

# Do You Know Human Pythiosis?

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## INTRODUCTION

Pythiosis is an emerging, life-threatening infectious disease caused by the fungus-like organism, *Pythium insidiosum*.<sup>1-3</sup> The disease was first described in horses in 1902, and then it has been reported in some other animals such as dogs, cats, and cattle. The common sites of infection in animals are cutaneous/subcutaneous tissues and the gastrointestinal tract. Pythiosis was recognized by several different names such as bursattee, cutaneous habronemiasis, espundia, kunker, granular dermatitis, leeches, swamp cancer, and phycomycosis.<sup>1,3</sup>

Human pythiosis was first reported from Thailand in 1985.<sup>3,4</sup> Since then, several cases have been described. Patients usually come with infection of the arteries, eyes, or cutaneous/subcutaneous tissues. Most healthcare professionals are not familiar with the disease and its causative agent. A diagnosis of pythiosis is time consuming, and requires skilled personnel. There are some putative cases of pythiosis that were misdiagnosed as aspergillosis or mucormycosis<sup>5</sup>, due to similar microscopic morphology of *P. insidiosum* to

*Aspergillus* species or Zygomycetes. A management of patients with pythiosis is difficult. Here, we describe human pythiosis in Thailand, in regarding the pathogen and its ecology, epidemiology, pathogenesis, pathology, clinical manifestations, diagnosis, and treatment.

## THE PATHOGEN

*P. insidiosum* is a member of the Oomycetes, which belongs to the kingdom Chromista (Stramenopila).<sup>1,6</sup> The Oomycetes is a unique group of pathogens that differs from fungi, bacteria, parasites, and viruses. *P. insidiosum* is the only *Pythium* species known to infect humans.<sup>7</sup> The organism presents in two forms including right-angle branching and broad hyphae and aquatic motile biflagellate zoospore, which is a specific characteristic of the Oomycetes. Although the microscopic morphology of *P. insidiosum* is similar to filamentous fungi, *P. insidiosum* is not considered as a true fungus based on biochemical, physiological, and phylogenetic analyses.<sup>8,9</sup> It is more closely related to diatoms and algae than to true fungi.

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## EPIDEMIOLOGY AND ECOLOGY

Pythiosis in animals has been increasingly found in tropical and subtropical countries.<sup>1</sup> Surprisingly, pythiosis in humans has been reported almost exclusively from Thailand.<sup>3,4,10-18</sup> Patients with pythiosis in Thailand were found nationwide, indicating a wide distribution of the pathogen throughout the country.<sup>18</sup> Due to similar geographic and climate conditions, *P. insidiosum* should also inhabit in Southeast Asian countries, and pythiosis cases may actually exist in these countries.

Based on the recent study<sup>18</sup>, patients with pythiosis had the age range of 20-60 years (86% of all reported cases), were male (71%), and had agricultural occupations (farmer, fisherman, and domestic husbandry, 75%). Together with the fact that *P. insidiosum* inhabits in swampy areas (farming field, river, and pond) where it can colonize on glass leaf or water plants<sup>1,3,19-21</sup>, such demographic characteristics would increase the chance of an individual to contact the pathogen and acquire the infection.

Interestingly, human pythiosis was usually reported in individuals with underlying hematological diseases.<sup>4,12,18</sup> The majority of them were thalassemia and paroxysmal nocturnal hemoglobinuria.<sup>18</sup> A strong association between human pythiosis and these hemolytic diseases was observed mainly in patients with cutaneous/subcutaneous, vascular, and disseminated pythiosis. This may suggest that hemolysis-relating conditions are essential for the infection in these tissues. In contrast, a majority of patients with ocular pythiosis had no underlying disease and were healthy.

## PATHOGENESIS AND PATHOLOGY

The zoospore is the infective unit of *P. insidiosum* since it can swim, attach to the host surface, germinate as hyphae, and cause pathology in various host tissues.<sup>18,19,22</sup> Therefore, a direct contact of the pathogen to the host surface (skin and eye) is an initial

step of infection. Unlike most of *Pythium* species, *P. insidiosum* can grow at host body temperature (37°C), which is an essential factor for its virulence.

Histopathological examination of the infection in cutaneous/subcutaneous tissues typically shows chronic infection with eosinophilia.<sup>3,18</sup> In arterial or vascular infection, angiographic and pathological findings usually demonstrate the occlusion or aneurysm of medium- to large-sized arteries of the lower extremities or trunk, such as peroneal, tibial, popliteal, femoral, iliac arteries, and abdominal aorta. The venous tissue is unremarkable. It is notable that the infection progresses proximally along the arterial wall, resulting in the inflammation, fibrosis, and aneurismal change of an affected artery. Histopathologically, the pathogen confines in the arterial wall or in the obstructive thrombi. In ocular pythiosis, the hyphae of *P. insidiosum* can be observed microscopically by KOH preparation of the corneal scraps or discharges. Histopathology usually shows that the causative pathogen locates in the corneal stroma.

## CLINICAL MANIFESTATIONS

### 1) Cutaneous/subcutaneous pythiosis

Patients usually come with an infection confined in the cutaneous/subcutaneous tissues, and characterized by chronic swelling and painful subcutaneous granulomatous infiltrative lump and ulcer, usually at the arm or leg. Acute infection is also documented with a case of acute necrotizing cellulitis of both legs.<sup>17</sup>

### 2) Vascular pythiosis

Chronic arterial insufficiency syndrome of the lower extremity was the major presentation of vascular pythiosis. The syndrome ranges from chronic intermittent claudication or resting pain of the calf to gangrenous ulceration of the foot or leg. Fever, paresthesia, itching, vesicle, skin ulcer, cellulitis,

necrotizing fasciitis, leg swelling, absence of arterial pulse, groin mass (aneurysm or lymph node), or abdominal mass (aortic aneurysm) is also noted. The symptoms take months to develop, and patients usually come to a hospitalary late in the course, usually three months after the initial symptom. A patient history of exposure to swampy area shortly prior to the illness is usually obtained. The infection occurs at one or both legs. A major cause of death is from the ruptured aortic aneurysm.

### 3) Ocular pythiosis

Patients usually present with the corneal ulcer or keratitis. Pain, irritation, decreasing of visual acuity, eye lid swelling, conjunctival injection, corneal infiltrates, perforated cornea, or hypopyon is observed in some patients. Endophthalmitis can occur in severe cases. To date, there have been no patients with ocular pythiosis involving beyond the ocular globe. Patients usually come to hospital early, within a few weeks after initial eye symptom. A recent history of eye trauma or corneal abrasion is usually obtained.

### 4) Miscellaneous pythiosis or pythiosis of unusual sites

There are some human pythiosis with unusual sites of infection including the gastrointestinal tract, brain, and rhinosinus.<sup>18</sup> This indicates that *P. insidiosum* can infect various types of host tissues. Gastrointestinal tract infection, a common form in animal pythiosis<sup>1</sup>, is likely to occur from consuming the zoospore-contaminated water. For infections in the brain and rhinosinus, the pathogen may invade directly to these sites through the nasal cavity.

## DIAGNOSIS

### 1) Isolation of *P. insidiosum* from the infected tissue

To our experiences, *P. insidiosum* is sensitive to

the low temperature, since an attempt to isolate the pathogen from a specimen transferred on ice usually has the high failure rate. The specimen for cultures should be stored in sterile distilled water at the room temperature while transferring to a clinical microbiology laboratory.

*P. insidiosum* can be cultured at the room temperature or at 37°C, in various types of agar including Sabouraud dextrose agar, potato dextrose agar, corn meal agar, soil extract agar, Czapek-Dox agar, and the tissue culture medium.<sup>23</sup> *P. insidiosum* grows vary fast, as the appearance of colonies within a few days of incubation, and its radial growth rate is 10-12 mm/day.<sup>23</sup> The colonies of *P. insidiosum* are flat, have no aerial hyphae, and look whitish, yellowish, or brownish. To confirm an isolate of *P. insidiosum*, an induction of the zoospore formation is needed. The methods for zoospore induction have been described in details elsewhere.<sup>20,23</sup>

### 2) Detection of anti-*P. insidiosum* antibodies in patient's serum

The serodiagnostic tests including the immunodiffusion (ID) test<sup>24-26</sup>, enzyme-linked immunosorbant assay (ELISA)<sup>27-29</sup>, and Western blot method<sup>30</sup>, have been developed to facilitate the diagnosis of pythiosis. For the ID test, the positive result is characterized by the precipitation lines generated by the diffusion across two-percent water agar between the patient's serum and *P. insidiosum* culture filtrate. Although the ID test has a high specificity, it has a very low sensitivity.<sup>18</sup> ELISA and Western blot method have been developed to overcome the low sensitivity of the ID test, with the test specificity being high.<sup>27-30</sup>

### 3) Polymerase chain reaction (PCR) amplification and sequencing analysis

The PCR amplification of the 18s rRNA gene of *P. insidiosum*, using the specific primers, is now

available and useful for an identification of the pathogen in the clinical specimens or in the hyphal mat from the culture specimens.<sup>31,32</sup> The PCR product of the 18s rRNA gene can be sequenced and blasted against the NCBI genome database for determining the species of *Pythium*.<sup>33</sup>

## MANAGEMENTS

Cutaneous/subcutaneous pythiosis usually has a good response after the administration of saturated solution of potassium iodide (SSKI), along with the surgical debridement. Conventional antifungal agents and SSKI have no favorable effectiveness in vascular, ocular, and miscellaneous pythiosis.<sup>18</sup> The radical excision is the main treatment option for cure. However, the infection can recur, if there is a remnant infection following the surgical debridement.

In vascular pythiosis, an infected tissue can be removed by the resection of the infected artery, below-knee amputation, above-knee amputation, and/or aneurysmectomy, depending on the level and extent of the affected arteries. Thromboembolectomy is not recommended since the infection can be easily spread while an infective clot is removed out from the distal to the proximal part of the artery. Most cases of vascular pythiosis undergo the limb amputation. Forty percent of patients with vascular pythiosis die from the infection, while 60 percent survive with some handicaps.

In ocular pythiosis, the main treatment is also the radical surgery to remove all infected tissue. An eye removal by enucleation or evisceration is reserved following the failure to control the infection by keratectomy. A majority of the patients eventually undergo an enucleation or evisceration. There is no fatal outcome in ocular pythiosis.

*P. insidiosum* immunotherapeutic vaccine has been recently developed, and used as a non-invasive treatment of pythiosis in humans and animals.<sup>13,16,34-36</sup> The vaccine is prepared from the crude extract antigens

of *P. insidiosum* cultured in laboratory. It has been shown that 60 percent of the horses, 97 percent of the cattle, and 33 percent of the dogs with pythiosis responded favorably after the administration of the vaccine.<sup>37</sup> In human pythiosis, *P. insidiosum* vaccine is usually reserved as the last resort in patients who have no response to the surgery and antifungal treatment or with inability to undergo the surgical debridement. In our recent study, the vaccine was injected in 12 patients with vascular pythiosis. Of these, 5 patients survived, 2 died, 2 still had persistent infection, and 3 had no available outcome.<sup>18</sup>

## SUMMARY AND FUTURE RESEARCH DIRECTIONS

Human pythiosis has significant morbidity and mortality. Vascular and ocular pythiosis are the most common forms of the infection, accounting for 95 percent of the cases.<sup>18</sup> Both early diagnosis and appropriate treatment are critical to ensure the better prognosis. The serodiagnostic tests, including the ID test, ELISA, and Western blot method are available, but have a limited use due to a low sensitivity (especially in ID test) or a requirement of skilled personnel and special equipments (in ELISA and Western blot method). A development of highly sensitive and specific, but more convenient tests including the agglutination and immunochromatography tests are urgently needed to facilitate an early diagnosis of pythiosis.

Conventional antifungal drugs are ineffective for the treatment of pythiosis, because *Pythium* species may lack the drug-target ergosterol.<sup>38</sup> Recently, a new group of antifungal drugs that inhibits the  $\beta$ -glucan synthesis has been available.<sup>39</sup> Since  $\beta$ -glucan is a major component of the cell wall of *P. insidiosum*, a use of  $\beta$ -glucan inhibitor including caspofungin, micafungin, or anidulafungin may be effective in the treatment of pythiosis. The immunotherapeutic vaccine

is another treatment option because the favorable outcomes were observed in some patients and animals.<sup>13,16,18,34-36</sup> However, the vaccine efficacy is usually limited. A development of a more effective immunotherapeutic vaccine would improve the treatment outcome.

Recently, the humoral immune response and immunogenic profile of *P. insidiosum* have been analyzed by Western blot method.<sup>30</sup> A specific 74-kDa immunodominant antigen of the pathogen has been identified.<sup>30</sup> This immunogen is a potential candidate for the development of a reliable and convenient serodiagnostic test as well as a more effective immunotherapeutic vaccine. In addition, the 74-kDa immunogen may involve in the pathogenesis of human pythiosis since this protein is not present in non-human pathogenic *Pythium* species (*P. deliense* and *P. aphanidermatum*) which are closely related to *P. insidiosum*.<sup>8,40</sup> The identification and characterization of the gene encoding this immunogen could provide an insight in basic biology and pathogenesis of pythiosis.

Human pythiosis is strongly associated with thalassemia.<sup>18</sup> However, the underlying mechanism still remains unknown. A major pathological change of thalassemia, especially the iron overload, might increase the host susceptibility to pythiosis by promoting the infectivity of the pathogen or impairing the host immunities.<sup>41-43</sup> Some other conditions associated with thalassaemic patients including chronic anemia and hemolysis may involve in the pathogenesis of pythiosis.<sup>18</sup> The extensive investigations are needed to elucidate this mystery.

Human pythiosis is endemic in Thailand. An awareness of human pythiosis is now increasing, because new cases have been diagnosed more often.<sup>18</sup> Patients with contributing factors should be educated to prevent themselves from the direct skin inoculation of the organism such as wearing boots when working outdoors. Since the habitat of *P. insidiosum* seems

widespread<sup>18</sup>, both attention and awareness for human pythiosis should be promoted in Thailand.

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