Clinical and Epidemiological Analyses of Human Pythiosis in Thailand


Departments of 1Pathology and 2Medicine, Faculty of Medicine, Ramathibodi Hospital, Departments of 3Medicine, 4Microbiology, and 5Otolaryngology, and 6Clinical Pathology, Faculty of Medicine, Siriraj Hospital, Mahidol University, 7Department of Medicine, Faculty of Medicine, Chulalongkorn University, and 8Surgery Unit, Lerdsin Hospital, Bangkok, 9Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Departments of 10Medicine and 11Pathology, Faculty of Medicine, Prince of Songkla University, Songkhla, Departments of 12Medicine and 13Ophthalmology, Faculty of Medicine, Chiang Mai University, Chiang Mai, 14Surgery Unit, Sapasittiprasong Hospital, Ubonrachathani, and 15Ophthalmology Unit, Metapracharak Hospital, Nakornpatom, Thailand

Background. Pythiosis is an emerging and life-threatening infectious disease in humans and animals that is caused by the pathogenic oomycete Pythium insidiosum. Human pythiosis is found mostly in Thailand, although disease in animals has been increasingly reported worldwide. Clinical information on human pythiosis is limited, and health care professionals are unfamiliar with the disease, leading to underdiagnosis, delayed treatment, and poor prognosis.

Methods. To retrospectively study the clinical and epidemiological features of human pythiosis, we analyzed clinical data from patients with pythiosis diagnosed during the period of January 1985 through June 2003 at 9 tertiary care hospitals throughout Thailand.

Results. A total of 102 cases of human pythiosis were documented nationwide. A substantial proportion (40%) of cases occurred in the last 4 years of the 18-year study interval. Clinical presentations fell into 4 groups: cutaneous/subcutaneous cases (5% of cases), vascular cases (59%), ocular cases (33%), and disseminated cases (3%). Almost all patients with cutaneous/subcutaneous, vascular, and disseminated pythiosis (85%) had underlying thalassemia-hemoglobinopathy syndrome. Most ocular cases (84%) were associated with no underlying disease. A majority of the patients were male (71%), were aged 20–60 years (86%), and reported an agricultural occupation (75%). Regarding treatment outcomes, all patients with disseminated infection died; 78% of patients with vascular disease required limb amputation, and 40% of these patients died; and 79% of patients with ocular pythiosis required enucleation/evisceration.

Conclusions. Here, we report, to our knowledge, the largest case study of human pythiosis. The disease has high rates of morbidity and mortality. Early diagnosis and effective treatment are urgently needed to improve clinical outcomes. Because P. insidiosum is distributed worldwide and can infect healthy individuals, an awareness of human pythiosis should be promoted in Thailand and in other countries.

Pythiosis is an emerging, life-threatening infectious disease caused by the oomycete Pythium insidiosum, which is the only Pythium species of the kingdom Chromista (Stramenopila) known to infect humans and animals in tropical, subtropical, and temperate countries [1–4]. P. insidiosum presents in 2 forms: perpendicular branching hyphae and biflagellate zoospore [5]. The zoospore swims to attach and invade host tissue [6]. Phylogenetic analysis has shown that Pythium species are more closely related to diatoms and algae than to true fungi [7].

Human pythiosis was first documented in 1985 [5, 8]. Since then, several human cases have been reported, and the disease is marked by high rates of morbidity and mortality [9–24]. Many health care professionals...
are unfamiliar with this devastating disease because clinical information about pythiosis and diagnostic tools are limited, leading to underrecognition and underdiagnosis of the disease, delayed treatment, and a poor prognosis for patients with pythiosis. Nevertheless, many new cases of human pythiosis have been diagnosed in 9 Thai tertiary care hospitals. We undertook this study to characterize the clinical and epidemiological features of human pythiosis in Thailand, by analyzing clinical data from patients with pythiosis that had been diagnosed at these hospitals.

**METHODS**

This retrospective study was performed by a collaboration of clinicians, pathologists, and microbiologists from 9 tertiary care hospitals located across Thailand (table 1). To identify cases, we screened microbiological and pathological records at these health care centers for the period from January 1985 through June 2003. To be included as a case, ≥ 1 of the following criteria had to be present: isolation of *P. insidiosum* from infected tissue, confirmed by induction and identification of zoospores [25, 26]; presence of anti-*P. insidiosum* antibodies in blood samples obtained from patients, as detected by in-house serodiagnostic tests (immunodiffusion or ELISA [27–29]); and demonstration of the typical clinical and pathological features of vascular pythiosis, as described elsewhere [9, 11, 14, 22]. These diagnostic clinical features are illustrated in figure 1. In brief, the features are (1) clinical presentation of arterial insufficiency or gangrenous ulcer due to the occlusion of an infected artery of the lower extremities; (2) hyphae that are uniform in size and

<table>
<thead>
<tr>
<th>Study variable</th>
<th>Vascular</th>
<th>Disseminated</th>
<th>Ocular</th>
<th>Cutaneous/subcutaneous</th>
<th>All forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%) of patients</td>
<td>60 (69)</td>
<td>3 (3)</td>
<td>34 (33)</td>
<td>5 (5)</td>
<td>102 (100)</td>
</tr>
<tr>
<td>Ratio of male to female patients</td>
<td>2.4:1</td>
<td>2:1</td>
<td>2:1</td>
<td>...</td>
<td>2.4:1</td>
</tr>
<tr>
<td>Age, mean years (range)</td>
<td>38 (14–71)</td>
<td>28 (12–44)</td>
<td>44 (20–78)</td>
<td>27 (15–40)</td>
<td>39 (12–78)</td>
</tr>
<tr>
<td>Occupation, n/N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agriculture</td>
<td>41/50 (82)</td>
<td>0/3 (0)</td>
<td>14/20 (70)</td>
<td>2/3 (67)</td>
<td>57/76 (75)</td>
</tr>
<tr>
<td>Nonagriculture</td>
<td>9/50 (18)</td>
<td>3/3 (100)</td>
<td>6/20 (30)</td>
<td>1/3 (33)</td>
<td>19/76 (25)</td>
</tr>
<tr>
<td>Principal diagnostic method, no. of patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture identification</td>
<td>26</td>
<td>2</td>
<td>34</td>
<td>5</td>
<td>67</td>
</tr>
<tr>
<td>Serodiagnosis</td>
<td>13</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>14</td>
</tr>
<tr>
<td>Clinicopathological features</td>
<td>21</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>3</td>
<td>34</td>
<td>5</td>
<td>102</td>
</tr>
<tr>
<td>Duration of symptoms, mean days (range)</td>
<td>91 (7–365)</td>
<td>11 (1–21)</td>
<td>17 (1–60)</td>
<td>41 (4–90)</td>
<td>66 (1–365)</td>
</tr>
<tr>
<td>Underlying disease, n/N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematological disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalassemia syndrome</td>
<td>53/59 (90)</td>
<td>1/3 (33)</td>
<td>2/19 (11)</td>
<td>3/5 (60)</td>
<td>59/86 (69)</td>
</tr>
<tr>
<td>Nonthalassemia</td>
<td>6/59 (10)</td>
<td>2/3 (67)</td>
<td>0/19 (0)</td>
<td>2/5 (40)</td>
<td>10/86 (11)</td>
</tr>
<tr>
<td>Nonhematological disease</td>
<td>0/59 (0)</td>
<td>0/3 (0)</td>
<td>1/19 (5)</td>
<td>0/6 (0)</td>
<td>1/86 (1)</td>
</tr>
<tr>
<td>No underlying disease</td>
<td>0/59 (0)</td>
<td>0/3 (0)</td>
<td>16/19 (84)</td>
<td>0/5 (0)</td>
<td>16/86 (19)</td>
</tr>
<tr>
<td>Final treatment outcome, n/N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cured</td>
<td>26/43 (60)</td>
<td>0/3 (0)</td>
<td>19/19 (100)</td>
<td>3/3 (100)</td>
<td>48/68 (71)</td>
</tr>
<tr>
<td>Limb amputated</td>
<td>26/43 (60)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Eye lost</td>
<td>...</td>
<td>...</td>
<td>15/19 (79)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Eye saved</td>
<td>...</td>
<td>...</td>
<td>4/19 (21)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Died</td>
<td>17/43 (40)</td>
<td>3/3 (100)</td>
<td>0/19 (0)</td>
<td>0/3 (0)</td>
<td>20/68 (29)</td>
</tr>
</tbody>
</table>

NOTE. The participating tertiary care departments and hospitals were Faculty of Medicine, Ramathibodi Hospital, and Faculty of Medicine, Siriraj Hospital, Mahidol University (Bangkok); Faculty of Medicine, Khon Kaen University (Khon Kaen); Faculty of Medicine, Prince of Songkla University (Songkla); Faculty of Medicine, Chiang Mai University (Chiang Mai); Faculty of Medicine, Chulalongkorn University (Bangkok); Sapasitprasong Hospital (Ubonratchathani); LerdSin Hospital (Bangkok); and Metapracharak Hospital (Nakornprathom).

a Data were available for each study variable as follows: sex, 102 patients (100%); age, 100 patients (98%); occupation, 76 patients (75%); principal diagnostic method, 102 patients (100%); mean duration of symptoms, 79 patients (77%); underlying disease, 86 patients (84%); and final treatment outcome, 68 patients (87%).

b All patients were male.

c Nonagriculture occupations included domestic laborer, 8 patients; student, 4 patients; car driver, 2 patients; grocery seller, 1 patient; government service agent, 1 patient; and monk, 1 patient. Two patients were unemployed.

d One patient had idiopathic thrombocytopenic purpura and was seropositive for HIV.

e Patient was HIV seropositive.
branching at right angles, as determined by histopathological examination of the infected artery with Gomori methenamine silver staining (but not hematoxylin and eosin staining); and (3) no clinical improvement or worsening of the condition after administration of conventional antifungal chemotherapy.

We collected the following demographic and clinical information from patients: sex, age, place of residence (province), occupation, year of diagnosis, clinical presentation, duration of symptom/sign before seeking medical care, underlying disease, and management and outcome of infection. Because this is a retrospective study, and because clinical information was incomplete for some patients, the percentage and total number of patients with available data are provided for each category (table 1).

RESULTS

Number and Epidemiological Features of Cases

One hundred two cases of human pythiosis, including 43 previously reported cases [9–11, 14, 16, 19–22, 24], were diagnosed during the period from January 1985 through June 2003 (figure 2). A mean of 5.5 cases (range, 1–13 cases) were documented per year. The cases were found nationwide, and the distribution of cases can be regionally divided as follows: central region, 46% of cases; northeastern region, 27%; northern region, 16%; southern region, 8%; and eastern region, 3% (figure 3).

Patients’ ages spanned a broad range (figure 4): 6% of patients were aged 12–19 years, 49% were aged 20–39 years, 37% were aged 40–59 years, and 8% were aged ≥60 years. The number of male patients was 2.4-fold higher than that of female patients. Seventy-five percent of patients reported an agricultural occupation (table 1); most of these patients grew rice, corn, or vegetable crops.

Methods of Diagnosis

A majority (66%) of cases (table 1) were diagnosed by identification of *P. insidiosum* on culture, as described elsewhere [25, 26], with use of such specimens as swab samples of discharge, corneal scrapings, blood clots, and tissue biopsy samples.

In-house, noncommercial serological tests for detection of antibodies to *P. insidiosum* included immunodiffusion and ELISA (available since 1989 and 2002, respectively), which have been shown to be specific for pythiosis [27–29]. They were used to confirm the diagnoses of 14 culture-negative cases in this study. Among 38 patients (28 with vascular pythiosis, 7 with ocular pythiosis, 2 with cutaneous/subcutaneous pythiosis, and 1 with disseminated pythiosis) whose serum samples were available for testing, 33 patients (27 with vascular pythiosis, 3 with ocular pythiosis, 2 with cutaneous/subcutaneous pythiosis, and 1 with disseminated pythiosis) were found to be seropositive for *P. insidiosum* by at least one of the methods. There were 21 patients with vascular cases included in this study who had no culture-based or serological proof of infection, but these patients had the characteristic clinicopathological features of vascular pythiosis, as described elsewhere [9, 11, 14, 22].
Clinical Manifestations of Disease, Management, and Outcome

Clinical presentations were categorized into 4 groups (table 1), as shown below.

Cutaneous/subcutaneous pythiosis. Five patients were determined to have cutaneous/subcutaneous pythiosis, in which infection was confined to cutaneous/subcutaneous tissue. The first 4 patients had similar lesions that were characterized by chronic swelling (duration, 1–3 months) and a painful, subcutaneous, granulomatous, infiltrative lump and ulcer on the arm or leg. Histopathological examination revealed chronic infection with eosinophilia in the dermis and subcutaneous tissue. Treatment with antifungal agents, such as amphotericin B, 5-fluorocytosine, and ketoconazole, was ineffective. These patients had good responses to saturated solution of potassium iodide (SSKI; 1 mL orally 3 times per day for up to 3 months). Three patients were cured of pythiosis, and 1 patient was lost to follow-up. The fifth patient had a different presentation [24]: he was a 15-year-old boy with thalassemia, and he presented with 4-day history of necrotizing cellulitis of both legs, which he experienced shortly after swimming in a river. The findings of an examination of muscles and vascular tissue were unremarkable. His condition improved after surgical debridement and the administration of SSKI, itraconazole, and terbinafine in combination.

Vascular pythiosis. Sixty patients received a diagnosis of vascular pythiosis on the basis of identification of *P. insidiosum* on culture (26 patients), on the basis of serological testing (13 patients), and on the basis of clinicopathological features (21 patients). These infections were confined mainly to arterial tissues. Patients with vascular pythiosis mostly presented with arterial insufficiency syndrome of the lower extremities. Clinical presentations varied from intermittent claudication to gangrenous ulceration. Other symptoms and signs were observed, such as fever, paresthesia, itching, vesicle/bulla, skin ulcer, cellulitis, necrotizing fasciitis, leg swelling, absence of arterial pulse, groin mass (iliac or femoral aneurysm), and abdominal mass (aortic aneurysm). The mean duration of symptoms before seeking medical care was 3 months. Of 59 patients with available data, 53 had thalassemia-hemoglobinopathy syndrome, 4 had paroxysmal nocturnal hemoglobinuria, and 1 patient each had chronic anemia and acute myelogenous leukemia.

Angiographic findings showed an occlusion or dilatation of infected medium-to-large-sized arteries of the lower extremities. In some patients with advanced pythiosis, aortic aneurysm was observed. External and internal carotid arteritis was reported in 1 case [16]. Pathological findings revealed hyphae invading the arterial wall, with eosinophil infiltration, focal supplicative granuloma, and giant cells surrounding the hyphae.
as well as a blood clot containing hyphae in the arterial lumen. No infection in venous tissue was noted. The infection progressed proximally along the arterial wall and caused aneurysm or arterial occlusion from a thrombus or fibrosis. Embolism to the other leg occurred when the bifurcation of aorta was affected, as also reported in detail elsewhere [9]. Patients were usually affected unilaterally. Four patients had bilateral involvement of lower extremities. From data available on 26 patients with vascular pythiosis, 7 underwent splenectomy as treatment for hypersplenism in thalassemia.

Administration of SSKI and conventional antifungal agents (including amphotericin B, ketoconazole, itraconazole, and terbinafine) had no favorable effect on vascular pythiosis. With consideration of the level and severity of affected arteries, infected tissue was removed by one or a combination of the following operations: below-knee amputation, above-knee amputation, resection of infected artery, thromboembolectomy, aneurysmectomy, or hip disarticulation. Limb amputation was performed for at least 47 (78%) of the patients with vascular pythiosis. Nine patients underwent both below-knee and above-knee amputation of the same leg because of progression of infection. The patients with infection in both legs underwent bilateral amputations.

Among 43 patients with vascular pythiosis who had known final outcomes, 40% died, whereas 60% were cured of the disease, but only after undergoing amputation. P. insidiosum vaccine prepared from cytoplasmic and secretory antigens of P. insidiosum [16, 22, 30, 31] was used as immunotherapy for 12 patients with inoperable vascular pythiosis. At least 2 injections of 100–200μL each of antigen, 2 mg/mL, at a 14-day interval were given. After vaccination, 5 cases were cured, 2 patients died, 2 patients had persistent infection, and 3 patients were lost to follow-up. Ruptured aneurysm (especially of the aorta) was a major cause of death. The mean duration of symptoms before patients sought medical care was 2 months (range, 0.5–6 months), whereas the mean duration for patients who died of pythiosis subsequently cured was 2 months (range, 0.5–6 months). Patients who died, 2 patients had persistent infection, and 3 patients were lost to follow-up. Ruptured aneurysm (especially of the aorta) was a major cause of death. The mean duration of symptoms before patients sought medical care for those whose was obliterated was 2 months (range, 0.5–6 months), whereas the mean duration for patients who died of the disease was 4 months (range, 0.25–12 months).

Ocular pythiosis. Thirty-four patients were determined to have ocular pythiosis. Patients usually presented with corneal ulcer or keratitis. Other signs and symptoms included pain, irritation, decreased visual acuity, eyelid swelling, conjunctival injection, corneal infiltrates, and hypopyon. In some cases, a perforated cornea was found at presentation. The mean duration of symptoms before patients sought medical care was 17 days. Shortly before symptoms began, 10 patients had obvious histories of eye trauma, such as a foreign body or corneal abrasion by plant materials. Hyphae were observed microscopically by potassium hydroxide preparation of corneal scrapings or discharges. In all cases, P. insidiosum was successfully cultured from corneal specimens. Of 7 patients with ocular cases whose serum samples were sent elsewhere (Department of Pathology, Ramathibodi Hospital, and Department of Microbiology, Siriraj Hospital, Bangkok) for serological testing, only 3 were found to be seropositive for pythiosis. The causative organism was demonstrated histologically in corneal specimens by staining with Gomori methenamine silver, but not with hematoxylin and eosin staining. In some cases, the infection progressed deeply and caused endophthalmitis. Neither infection beyond the eye nor bilateral eye involvement was found.

Topical and systemic conventional antifungal agents (amphotericin B, ketoconazole, miconazole, and itraconazole) and SSKI were ineffective for ocular pythiosis. Therefore, radical surgery was employed to remove infected tissue. Enucleation or evisceration was performed if keratectomy or keratoplasty failed to control the infection. Of 19 patients with ocular pythiosis whose final outcomes were known, 4 patients (21%) had eyes saved by anterior lamella or penetrating keratoplasty, whereas 15 patients (79%) lost eyes after enucleation or evisceration was necessary to control the infection. The mean duration of symptoms before patients sought medical care was the same (13 days) for patients whose eyes were saved and for those who lost eyes. All patients survived.

Disseminated pythiosis. Three patients were determined to have disseminated pythiosis. The first patient was a 14-year-old boy with hemophilia A and acute lymphoblastic leukemia. He had upper gastrointestinal bleeding, peritonitis, and bloody mucous stools. He underwent gastrectomy and removal of an ischemic ileum. Pathological examination revealed perforation of both stomach and ileum. Clusters of fungal elements were histologically demonstrated in the gastric wall. P. insidiosum was later isolated from an ascitic fluid specimen. The patient had hemodynamic instability and died of sepsis.

The second patient was 26-year-old woman with thalassemia. She presented with a 1-day history of severe left-side headache, without fever or signs of meningeal irritation. MRI revealed a hemorrhagic mass in the frontal brain. Craniotomy was performed as an emergency procedure. P. insidiosum was isolated from a brain tissue sample. The patient died a few days after the operation.

The third patient was a 44-year-old man with paroxysmal nocturnal hemoglobinuria. He presented with headache and left-side ocular pain due to orbital cellulitis and acute rhinosinusitis of 3 weeks’ duration. This case was diagnosed serologically by immunodiffusion. Antifungal agents and the P. insidiosum vaccine were given, and surgical drainage was performed. Unexpectedly, the patient developed pericardial effusion and died of cardiac arrest. Autopsy was not performed.

Underlying Diseases
Eighty-one percent of patients had underlying diseases, 99% of which were hematological disorders (table 1). Strikingly, 86%
of the hematological disorders were cases of thalassemia and included a broad spectrum of genotypes: β-thalassemia/hemoglobin E (in 25 patients), α-thalassemia (in 7 patients), hemoglobin H disease (in 5 patients), homozygous β-thalassemia (in 2 patients), AE Bart disease (in 2 patients), hemoglobin E trait (in 1 patient), and undefined thalassemia (in 17 patients). Other underlying hematological diseases (14%) were paroxysmal nocturnal hemoglobinuria (in 6 patients), anemia of undefined etiology (in 1 patient), idiopathic thrombocytopenic purpura (in 1 patient), acute myelogenous leukemia (in 1 patient), and hemophilia A with acute lymphoblastic leukemia (in 1 patient). One patient each in the cutaneous/subcutaneous pythiosis and ocular pythiosis groups was HIV seropositive. Patients without underlying disease (19%) were found only in the ocular pythiosis group.

DISCUSSION

Patients with pythiosis were found all over Thailand, suggesting that the pathogen is widely distributed throughout the country. Human pythiosis was associated with an agricultural career, an age of 20–60 years, and male sex. Because much of Thailand consists of swamps, which are suitable for the life cycle of P. insidiosum [6], people living in these areas are more likely to contact the pathogen and to acquire infection. Recently, 5 putative cases of human pythiosis were found to have been misdiagnosed as mucormycosis, aspergillosis, and penicillosis [32]. Because the causative organism is found worldwide [1–4], and because health care professionals are often unfamiliar with this emerging disease, it is important to promote awareness of human pythiosis and, perhaps, to undertake active surveillance in other countries as well. Pythiosis with high morbidity and mortality has also been increasingly reported worldwide in pets and domestic animals, such as horses, dogs, cats, cattle, and sheep [1–4].

Understanding the route of entry and pathogenesis of P. insidiosum infection could help to prevent and control the disease. The motile zoospore is the infective unit of P. insidiosum, and it can swim to invade host tissues as germinating hyphae [6]. Human hairs are known to facilitate attachment and encystment of the zoospore [6, 33] and, thus, infection. Contact with P. insidiosum–contaminated sources and/or having broken or unprotected skin in an exposure area could increase the chance of infection. In patients with cutaneous/subcutaneous or ocular pythiosis, direct contact with the pathogen is likely an initial step of these superficial infections. In patients with disseminated pythiosis, the different clinical features may reflect different routes of infection, such as gastrointestinal and rhinocerebral routes. In patients with vascular pythiosis, the lower extremities are often involved, because they tend to be exposure sites of inoculation. Some patients with vascular pythiosis recalled having soft-tissue swelling or irritation that occurred briefly several months before they had arterial insufficiency symptoms, whereas a few patients had severe soft-tissue infection at presentation, and later (within weeks) developed vascular infection at the affected region [16]. These observations suggest that the route of entry could be direct invasion through the skin to a targeted arterial tissue, and that the size of inoculum or strain of the pathogen may affect extension or severity of infection. Some cases of vascular pythiosis with bilateral leg infection could have been due to simultaneous bilateral inoculation.

Healthy individuals are vulnerable to ocular pythiosis, which is not the case for other forms of pythiosis in Thai patients. Most patients lose their sight shortly after infection, usually within a few months, demonstrating the aggressive nature of this disease. As is the case for infections with true fungi [34], farming exposure and direct eye trauma predispose persons to Pythium infection. Some cases of fungal corneal ulcer with unknown etiology may actually be ocular pythiosis, because P. insidiosum has morphology similar to some pathogenic filamentous fungi, such as Aspergillus species [21, 32]. Results of serodiagnostic tests for ocular pythiosis should be interpreted carefully, because false-negative results are common.

Interestingly, all of our patients with cutaneous/subcutaneous, vascular, and disseminated pythiosis had underlying hematological disorders. In contrast, most (84%) of the patients with ocular pythiosis had no such underlying conditions (table 1). From our data, we found that 90% of the patients in the vascular pythiosis group had thalassemia (table 1), suggesting a striking association between vascular pythiosis and thalassemia. In Thailand, it is estimated that 600,000 individuals (1% of total population) have thalassemia [35], which perhaps explains the endemicity of human pythiosis in Thailand. How thalassemia predisposes a patient to pythiosis is unknown. Splenectomy was performed for the minority of patients in our study. Although splenectomy increases the risk of acquiring some types of infections [35, 36], it is unlikely to play a major role in the pathogenesis of pythiosis. Iron overload—a major pathological change in patients with thalassemia—could increase host susceptibility to pythiosis by promoting infectivity of the pathogen or by impairing host immunity [36–38]. However, we found 6 patients with pythiosis who had paroxysmal nocturnal hemoglobinuria and its associated iron deficiency [39]. Thus, iron overload alone may not explain susceptibility to the infection. Thalassemia and paroxysmal nocturnal hemoglobinuria do share the common feature of chronic hemolysis. Additional investigation is required to identify underlying factors that contribute to the host susceptibility to pythiosis.

Conventional antifungal therapy was usually unsuccessful in patients with pythiosis. Pythium species may lack the antifungal-target ergosterol [40]. Nevertheless, 2 Australian patients with subcutaneous pythiosis responded well to amphotericin B treatment with extensive surgical debridement [13], and an
American patient with deeply invasive facial *Pythium* infection was cured after receiving itraconazole and terbinafine, guided by an in vitro susceptibility test [17]. Variation in host factors and *P. insidiosum* strains might have contributed to these different treatment outcomes. Because *Pythium* cell walls are known to harbor the polysaccharide β-glucan [41], new antifungal drugs that inhibit glucan synthesis, such as caspofungin, may be useful for treating patients with pythiosis [42]. In our study, radical surgery remained the main effective treatment for this infection. The disease can relapse if organisms are left behind. Because a grossly normal looking artery at the surgical margin may not indicate an adequate excision, microscopic demonstration of an organism-free surgical margin is needed to ensure a good outcome. Regarding immunotherapy, Mendoza et al. [30, 31] and Dixon et al. [43] reported the efficacy of a *P. insidiosum* vaccine as immunotherapy in equine and canine pythiosis, for which the cure rate was approximately 50%–72%. The same vaccine (provided by Leonel Mendoza, College of Natural Sciences, Medical Technology Program, Michigan State University, East Lansing) was used as a last resort as compassionate therapy for some inoperable human cases reported here. Five of 9 vaccinated patients with known outcomes responded favorably.

In conclusion, pythiosis is associated with high rates of morbidity and mortality. Early diagnosis and treatment improve the prognosis. The disease is not uncommon in Thailand and Southeast Asia. Because serological tests and recently developed PCR and DNA sequence analysis diagnostic tests have become available [18, 27–29, 44], discovery of additional cases of pythiosis would be expected. People with predisposing factors, such as thalassemia and agricultural occupation, should be educated to protect themselves by wearing boots when exposed to swampy areas. Efforts from various disciplines are needed to address remaining questions regarding the pathogenesis of pythiosis, the spectrum of clinical manifestations, and the effectiveness of antimicrobial therapy and immunotherapy for pythiosis.

Acknowledgments

We thank all medical staffs and laboratory technicians who were involved in this study, as well as those who cared for the patients at the 9 centers. We also thank Thomas D. Sullivan and Bruce S. Klein for reviewing this manuscript.

Financial support. Ramathibodi Foundation (to T.K.).

Potential conflicts of interest. All authors: no conflicts.

References

27. Krajaejun T, Kunakorn M, Niemhom S, Chongtrakool P, Prachatkam R. Development and evaluation of an in-house enzyme-linked im-


