MMTN
MEDICAL MYCOLOGY TRAINING NETWORK

Mimic fungal infections in Asia

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ASIA FUNGAL WORKING GROUP an ISHAM working group
ISHAM

Taipei, Taiwan
Nov 16, 2018, 11:45 am.

Outline:

- Introduction
- Clinical manifestation – human & animals
- Diagnosis
- Treatment
- Epidemiology
- Message

Rare Fungal infections in Asia

- **Yeast**
  - *Fereydounia khargensis*
  - *Pichia anomala*
  - *Kodamaea ohmeri*
  - *Trichosporon inkin*
  - *T. mucoides*
  - *Rhodotorula mucilaginosa*
  - *Saccharomyces cerevisiae*
  - *Blastoschizomyces capitatus*
- **Septate mould**
  - *Alternaria* spp.
  - *A. alternate*
  - *A. malorum*
  - *Chaetomium globosum*
  - *Exserohilum* spp.
  - *Paecilomyces formosus*
  - *Pyrenochaeta romeroi*
  - *Scedosporium apiospermum*
  - *S. prolificans*
- **Non-septate mould**
  - *Conidiobolus coronatus*
  - *Cunninghamella bertholletiae*
  - *Rhizomucor* spp.
  - *Saksenaea erythrospora*
- **Dimorphic fungi**
  - *Emergomyces*
- **Fungus-like microbes**
  - *Lagenidium albertoi*
  - *Prototheca wickerhamii*
  - *Rhinosporidium seeberi*
  - *Pythium insidiosum*

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Cause of emergence

- Fungi adapting higher temperature and acquire virulence factors
- Advancement of medical devices and management
- Broad-spectrum and steroid use
- International travel and natural disasters

Challenges

- Epidemiology not well understood with regard to environmental reservoirs, modes of transmission, & ways to detect them
- Their relative rarity, laboratory diagnosis of these potential pathogens is challenging..
- Specific identification requires expertise
- Antifungal susceptibility testing challenging because reliable methodology & antifungal breakpoints not available.
- Quality-assured diagnosis requires reference laboratories.
- Reference laboratory facilities are not available in majority of Asian countries.

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Rare yeast infections in Asian countries with misdiagnosis issues

Organisms	Specimens (n)	Disease spectrum (n)	Underlying conditions/History (n)	Microbiological Laboratory Diagnosis	Misdiagnosed by common identification methods
Fereydounia khargensis	Blood (1), pleural fluid (1)	Bloodstream infection (1), respiratory track (1)	Low CD4 count (1), complicated medical conditions, DM and hepatitis B (1)	Macro: dry, slightly wrinkled and fringed margins colony at 48h on SDA then turn darker after 72-120h Micro: vegetative cells W or W/O blastospores produced by polar budding on short stalks. Pseudohyphae is occasionally observed.	*F. khargensis* can be misdiagnosed by API 20C (*Cr. neoformans*) and VITEK 2 (*Cr. laurentii*)

***F. khargensis* can be misdiagnosed by**
- **API 20C → *Cr. neoformans***
- **VITEK 2 → *Cr. laurentii***

Organisms	The MICs values (µg/ml)*							
	Azoles			Polyenes		Echinocandins		
	FLC	VOR	POS	ITR	AMB	CAS	MIC	ANI
Fereydounia khargensis	2-8	0.03-2	ND	0.09-0.125	>32	4->32	ND	>32

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Fungus-like infections in Asian countries with less effective of antifungal treatment

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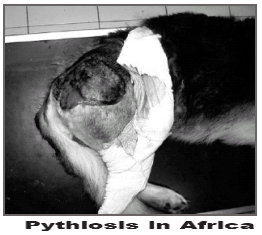
Infected Animals

Vet Pathol 41:412–415 (2004)

Vet Micro. 2005

Fig. 1. GOAT: Extensive and ulcerative cutaneous lesion in the metatarsal-phalangeal region of the left hind limb

J Comp Pathol 2015 ; 152 : 103-5.



Pythiosis in Africa
EID Vol. 11, No. 3, 2005

Courtesy from Dr. Leonel Mendoza, MSU

Equine, Bovine, Canine, Beef Calve, Tiger, Sheep, Goat, Bird

The First Human Case

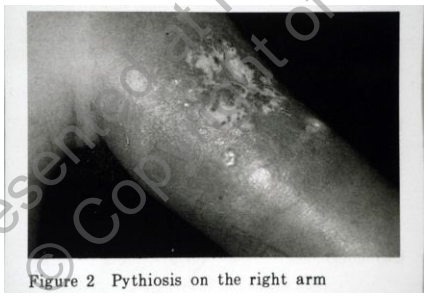


Figure 2 Pythiosis on the right arm

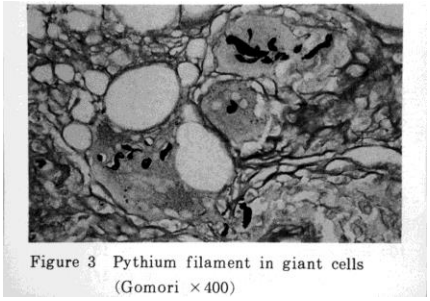


Figure 3 Pythium filament in giant cells (Gomori ×400)

Courtesy from Sathapatayavongs B., Mahidol U.

- Prof. M. Thianprasit, 1985
- Thai male
- Painful subcutaneous granulomatous infiltrative lumps with ulcer
- Treated SSKI

Type of Infection in Animal & Human

Type	Animals/Human	Note
(Sub) cutaneous	Equine (most) Dog, Cat, Cattle, Sheep, Bird, Bear, Camel / 5%	Tissue containing eosinophil and broad non-setate hyphae
Vascular type	59%	Abnormality of RBC
Ocular type	33%	No underlying disease
Disseminated pythiosis, GI	Equine (rarely for GI) Dog (most), Bear, Bengal tiger (GI), Jaguar tiger (Pulmonary) / 3%	Vomiting, weight loss, diarrhea, some mass in abdomen

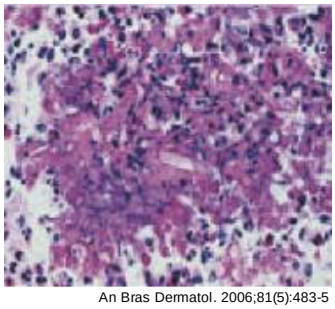
Modified from Krajaejun T et al. CID 2006;43:569-76, Wim Gaastra *et al.*, 2010

Localized Type: Cutaneous / Subcutaneous Pythiosis

1st human subcutaneous case in Thailand (Thianprasit,1985)
1st human subcu. in Brazil (Marques, SA. et al. 2006)

Abstract: Pythiosis is caused by an aquatic fungus-like organism, *Pythium insidiosum*, pathogenic to men and animals. A patient with a phagedenic ulcer on the leg is reported. Histopathological examination was suggestive of zygomycosis, response to antifungal drugs was poor and cure was obtained by means of wide surgical excision. Etiologic diagnosis was confirmed by molecular amplification and DNA sequencing of colonies isolated in Sabouraud agar. After BLAST analysis, the sequence showed 100% identity with those of P. insidiosum deposited on the GenBank.

FIGURE 1: Phagedenic ulcer (A) with grossly granulated bed, purulent secretion, located on the right pre-tibial region; (B) after treatment with itraconazol, partial resolution of the ulcerated ulcer, nodules and nodosities on the proximal region; (C) six months after excision and grafting, displaying apparent cure



An Bras Dermatol. 2006;81(5):483-5

- Onset 1-3 months
- Present as lump/ulcer
- H&E: rare septate hyphae
- Might be misdiagnosed as zygomycotic infection
- Suppurative granulomatous inflammation

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Cutaneous pythiosis in a goat.
do Carmo PM[1] et al. J Comp Pathol 2015: 103-5

Subcu. Pythiosis vs Zygomycosis

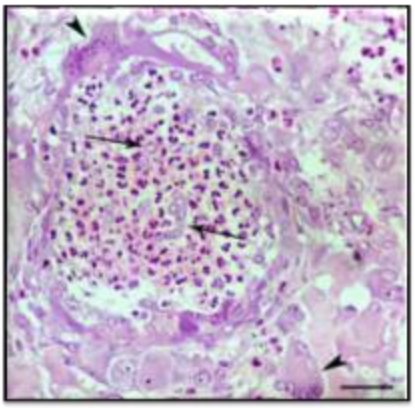
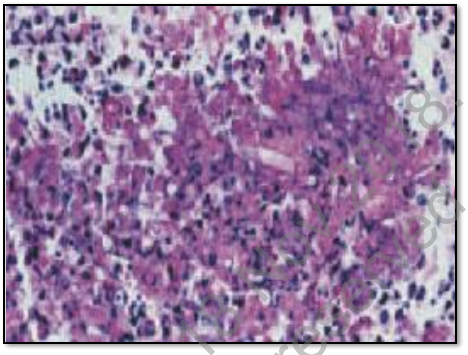


Fig. 2. Section of the lesion showing a central area with neutrophils, eosinophils and hyphae (arrows), surrounded by a granulomatous exudate with some multinucleate giant cells (arrowheads). HE. Bar, 50 mm.

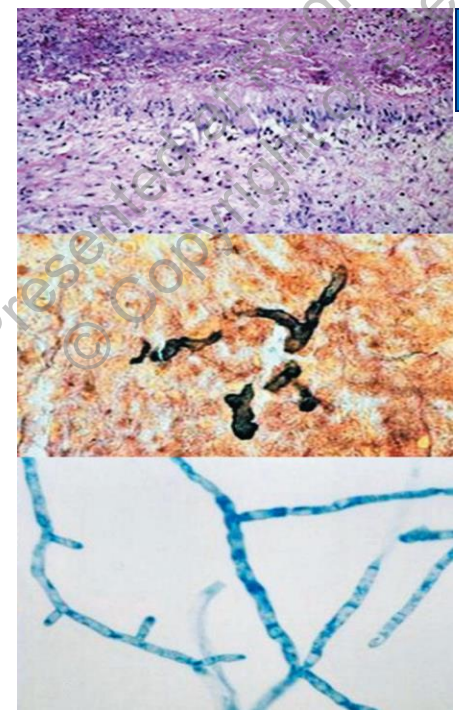
Splendore-Hoeppli – phenomenon (H&E)

Human Pythiosis, Brazil



EID. V.11 (5), 715-8, 2005

J Comp Pathol. 2015 Feb-Apr;152(2-3):103-5. doi: 10.1016/j.jcpa.2014.11.005. Epub 2014 Dec 31



Pathological finding:

Cutaneous/ Subcutaneous infection

Granuloma with palisade-like Arrangement (H&E 200x)

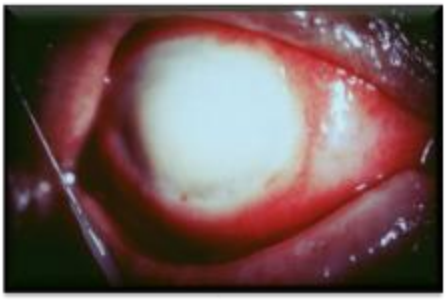
Wide, distorted, rare septate hyphae

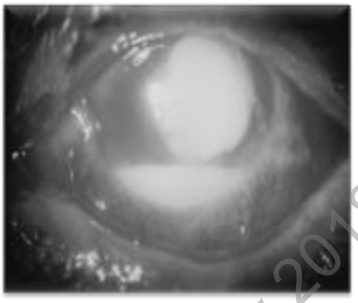
Non-septated hyaline filaments, in PDA agar (cotton blue 40x)

Marques S el al, An Bras Dermatol 2006;81

Clinical manifestation in Human

Localized type: Cornea



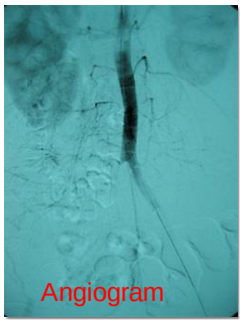


- Immunocompetent
- Corneal ulcer
- Hypopyon
- Endomophthalmitis
- Corneal perforation

Clinical manifestation in Human

Vascular type

J Infect Dis 1989; 159:274



Pupaibun P. et al., 20

Cerebral Pythiosis: A Case Report of *Pythium insidiosum* Infection Presented with Brain Abscess

ABSTRACT

Pythium insidiosum, a fungus-like organism, infects both animals and humans, causing a life-threatening infectious condition called pythiosis. Human pythiosis is an uncommon condition, but an emerging disease in the tropical regions of the world. It can be both localized and systemic forms, involving vascular structures. Most patients with arterial pythiosis have underlying hemoglobinopathy, i.e. thalassemia. We here reported a thalassemic patient suffering from cerebral pythiosis (left common carotid pythiosis arterial aneurysms, septic embolisms evolving to brain abscess over the left anterior cerebral artery territory). The prognosis of cerebral pythiosis is very poor because this condition is rapidly progressive in nature, no effective antibiotics, and requires an adequate surgical debridement to control infection. (*J Infect Dis Antimicrob Agents 2011;28:129-32.*)

Note: This case had been presented and discussed in the Interhospital Case Conference on Infectious Diseases (ICCID), 23 December 2010, Bangkok, Thailand.

- 27 yr Thai male, w orker in Forestry department
- beta-thalassemia haemoglobin E
- 3 mo. before: toothache – left upper molars, nasal congestion w ith w hitish discharge from left nostril
- 3 w k prior adm, high grade fever
- 1 w k later, occipital headache & funny feelings , focal seizure lasting for a min. & 2nd generalized tonic-conic seisure for a min.
- Abt treatment, referred to another university hospital
- 39-41C fever, drow siness, significant left carotid bruit, rt. facial palsy & spastic rt. Hemiparesis
- Initial diag: maxillary sinus --MRI ©
- Pythium antibody, culture isolation and PCR : ***Pythium insidiosum***

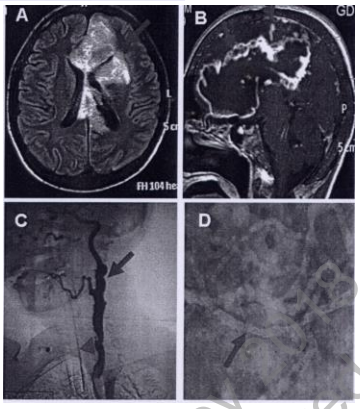


Figure 1. Showing MRI, left carotid angiography and Pus Wright stain. 1A: Axial FLAIR MRI brain demonstrates hypersignal intensity lesion over the left frontoparietal region (arrow) and extensively involved corpus collosum (arrow head). 1B: Saggital T1W MRI brain with gadolinium enhancement reveals a large irregularly rim enhancing lesion (arrow) over the left cerebral hemisphere with satellite surrounding small lesions (arrow head). 1C: Left carotid angiography shows multiple aneurysms (arrow) and a dissection (arrow head) over the left common and proximal internal carotid arteries. 1D: Pus Wright's stain highlights multiple thin-walled hyphae with infrequently septate and branching (arrow).

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Trakulhun T. et al., J Infectious Diseases and Antimicrobial Agents. V28:2, MayAug2011

Laboratory Diagnosis

Collecting specimens
- Clinical manifestation

Transportation:
- RT, not on ice, asap.

Serological approach:
Antibody detection
- Immunodiffusion
- Immunoperoxidase
- Lateral flow
- Hemaglutination test
- FA
- ELISA
- Western Blot

Conventional approach
- **Direct examination**
  - KOH-preparation
  - GMS stain
  - H&E, PAS
  - Calcoflour
- **Cultivation**
  - Temperature
  - Moisture
- Media : any
- Identification:
  - Zoospore production
  - Molec. identification

Molecular approach
- **PCR**
  (Diagnosis & Identification)

How to due with suspected Vascular type??

Host :

- symptom limb: gangrene
- failure from antifungal treatment
- location: tropical / subtropical
- history & occupation
  : swampy / agricultural area
- abnormal red blood cells: thalassemia, PNH

Physician : Angiogram, CTA - aneurysm

Serology : antibody

Specimens & Diag. :
serum : antibody testing
artery, thrombus :
KOH, staining, culture

~~**Skin, muscle**~~

Conventional approach

KOH-preparation: rare septate hyphae

KOH-Calcofluor preparation

Histopathology: GMS

Culture on common media: SDA, PDA, Blood agar:
1-2 days growth

&

Preliminary screening by induction of Zoospores

Treatment

- NO standard treatment
- Amphotericin B, Itraconazole & terbinafine
- SSKI
- Surgery
- Immunotherapy with *P. insidiosum* antigen (PIA)
  - 50-60% successful rate in human

(Wanachiwanawin W. et al., 2004, Permpalung N et. al., 2015)

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Efficacy of immunotherapy using antigens of *Pythium insidiosum* in the treatment of vascular pythiosis in humans

Wanchai Wanachiwanawin [a,*], Leonel Mendoza [b], Sanan Visuthisakchai [a], Piroon Mutsikapan [c], Boonmee Sathapatayavongs [d], Angkana Chaiprasert [a], Parvinee Suwanagool [a], Worapong Manuskiatti [a], Chanian Ruangsetakit [a], Libero Ajello [e]

Patient	Sex/age (years)	Underlying disease	Extent of arteritis and thrombosis	Diagnosis of pythiosis	Duration of infection	Previous treatment	
						Treatment	Result
1 KS	M/16	Aplastic anemia/PNH syndrome	Right poptiteal, femoral and iliac arteries	Culture and ID[a]	2 months	Itraconazole, anticoagulation, transfemoral embolectomy, AK amputation	Evidence of arteritis and thrombosis above the amputated level.
2 LP	M/72	Hb H disease	Left popliteal, superficial femoral arteries	Culture and histopathology	3 months	Anticoagulation, popliteal embolectomy, BK and AK amputations	Presence of arterities and *Pythium* at AK amputated level
3 KC	F/38	β-Thal/Hb E	Left femoral and iliac arteries	Culture and histopathology	6 months	Anticoagulation, aortofemoral by pass	Reocclusion, *Pythium* identified from graft.
4 BJ	M/40	Aplastic anemia/PNH syndrome	Right tibial and popliteal arteries	Culture and ID	2 weeks	SSKI	PIA was started within 4 days after admission
5 DD	M/60	α-Thal 1/Hb E heterozygote	Right common femoral artery	Histopathology	3 months	Thromboembolectomy at superficial femoral artery	Persistent ischemia with gangrenous ulcer at right leg
6 HB	M/29	Hb H disease	Left popliteal artery	Histopathology	6 months	AK amputation, dissection of the proximal part of the diseased superficial femoral artery	Further occlusion to the left superficial femoral artery, presence of fungal elements at the most proximal part
7 AT	M/26	β-Thal/Hb E postsplenectomy	Right popliteal and anterior tibial arteries (with aneurysmal formation)	Histopathology	4 months	Aneurysmectomy and saphenous vein graft interposition	Persistence of ischemic pain
8 SK	F/26	AE Bart's disease	Left superficial femoral artery	Histopathology and culture	2 months	BK amputation and resection of proximal part of the occluded artery	Well-healed BK stump, but presence of organisms above the stump

*ID represents immuno-diffusion test; PNH: paroxysmal nocturnal hemoglobinuria; Hb H disease: hemoglobin H disease; β-thal/HbE: β-thalassemia/hemoglobin E disease; AK: above knee; BK: below knee; SSKI: saturated solution of potassium iodide.

Treatment outcome of surgery, antifungal therapy and immunotherapy in ocular & vascular human pythiosis: a retrospective study of 18 patients

Nitipong Permpalung[1,2†], Navaporn Worasilchai[1†], Rongpong Plongla[1,3], Sikarin Upala[2,4], Anawin Sanguankeo[2,4], Leilani Paitoonpong[1,3], Leonel Mendoza[5] and Ariya Chindamporn[1*]

JAC 2015, Jan 27.

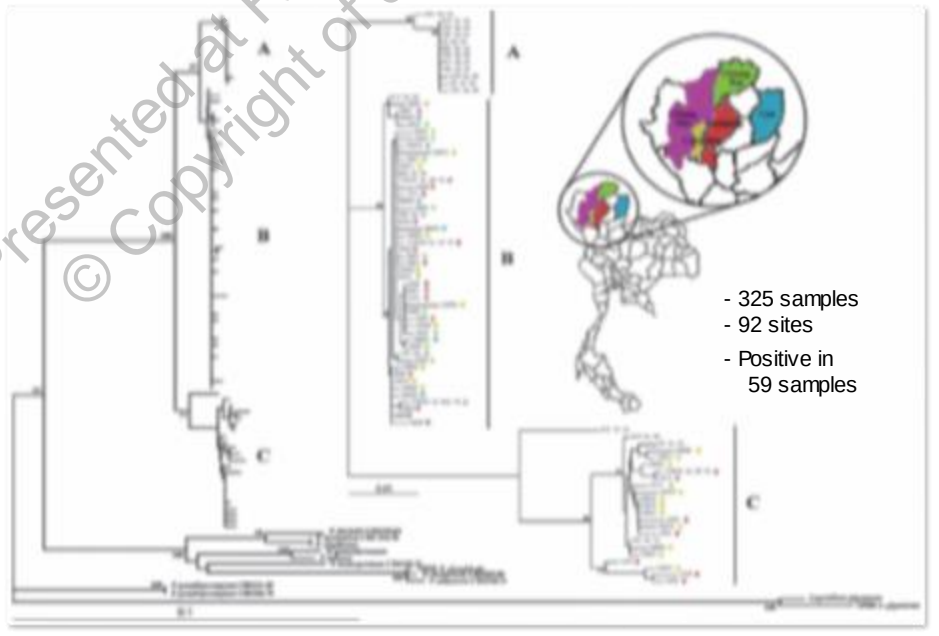
Table 1. Characteristics of patients with vascular pythiosis

Case (sex/age, years)	Underlying disease	Ferritin (ng/mL)	Lesions	Treatment: surgery (time to surgery, months)	Treatment: free margin	Treatment: drugs (duration, months)	Treatment: PIA vaccine schedule	Treatment: PIA type	Result
V1 (M/26)	β-thalassaemia post-splenectomy	1910	right external iliac artery	right AK amputation (2)	no	ITC (5) TRB (5)	D 0, 2500 μL id D 15, 200 μL sc D 30, 200 μL sc D 44, 200 μL sc	PIA-1	died
V2 (M/46)	β-thalassaemia post-splenectomy	NA	bilateral common iliac artery	left AK amputation (4)	no	ITC (1) TRB (1)	D 0, 1 mL sc D 7, 1 mL sc D 14, 1 mL sc	PIA-2	died
V3 (M/41)	haemoglobin H disease	1971	right external iliac artery	right hip disarticulation (5)	no	ITC (1) TRB (1)	D 0, 1 mL sc D 8, 1 mL sc D 14, 1 mL sc D 25, 1 mL sc	PIA-2	died
V4 (M/39)	haemoglobin E disease	221	right common iliac artery	right hemipelvectomy (48)	no	ITC (1) TRB (1)	D 0, 1 mL sc	PIA-1	died
V5 (M/44)	β-thalassaemia	2823	right dorsalis pedis	debridement (7)	yes	ITC (24) TRB (24)	D 0, 100 μL sc D 14, 100 μL sc D 28, 100 μL sc D 42, 100 μL sc D 56, 100 μL sc	PIA-1	survived
V6 (M/38)	β-thalassaemia post-splenectomy	4419	left superficial and deep femoral artery	left hip disarticulation (6)	yes	ITC (12) TRB (12)	D 0, 100 μL id D 14, 100 μL sc D 21, 100 μL sc D 29, 100 μL sc D 43, 100 μL sc	PIA-1	survived
V7 (M/49)	β-thalassaemia haemoglobin E disease	3947	left posterior tibial artery	left AK amputation (2)	yes	ITC (12) TRB (12)	D 0, 1 mL sc D 7, 1 mL sc D 14, 1 mL sc D 28, 1 mL sc D 42, 1 mL sc D 56, 1 mL sc	PIA-1	survived
V8 (M/31)	β-thalassaemia haemoglobin E disease	3097	left femoral artery	left AK amputation (2)	yes	ITC (12) TRB (12)	D 0, 1 mL sc D 7, 1 mL sc D 14, 1 mL sc D 28, 1 mL sc D 35, 1 mL sc D 42, 1 mL sc D 49, 1 mL sc D 56, 1 mL sc	PIA-2	survived
V9 (M/29)	β-thalassaemia haemoglobin E disease	1929	right superficial femoral artery	right hip disarticulation (7)	yes	ITC (12) TRB (12)	D 0, 100 μL sc D 8, 100 μL sc D 14, 100 μL sc D 113, 100 μL sc D 205, 100 μL sc	PIA-1	survived

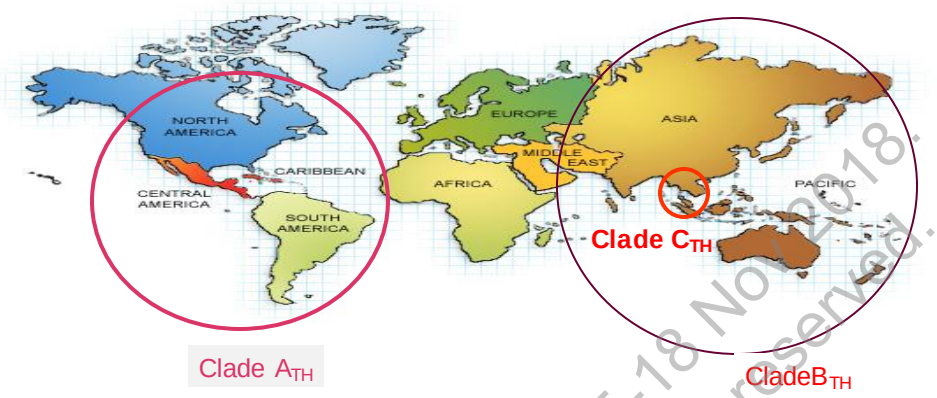
AK, above knee; D, day; id, intradermally; ITC, itraconazole; M, male; NA, not available; TRB, terbinafine; sc, subcutaneously.

Environmental isolated *P. insidiosum:* Northern part, Thailand

Medical Mycology February 2008, 46, 41–52



Phylogenetic distribution of *P.insidiosum* isolated from different geographic regions based on ITS

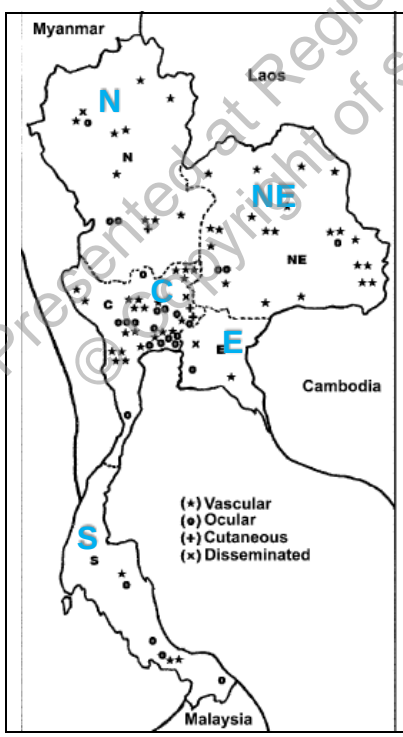


Clade A_{TH}

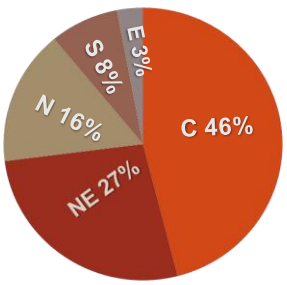
CladeB_{TH}

Ref: http://cdn.gotoknow.org

Schurko *et al*. 2003. *Mycol Res*, Kammarnjessadakul *et al*. *Med Mycol*. 2010,49:289-295



Clinical and Epidemiological Analyses of Human Pythiosis in Thailand

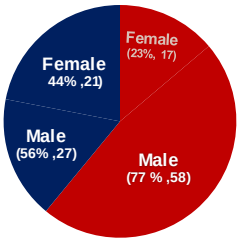


- Jan.1985-June 2003; 102 cases/18 yr
- 9 tertiary care hospitals
- 1-13 case (Mean 5.5 case/yr.)
- Age 20-60 year
- Male : Female 2.4:1
- Agricultural occupation 75%
- Type of infection: Localized, Systemic

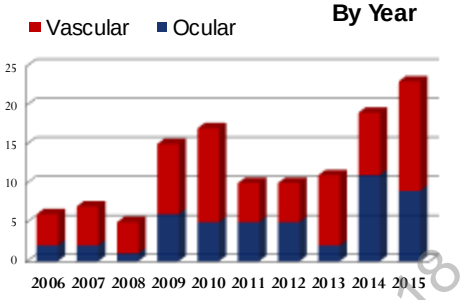
Human Pythiosis in Thailand • CID 2006:43 (1 September)

Yr. 2006-15: KCMH (N =123) - Vas. (75); Ocular(48)

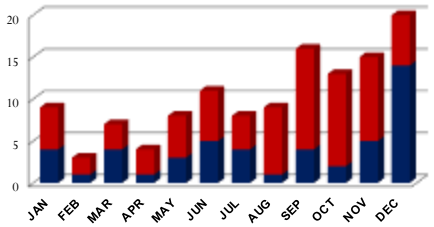
By gender



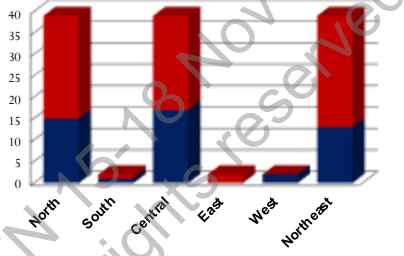
By Year



By month



By Hometown



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MYCOLOGY

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Monitoring Anti-*Pythium insidiosum* IgG Antibodies and (1→3)-β-D-Glucan in Vascular Pythiosis

Navaporn Worasilchai,[a] Nitipong Permpalung,[b,c] Pakawat Chongsathidkiet,[d] Asada Leelahavanichkul,[e,f] Alberto Leonel Mendoza,[g] Tanapat Palaga,[h] Rangsima Reantragoon,[e] Malcolm Finkelman,[i] Pranee Sutcharitchan,[j,k] Ariya Chindamporn[l]

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Factors relates to survival rate

Criteria of 1 year survival	Survival group (45)	Deceased group (5)	P-value*
Patient related parameters			
Age (years)	33.6 ± 10.7	49.2 ± 17.2	0.006
Male gender	25 (56%)	4 (80%)	0.29
Occupation			0.39
- Agriculture related	39 (86.7%)	5 (100%)	
- Non-agriculture related	6 (13.3%)	-	
History of water exposure within 3 months			0.49
- Exposed water	41 (91.1%)	5 (100%)	
- Non exposed water	4 (8.9%)	-	
Underlying disease			0.87
- α-thalassemia	3 (6.7%)	-	
- β-thalassemia	3 (6.7%)	-	
- β-thalassemia Hemoglobin E disease	32 (71.1%)	4 (80%)	
- H-constant spring	2 (4.4%)	-	
- Hemoglobin H disease	5 (11.1%)	1 (20%)	
Serum ferritin (ng/ml)	1,388.40± 653	1,676 ± 402.4	0.34
Duration from disease onset to 1st medical attention (mo.)	1.9 ± 0.7	4.4 ± 0.7	<0.0001
Duration from diagnosis to first definitive surgery (mo.)	0.6 ± 0.2	1.5 ± 1.2	<0.0001

* By chi-square or Fisher's exact test

Age

Duration time (disease onset to surgery)

Factors relates to survival rate

Criteria of 1 year survived	Survival group (45)	Deceased group (5)	P-value
Disease and treatment related parameters			
Lesions			0.05
- Brachial artery	1 (2.2%)	-	
- Radial artery	11 (24.4%)	-	
- Ulnar artery	1 (2.2%)	-	
- Femoral artery	17 (37.8%)	4 (80%)	
- Anterior tibial artery	9 (20%)	-	
- Posterior tibial artery	3 (6.7%)	-	
- Iliac artery	3 (6.7%)	-	
- External carotid artery	-	1 (20%)	
Surgery			<0.0001
- Amputation	45 (100%)	2 (40%)	
- Debridement		3 (60%)	
Antifungal agent			0.17
- Itraconazole alone	5 (11.1%)	2 (40%)	
- Itraconazole + terbinafine	34 (75.6%)	3 (60%)	
- SSKI + terbinafine	6 (13.3%)	-	
Duration of antifungal treatment (months)	5.9 ± 4.6	1.4 ± 0.9	0.04
Iron chelation drug	45 (100%)	5 (100%)	-

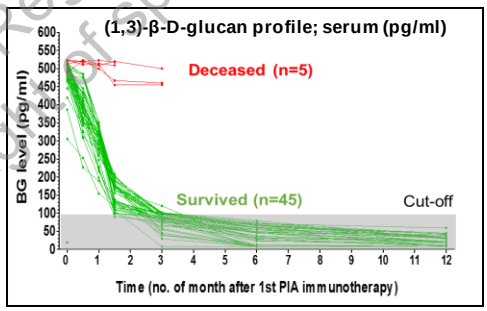
Type of Surgery

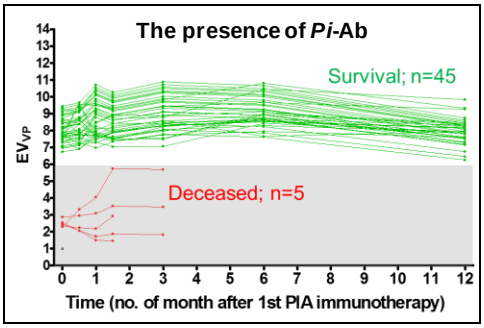
Factors relates to survival rate

Criteria of 1 year survived	Survival group (45)	Deceased group (5)	*P-value*
Clinical sign/symptoms post treatment initiation			
- Fever > 38.2°C	-	3 (60%)	0.008
- Arterial insufficiency syndrome (claudication, paresthesia, gangrenous ulceration)	-	2 (40%)	0.008
- Mass at surgical sites (arterial aneurysm)	-	1 (20%)	0.1
- New skin lesions	-	-	-
- Inflammation/infection at surgical sites	-	3 (60%)	0.008

Clinical symptoms post treatment initiation

Biomarker





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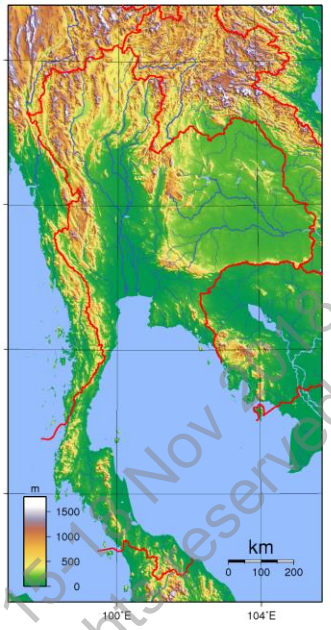

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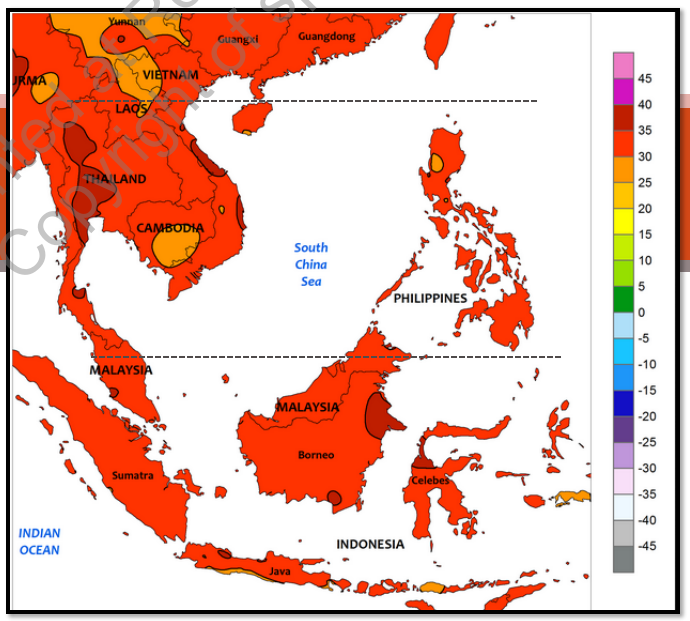

National Oceanic and Atmospheric Administration

Ref: Climate prediction center, NOAA

- King Chulalongkorn Memorial Hospital (Thai Redcross Hospital)

- Faculty of Medicine, Chulalongkorn University

Our team +
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Thank you for your attention