 2 trường hợp BN bị nhiễm nấm Histoplasma đã được chẩn đoán và điều trị tại bệnh viện Chợ Rẫy trong năm 2006- 2007. Tuổi BN từ 55-57 tuổi. Cả 2 BN có tiền căn lao phổi và điều trị theo chương trình chống lao quốc gia. Một bệnh nhân bị nhiễm Histoplasma lan tràn nội tạng và có viêm tuỷ xương đòn phải, bệnh nhân còn lại bị nhiễm Histoplasma thể phổi cấp tính. Cả hai đều bị viêm phổi nặng, gan, hạch to và nổi các nốt cục và hồng ban đa dạng ở khắp người.Không có BN nào có xét nghiệm HIV (+), các xét nghiệm nhuộm BK và cấy đàm, mủ, mô xương sinh thiết âm tính. Xét nghiệm tế bào học của mô đa sinh thiết, mô xương phát hiện Histoplasma. Cả hai BN được điều trị thuốc kháng sinh chống nấm thành công.

ASTRACT

TWO CASES OF HISTOPLASMOSIS AT CHO RAY HOSPITAL IN 2006 – 2007

Nguyen Thi To Nhu * Y Hoc TP. Ho Chi Minh * Vol. 12 – Supplement of No 1 - 2008: 37 - 43

2 cases of Histoplasmosis were diagnosed and treated at ChoRayHospital HCMC.

Their ages ranged: 55-57 years. Both of patients had lung tuberculosis, were treated by regimen of National TB control program in the past history of disease. One patient had progressive diseminated histoplasmosis, had right clavicular osteomyelitis, one had acute pulmonary histoplasmosis. Both of them had severe pneumonitis, peripheral lymphadenopathy, hepatomegaly and scattered papulo-nodules and erythema multiform over body. None of patients were HIV positive. BK smears and bactrial culture of sputum, pus, bone biopsy were negative. Cytology ofbone and skin biopsy revealed Histoplasma. Two patients were treated successfully by Amphotricin và Itraconazol.

MỞ ĐẦU

Bệnh do nhiễm nấm *Histoplasma* gọi là histoplasmosis, bệnh này thường được đề cập trong y văn nước ngoài, nhưng ở Việt Nam khá hiếm gặp, Chúng tôi xin trình 2 trường hợp nhiễm nấm này ở 2 bệnh nhân đã được điều trị tại bệnh viện Chợ Rẫy trong thời gian vừa qua.

TRƯỜNG HỢP LÂM SÀNG

Bệnh nhân 1

Phần hành chính

Nguyễn T- A., sanh năm 1952- Điều trị từ 31/10/2006 đến 8/12/2006

Nghề nghiệp: Làm rẫy. Dân tộc: Châu Ro

* Bộ môn Nội Tổng Quát - Đại học Y Dược Tp.HCM

Địa Chỉ: tổ 5, ấp 5, xã Thanh sơn, huyện Định Quán, Tỉnh Đồng Nai.

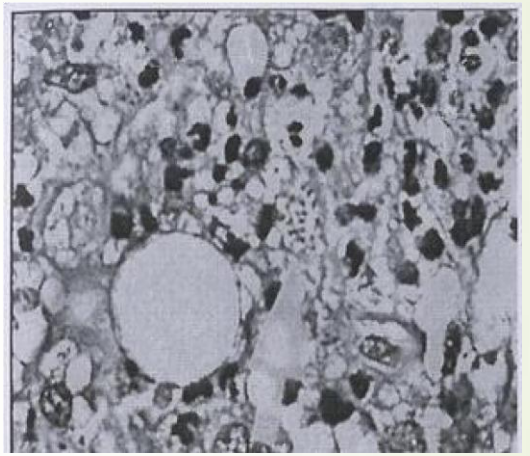
Lý do nhập viện: Áp xe phần mềm 2 vai, viêm phổi (BV Chấn thương Chỉnh Hình chuyển).

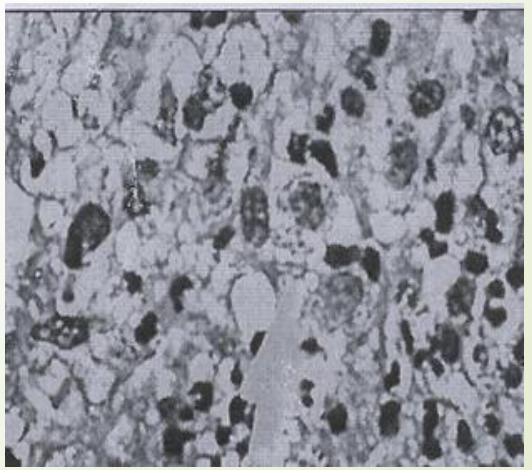
Bệnh sử

BN mới vừa ngừng điều trị lao phổi theo CT kháng lao quốc gia 1 tháng. Nhưng BN vẫn sốt về chiều, còn ho có đàm, rãi rác trong người có nổi u cục nhỏ, có mủ vàng. Đau nhức, sưng,đỏ hai vai và ngày càng sụt cân, suy kiệt nên nhập vào BV Phạm Ngọc. tại đây BN được truyền máu vì thiếu máu nặng và gởi qua BV ChấnThương Chỉnh hình. BV Chấn thương Chỉnh Hình gởi BN qua BV Chợ Rẫy với chẩn đoán như trên.

Khám lúc nhập viện Chợ Rẫy

BN suy kiệt, da niêm xanh nhợt, HA =90/60mmHg, T° = 39 ° C,Phổi có ran nổ 2 bên, nhiều ở 2 đáy phổi. Vết thương vùng vai có nhét mèche ra mủ vàng.





Introduction - aims

- To utilize a rapid diagnostic technique for histoplasmosis in Vietnam

- To identify the proportion of Histoplasmosis among the patients with lung infection in Vietnam

  (combination of PCR, serological test and culture isolation)

Methods

- Sample collection:
  - Time: 2012 – 2013
  - Study object: Lung infection patients
  - Sample type:

    Bronchoalveolar lavage fluid (BALF) : 158

    Peripheral blood: 206

Methods

- Lab analysis:
  - ELISA: Histoplasma Dx™Select kit (Focus – USA)
  - Culture: BHI + chloramphenicol 10% and incubation for 30^0C/month
  - Nested PCR:

    H. capsulatum M antigen gene (Msp1F/Msp2R and Msp2F/Msp3R) (Hideaki et al., 2013)
  - Sequencing

    Nucleotide sequences for the 269bp products were verified using the National Center for Biotechnology Information, Basic Local Alignment Search Tool.
  - Ethical approval

    The study was approved by the ethics committee of National Institute of Hygiene and Epidemiology, Hanoi, Vietnam (No. 01 IRB, dated January 6, 2012).

Results

- Lab analysis:
  - ELISA: 27/206 (13.1%)
  - Culture: negative
  - Nested PCR: 9/158 (5.7%)
  - Sequencing: 9/9 (100%)

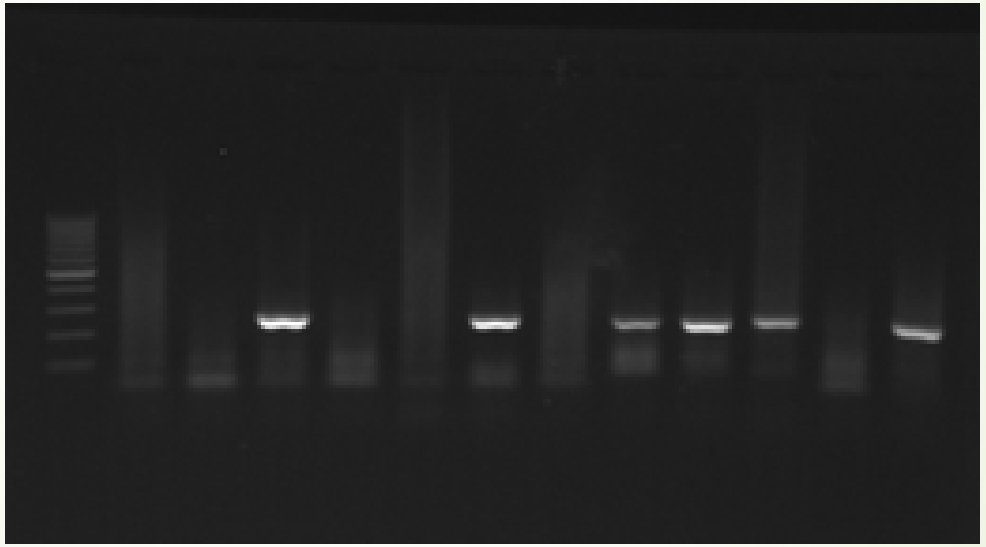
Results - Characteristics of patients

Sex		Age (yr)		Occupational			Clinical manifestations	
Male	Female	20-40	41-60	Farmer	Worker	Others	Acute pulmonary	Chronic pulmonary
119	87	66	140	107	77	22	90	116

Results – Comparision of ELISA findings in the patients group

Patients group (n)	Patients positive/total number (%)
Acute pulmonary	9/206 (6.25)
Chronic pulmonary	17/206 (11.8)

Results - Nested PCR results in lung infected patient



269 bp

Lane 1: Marker 100bp

Lane 2-11: clinical samples

Lane 12: Negative control

Lane 13: positive control

Discussion

- Seroprevalence of histoplasmosis among lung infection patients was corresponded with other studies done in Asian countries (4-15%)

  Cross react with other fungus (*Cryptococcus neoformans and Penicillium marneffei)*

- Culture: gold standard

  Time consuming (2-4 weeks) and inappropriate for patients having progressively disseminated histoplasmosis

- The PCR is able to detect the *H. capsulatum* M antigen in serum and parafilm embedded tissue

  Potential powerful tool for diagnosis of histoplasmosis in the clinical lab

Conclusions

- Perform diagnostic tools to the hospitals
- Continue to detect *Histoplasma* from both clinical and environmental samples in order to clearly understand the route of transmission
- Carry out the epidemiological studies on fungal disease from medical facilities in Vietnam

not only histoplasmosis but also target large range:

Candidiasis, Cryptococcosis, Pneumocytis pneumonia…

Conclusions

- isolates indicate that patients were infected with *H. capsulatum*
- Important to update on histoplasmosis infection and the manner of exposure to fungi
- Emphasize molecular characterization of infected cases located in the area
- People residing or visiting the endemic area should be aware of the health risks of histoplamosis infection
- health care personel must exersice the necessary preventive measures.

Acknowledgement

- This research is supported by
  - the grant-in-aid of Ministry of Health, Labor and Welfare, the Government of Japan (H23-Shinkou-shitei-020)
  - the Research Program on Emerging and Re-emerging Infectious Diseases from Japan Agency for Medical Research and development, AMED

Thank you for your attention