Pythiosis: An Emerging Oomycetic Disease of Humans and Animals

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Abstract

Pythiosis caused by Pythium insidiosum, is an emerging, life threatening infectious oomycetic disease of humans, and animals. Among several species of animals, equine is the main species affected by this oomycete. Disease can occur in sporadic and epidemic form, and has been reported from many counties of the world. The source of infection is exogenous. It is believed that humans, and animals acquire the infection from the aquatic environment where the organism live as a saprobe. Hence, pythiosis can be considered as a waterborne parafungal disease. In humans, various forms of disease including ocular, vascular, systemic, and cutaneous/subcutaneous are observed. Vascular and ocular pythiosis are the most common forms of the infection, accounting for about 95% of cases. In equines, pythiosis is characterized by the formation of irregular masses within the cutaneous lesions, called kunkers. Microbiological, histopathological, immunological, and molecular techniques are employed to diagnosis the disease. The conventional antifungal drugs are ineffective for the treatment of pythiosis, because Pythium species may lack the drug-target ergosterol. Recently, a new group of antifungal drugs such as caspofungin, micafungin, or anidulafungin may be effective in the treatment of pythiosis. The immunotherapeutic vaccine is another treatment option because the favourable outcomes were observed in some patients and animals. However, the vaccine efficacy is usually limited. Pythiosis is an occupational disease of fisherman, agricultural farmers, livestock handlers, and others who work in aquatic environment. Patients should be educated to prevent themselves from the direct skin inoculation of the organism by wearing boots when working outdoors. As prognosis is guarded, timely recognition, and treatment are highly imperative for successful management of pythiosis. Furthermore, understanding the route of infection and pathogenesis of P.insidiosum infection can help in the control of disease both in humans as well as in animals. More research is warranted on the development of safe, cheap, and potent chemotherapeutic and immunoprophylactic agents to combat pythiosis in humans and animals.

Key words: Emerging disease, Equine, Human, Oomycete, Pythium insidiosum, Pythiosis

Introduction

Emerging infectious diseases of multiple etiologies due to public health, and economic consequences pose great challenges to the developing, and developed nations of the world (Pal, 2014). Among these, pythiosis (bursatee, cutaneous harbronemiasis, granular dermatitis swamp fever) is non-contagious,
oomycetic disease of humans as well as animals (Pal, 2007). It is an emerging, life-threatening, pyogranulomatous infectious disease caused by the fungus-like organism, Pythium insidiosum (Mendoza et al., 1996). Pythiosis is an emerging tropical disease silently killing the dogs in the US. The recorded history of pythiosis goes back to year 1884 when Smith, a Veterinarian, reported the disease for the first time among horses in India (Pal, 2007). However, human pytiosis was first recorded from Thailand in 1985 (Imwidthaya, 1994). The common sites of infection in animals are cutaneous/subcutaneous tissues, and the gastrointestinal tract. This filamentous organism initially affects the skin, and subcutaneous tissues, but can involve adjacent tissues like tendons, ligaments, bone, and invade the gastrointestinal tract or other tissues, and organs, resulting in multisystemic disease. The incubation period of pythiosis is not clearly defined but disease likely to develop weeks to months after exposure with contaminated environment (Pal, 2007).

Pythiosis has been reported in regions of tropical, subtropical, and temperate climate, more particularly in swamps or flooded areas. P. insidiosum requires a warm (30–40 °C), aquatic environment, and organic substrate to complete its life cycle (Chaffin et al., 1995). The Brazilian Pantanal perhaps presents the world’s highest incidence, and prevalence of equine pythiosis (Mendoza et al., 1996). In addition, the disease has been related in others regions of Brazil, representing a problem of relevance to horse breeding. Cases of pythiosis in other animals, such as canine, bovine, cats, sheep, and tropical animals held in captivity, are described around the world (Gaastra et al., 2010).

The equine is the species most affected by the disease usually presenting as cutaneous and subcutaneous form. The lesions are tumor-like, usually located on the extremities, with fast growth, and difficult for treatment. The lesion has abundant fibrous connective tissue, intersected by sinus and cores of necrotic yellow-gray material named as kunkers, and they are easily shed from lesions (Mendoza et al., 1996). There is the possibility that kunkers detached from equine lesions can contaminate the environment, and they can play an important role in the epidemiology of pythiosis, generating a new biological cycle of this oomycete (Mendoza et al. 1996).

It is believed that susceptible hosts get the disease when they enter an endemic swampy area contaminated with P. insidiosum. The disease is commonly observed in the individuals who remain for long periods in contact with standing water in lakes, ponds, or marshy places (Chaffin et al., 1995). Like other zoosporic parafungal organisms, P. insidiosum may use plants to produce sporangia that discharge zoospores, and colonize new plants, expanding its ecological niche. The disease occurs when these zoospores are attracted by injured human and animal tissues. The present paper highlights the increasing significance of P. insidiosum in humans and animals infections. The greater emphasis is given on the clinical spectrum, diagnostic procedures, and disease management.
Etiology
The etiologic agent was isolated for the first time from the cutaneous lesions of horses by De Haan and Hoogkamer in 1901 in Indonesia (Pal, 2007). The disease is caused by the fungus-like organism *Pythium insidiosum*, which is a member of the family Pythiaceae, order Pythiales, and class Oomycetes (Mendoza et al., 1996; Pal, 2007). The organism needs wet environment to carry out life cycle, and produce motile flagellated zoospores. Moreover, the pathogen has the ability to form resistant spores when conditions do not favour zoospore formation. Naturally, *P. insidiosum* inhabits swampy areas, where it is present in the form of mycelium or biflagellate zoospores. The zoospore is an infective stage where it can swim, attach to, and penetrate host tissue, and then slowly progress into blood circulation (Mendoza et al., 1993). The Oomycetes is a unique group of pathogens that differs from fungi, bacteria, parasites, and viruses. Phylogenetic analysis shows that Pythium species are more closely related to diatoms and algae than fungi. There are about 120 species of the genus Pythium of which *P. insidiosum* is the only species known to infect humans, and animals (Kamoun, 2003; Pal, 2007). 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. The pathogen can survive in environment for a considerable length of time (Pal, 2007).

Host
Natural infection due to *P. insidiosum* has been described in human beings, and many species of animals such as bears, birds, camels, cats, cattle, dogs, donkeys, horses, sheep, and zebras (Helman and Oliver, 1999; Mendoza et al., 2004; Tabosa et al., 2004; Pal, 2007; Gaastra et al., 2010). Among the animals, equines are most affected followed by canines (Pal, 2007).

Pathogenesis
Zoospores present in the environment serves as the source of infection to humans as well as animals (Pal, 2007). The zoospore is the infective unit of *P. Insidiosum* which develops only in water. Zoospores can swim, attach to the host surface, germinate as hyphae at the optimum temperature of 34 to 36 C, and cause pathology in various tissues of humans, and animals (Mendoza et al., 1993). Therefore, a direct contact of the pathogen to the host surface such as the skin, and eye is an initial step of infection. The organism can grow at host body temperature (37°C), which is an essential factor for its virulence (Krajacejun et al., 2008).
Clinical Spectrum

Humans
Clinical presentation of human pythiosis can be classified into four types. Cutaneous/subcutaneous infection can lead to vascular, and disseminated (systemic) disease. The majority of the patients affected with cutaneous/subcutaneous, vascular, and disseminated forms have underlying disease (Pal, 2007).

1. Cutaneous/subcutaneous form
The cutaneous/subcutaneous pythiosis is 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 (Pupaibool et al., 2006).

2. Ocular form
The patients with ocular pythiosis exhibit corneal ulcer or keratitis (Pal, 2007). In addition, pain, irritation, deminision of vision, eye lid swelling, hypopyon, conjunctival injection, corneal infiltrates, or perforated cornea is observed in some patients. However, endophthamitis can occur in severe cases. A recent history of eye trauma or corneal abrasion is narrated by most of the patients (Krajaejun et al., 2008).

3. Vascular form
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. The other clinical manifestations are fever, itching, paresthesia, leg swelling, vesicle, cellulitis, skin ulcer, necrotizing fasciitis, absence of arterial pulse, abdominal mass or groin mass (Pal, 2007; Krajaejun et al., 2008) The infection can involve one or both the legs. The symptoms take months to develop, and patients usually visit to a hospital late in the course, usually three months after the initial symptoms. Most of the patients reveal history of exposure to swampy area shortly prior to the illness. The ruptured aortic aneurysm is recognized as the main cause of death in vascular pythiosis (Krajaejun et al., 2008).

4. Systemic form
There are some cases of disseminated pythiosis with involvement of the gastrointestinal tract, brain, and rhinosinus. This indicates that pathogen can infect various types of tissues. The gastrointestinal tract infection, a common form in animal pythiosis, is likely to occur from consuming the zoospores contaminated water. The organism may invade directly to the brain, and rhinosinus through the nasal cavity (Pupaibool et al., 2006; Krajaejun et al., 2008).
Animals
The disease is most widely reported in horses, and dogs. In horse, the subcutaneous pythiosis is the most common form, and infection occurs through a wound while standing in contaminated water. Lesions are large, roughly circular, ulcerated, granulomatous, and fistulated nodules, and are mostly noticed on the legs, especially the lower limbs, abdomen, chest, and genitalia (Pal, 2007). The granulomas contain firm, yellowish coralliform masses of necrotic tissues known as kunkers. The involvement of bones may be a feature of chronic disease. Enteric pythiosis is also observed in equines. In dogs, the gastrointestinal involvement is the most common clinical presentation. The main clinical signs include vomiting, diarrhoea, weight loss, and anorexia (Helman and Oliver, 1999; Pal, 2007). The cutaneous lesions are also observed in dogs, and are characterized by extensive ulcerative pyogranulomatous dermatitis with multiple draining tracts. Lesions may be singular or multiple, affecting the limbs, ventral thorax, abdomen, perineal region, rump, and tail. The outbreaks of pythiosis are recorded in sheep (Tabosa et al., 2004).

Epidemiology
Over the past two decades, human pythiosis has emerged as an important parafungal disease, Pythiosis can occur in sporadic form, and also in epidemic form; and is marked by high rates of morbidity and mortality (Tabosa et al., 2004; Pal, 2007). The disease in animals has been increasingly found in the tropical and subtropical countries (Mendoza et al., 1996). The cases have also been described from temperate regions (Pal, 2007). Surprisingly, pythiosis in humans has been reported almost exclusively from Thailand because people living in swampy areas are more likely to contract the pathogen, and acquire the infection (Thianprasit et al., 1996). About 90 % of patients with vascular pythiosis had thalessemia. In Thailand, 1 % of the total population has thalessemia which may perhaps explain the endemicity of human pythiosis in the country. The patients with pythiosis were found throughout Thailand. Due to similar geographic and climate conditions, P. insidiosum should also inhabit in other countries of Southeast Asia, and the cases may actually exist in these countries but remain unidentified (Thianprasit et al., 1996; Pal, 2007). The disease is known as the swamp cancer because it is generally found in unmoving water such as swamps, and also it is possible to get the disease in areas with no water via oospores in the soil, and grass. Generally, pythiosis occurs during the summer after heavy amounts of rain. The healthy individuals are vulnerable to ocular pythiosis; and direct trauma to the eye predisposes the person to P.insidiosum infection. In patients with vascular pythiosis, the lower extremities are often involved because they tend to be the exposure sites of the infection. (Pal, 2007).

Maximum cases of pythiosis are recorded in males within the age range of 20-60 years, and most patients had agricultural occupations such as farmer, fisherman, and domestic husbandry. (Krajaejun, et al., 2008).
As *P. insidiosum* inhabits in swammy areas like farming field, river, and pond where it can colonize on glass leaf or water plants, hence, such demographic characteristics would increase the chance of an individual to contact the pathogen, and acquire the infection (Mendoza et al., 1996).

**Diagnosis**

There are no pathognomonic signs, from either the clinical or histopathological perspective, and hence the disease can often be leading to the misidentification as zygomycosis or lagenidiosis. A presumptive diagnosis of pythiosis is often made on the basis of clinical presentation in conjunction with supportive histopathological findings such as pyogranulomatous inflammation associated with broad, irregularly branching, infrequently septate hyphae with nonparallel walls (Thomas and Lewis, 1998). The hyphae of *P. insidiosum* can be demonstrated in corneal scrapings or ocular discharge by potassium hydroxide technique. However, the isolation, and identification of the pathogen from the clinical specimens of humans, and animals is highly imperative for making an unequivocal diagnosis (Pal, 2007).

**Microbiological Techniques**

As *P. insidiosum* is sensitive to cold, therefore, the specimen for cultures should be stored in sterile distilled water at the room temperature during transport to the laboratory. Various nutrient media such as brain heart infusion (BHI) agar, Sabouraud dextrose agar, corn meal agar, potato dextrose agar (PDA), Czapek-Dox agar, soil extract agar, and the tissue culture medium are employed for the cultivation of *P. insidiosum* from clinical specimens (Pal, 2007; Krajaejun et al., 2008). The organism grows vary fast, as the colonies appear within 48 hours of incubation at 37 C. The colonies are flat, glabrous, whitish, yellowish, or brownish without aerial hyphae. An induction of the zoospore formation is necessary for the identification of the pathogen (Chaiprasert et al., 1990). The detailed microscopic morphology of the cultures recovered from humans and animals can be studied in PHOL (Pal, Hasegawa, Ono, Lee) stain (Pal et al., 1990). This stain contains methylene blue 0.3 ml (3% aqueous solution), glycerol 3.0 ml, and formalin (4%). In addition, this stain is found very useful to study of fungi and algae (Pal, 2007).

**Immunological Methods**

The several immunological assays such as immunodiffusion, enzyme-linked immunosorbant assay, immunochromatographic, and Western blot have been developed to help in the diagnosis of disease (Pracharktam et al., 1991; Krajaejun et al, 2002; Pal, 2007; Krajaejun et al., 2009). It is important to mention that false negative results can occur in patients with ocular pythiosis by using immunodiffusion.
test (Krajaejun et al, 2009). Recently, immunochromatographic test (ICT) is found very sensitive to diagnose cutaneous and vascular pythiosis in humans (Krajaejun et al., 2009). It is suggested that the efficacy of immunochromatographic method should be assessed in the diagnosis of animal pythiosis.

**Molecular Tools**

Very recently, PCR based molecular techniques are developed for the diagnosis of disease (Pal, 2007). The PCR amplification of the 18s rRNA gene of *P. insidiosum*, using the specific primers, is employed for an identification of the pathogen in clinical specimens as well as in culture (Grooters and Gee, 2002). The PCR product of the 18s rRNA gene can be sequenced, and blasted against the NCBI genome database to determine the organism at species level (Pal, 2007; Krajaejun et al., 2008).

**Treatment**

The treatment of pythiosis is extremely difficult due to its resistance to most antifungal drugs, and requirement for extensive surgical procedures depending on the anatomical location of the lesions. Laohapensang and co-workers (2009) reported that amphotericin B, potassium iodide, and terbinafine can be used to treat the cutaneous form of disease. The cutaneous/subcutaneous pythiosis usually has a good response after the administration of saturated solution of potassium iodide, along with the surgical debridement. Conventional antifungal agents and potassium iodide do not show encouraging results favourable in ocular, vascular, and systemic pythiosis. Hence, the radical excision is the main option for the better management of the disease. However, the infection can recur, even after the surgical intervention. Therefore, the surgical resection, and postoperative treatment with intraconazole, and terbinafine are imperative to decrease the chance of recurrence (Pal, 2007).

The main treatment of ocular pythiosis is the surgical removal of all the infected tissues from the eyes. However, the removal of the eye by enucleation or evisceration is reserved following the failure to control the infection by keratectomy. The prognosis is good as there is no mortality in ocular form (Krajaejun et al., 2008).

In vascular pythiosis, an infected tissue can be removed by the resection of the infected artery. As the infection can spread during the removal of an infective clot from the distal to the proximal part of the artery, thromboembolectomy is not advised. Unfortunately, majority of patients with vascular pythiosis undergo the amputation of limb. It is important to mention that 40% of patients with vascular pythiosis die from the infection, and 60% survive with some deformities (Krajaejun et al., 2008).

*P. insidiosum* immunotherapeutic vaccine has been recently developed, and used as a non-invasive treatment of pythiosis in humans and animals (Mendoza et al., 2003). 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 (Mendoza and Newton, 2005). In companion animals such as dogs and cats, aggressive surgical resection is the treatment of choice (Pal, 2007). It is emphasized that a long term follow up of patients is highly imperative in order to assess the efficacy of immunotherapy and antifungal drugs.

**Prevention and control**

As it is an environmental exposure, control is very difficult. However, the susceptible hosts must avoid the aquatic environment because zoospores of *P. insidiosum* has a strong tropism for the skin tissues (Pal, 2007). It is imperative to make an early diagnosis particularly in the immunocompromised patients to start the appropriate chemotherapy to prevent the further complications (Pal 2007).

**Conclusion**

*Pythium insidiosum*, a water borne pathogen, causes life threatening infections in humans and animals. Most cases occur in Thailand in the patients who suffer from chronic haemolytic anaemia; and thalassemia- haemoglobinopathy is the most common underlying disease. Pythiosis is frequently encountered in males in the age group of 20 to 60 years. The causative organism inhabits the swampy areas such as pond, river, and farming field. People in rural areas associated with agricultural operations like farming, animal husbandry, and fishing are at a greater risk of acquiring the infection. Direct contact of the organism to the host surface is the primary mode of transmission. Hitherto, there is no record of transmission of infection from animal to human or vice versa. As clinical signs are not characteristic, laboratory help is essential to establish an unequivocal diagnosis of pythiosis. Surgical excision, medication and immunotherapy are recommended for the better management of the disease in humans as well as in animals. Further research on the ecology, pathogenesis, epidemiology, diagnosis, and chemotherapy may be rewarding.

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**References**