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REVIEW

Update on pythiosis immunobiology and immunotherapy

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Abstract

Pythiosis is an invasive, ulcerative, pyogranulomatous disease caused by *Pythium insidiosum*, a fungus-like oomycete that has been reported to affect humans, horses, dogs, and other mammals mainly in tropical and subtropical areas of the world. The disease is characterized by an eosinophilic granulomatous and a Th2 immune response which in turn helps to protect the fungus from the host cells. Pythiosis can present clinically in subcutaneous, gastrointestinal, and vascular tissues or in a systemically disseminated form depending on the species and site of infection. Changes in iron metabolism and anemia are commonly observed. The diagnosis is accomplished through clinical and pathological features, laboratory characteristics of cultures, serological and molecular tests. Treatment includes radical surgery, antimicrobial drugs, immunotherapy or a combination of these treatments. Immunotherapy is a practical and non-invasive alternative for treating pythiosis which is believed to promote a switch from a Th2 to Th1 immune response, resulting in a favorable clinical response. This therapy has demonstrated cure rates above 70% and 55% in horses and humans but low cure rates in dogs and cats. Despite the curative properties of this type of immunotherapy, the antibodies that are produced do not prevent host reinfection. Thus, development of effective adjuvants and new diagnostic techniques for early disease diagnosis are of utmost importance. The aim of this review was to promote pythiosis awareness and to provide an update about the immunotherapy and immunobiology of this disease.

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Key words: *Pythium insidiosum*; Treatment; Pythiosis; Immunotherapy; Pathogenesis

Core tip: Pythiosis is a life-threatening disease for which there is no gold standard chemotherapy. Immunotherapy derived from killed mycelium from *Pythium insidiosum* is a non-invasive therapy that has demonstrated cure rates above 90% when associated with the surgical removal of the lesions and early disease diagnosis.

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INTRODUCTION

Pythium insidiosum (*P. insidiosum,* an oomycete also known as water mold) is a filamentous microorganism that shares many characteristics with fungi (*i.e.*, it grows by polarized hyphal extension, engages in an absorptive mode of nutrition, and it can form spores for reproduction). However, *P. insidiosum* is classified in a completely different taxonomic group, namely the Stramenopiles, together with diatoms and brown algae[1]. This classifica-

tion is the first essential information for understanding pythiosis in humans and other mammals; infections have similar clinical and histopathological characteristics as those of certain mycoses. Conversely, treating pythiosis with antifungal drugs is generally inefficient because the oomycetes do not synthesize ergosterol, which is a component of the plasma membrane of true fungi and the primary target of those drugs[2]. Similarly, the immune response of animals with pythiosis presents similar features to those of fungal infections, and there are peculiarities found only in infections caused by this oomycete[3]. The challenge of treating pythiosis is characterized by the severity of the disease in mammals and by the absence of a gold standard chemotherapy. Nevertheless, immunotherapy is a practical and non-invasive alternative for treating pythiosis, and there is a favorable clinical response. In this context, the aim of this review was to promote pythiosis awareness and to provide an update about the immunotherapy and immunobiology of this disease.

THE EPIDEMIOLOGY, IMMUNOBIOLOGY AND PATHOGENESIS OF PYTHIOSIS

The proposed life cycle of *P. insidiosum* is characterized by the colonization of aquatic plants and the soil of wetlands or swampy areas, which serve as a substrate for mycelial vegetative growth and the asexual formation of mobile biflagellate zoospores that move through the water, find another host, encyst and form a new mycelium that can then start a new colonization[4-6].

In view of this biological cycle, pythiosis cases are associated with human or animal contact with areas in which zoospore-containing water accumulates (such as wetlands and lakes) and environmental temperatures range between 30 ℃ and 40 ℃. Most reports of animal pythiosis are described in horses that live in swampy areas or periodically enter ponds or lakes. These cases are distributed primarily between the peak months of the rainy season in each region. A higher human disease frequency has been observed in thalassemic patients in Thailand, where it is common for people to work in flooded rice cultivation areas[7-9].

Human and animal pythiosis cases have been described in tropical and subtropical climatic regions. Although these cases are most often diagnosed in Australia, Asia, Latin America and United States, some cases have originated from temperate areas of Japan, South Korea, Oceania and Africa. There are no reports of animal-animal or animal-human transmission[10,11].

P. insidiosum hyphae do not exert sufficient pressure to penetrate undamaged skin through mechanical means alone[12], and they must effect a decisive reduction in tissue strength by proteinase secretion or by finding prior skin damage to invade their host. Indeed, *P. insidiosum* possesses a strong tropism for mammalian injured tissue[10]. Interestingly, *P. insidiosum* has been recovered from a mosquito larva (*Culex quinquefasciatus*)[13] and hematophagous insects prefer the same anatomical areas for blood feeding in which pythiosis lesions are more prevalent in horses[9]. Given this information, future studies should investigate if insect bites could favor the penetration of zoospores into injured skin or if infected mosquitoes could directly transmit the disease.

Once inside the host, pythiosis pathogenesis involves the Splendore-Hoeppli phenomenon (reaction), which is characterized by the presence of radiating, star-like asteroid or club-shaped eosinophilic material around the infectious agent[14]. Thus, *P. insidiosum* triggers an eosinophilic granulomatous reaction similar to other fungi, such as *Basidiobolus* spp. and *Conidiobolus* spp.[15], with characteristic histopathological features depending on the species and the clinical form[16].

Mendoza *et al*[17] proposed that the antigens released by *P. insidiosum* hyphae modulate the host's immune response and may be responsible by keeping an eosinophilic granulomatous response, locking the immune system into a Th2 immune response through the continuous stimulatory production of more eosinophils and mast cells, which in turn helps to protect the fungus from the host cells and leads to a worsening condition, and if not treated properly, can lead to host death. As a consequence, the *P. insidiosum* hyphae surrounded by degranulated eosinophils would be camouflaged inside the eosinophilic micro-abscesses, preventing their full presentation to the immune system and thereby ensuring their viable presence in infected tissues. These features and the subsequent finding of elevated IgE levels in humans and horses with the disease strongly validated the concept of a Th2 modulation by this pathogen during natural infection[3,17]. High Th2 interleukin levels [interleukin (IL)-4, IL-5 and IL-10] have also been detected in human patients with pythiosis, confirming a Th2 immune response[18,19].

The eosinophil degranulation in equids and camels with pythiosis is remarkable, forming around the hyphae cores of necrotic yellow-gray and firm materials called *kunkers*, which are easily shed from lesions[4,15,20,21]. The *kunkers* range from 2 to 10 mm in diameter, have an irregular shape and sandy aspect, may be branched and invade the granulation tissue within the sinus formed along its trajectory. This pronounced degranulation is also associated with extensive tissue damage and with the tumor-like appearance of lesions that can reach over 50 cm in diameter[22]. Horses are the mammals that are most affected by pythiosis, with no predisposition according to their age, race or sex. However, although young animals are also susceptible, the disease is rarely observed in animals under one year of age, and the bodily lesions are predominant in dark pigmented areas[9].

The lesions are subcutaneous and present primarily in the distal extremities, the ventral portion of the thoracoabdominal wall and face, which represent anatomical structures that remain in contact with contaminated water containing *P. insidiosum* zoospores[7,23,24]. The *kunkers* are considered to be pathognomonic of pythiosis in equids, and they have also been described in camels with vulvar pythiosis[21] and in a case of equine conidiobolo-

mycosis[25]. The disease was also described in cattle[26-29], cats[27,30], dogs[16,17,27,31-39], sheep[40,41] and occasionally in animals kept in captivity in zoos[42,43] and birds[44].

P. insidiosum can cause superficial infections in humans, namely keratitis with corneal involvement[45-47]; cutaneous and subcutaneous infections[48]; orbital pythiosis and bone involvement[49,50]; and systemic infections, namely arteritis of the lower limbs and/or dissemination[8,19,51]. Although pythiosis can affect apparently healthy individuals[48,50,51], most cases are reported in patients with thalassemia and other hematological diseases[8]. The same authors have argued that iron overload, which is a marked characteristic of patients with thalassemia, could increase host susceptibility to pythiosis by promoting the infectivity of the pathogen or by impairing host immunity.

In fact, both iron overload and deficiency can weaken the immune system[52-54]. Additionally, many microorganisms are known to be avid for iron during infection[53,55] and changes in iron metabolism may increase host susceptibility to infection by *P. insidiosum*. Krajaejun *et al*[56] described that *P. insidiosum* expresses a gene encoding a ferrochelatase and Krajaejun *et al*[57] reported, through the transcriptome analysis of this species, an extensive repertoire of proteins that may be involved as virulence factors during infection. Although the role of iron in pythiosis has not been fully explained, the disease is more frequently found in human patients with thalassemia and with other hemolytic anemias[18,19,58]. Anemia as a consequence of the disease has already been described in horses[59-65], dogs[33,34,37,39,66], cats[39,67], camels[21,68] and in a jaguar[42] and Bengal tiger[69]. Santos *et al*[9] also argue that the iron deficiency is common in lactating foals (< 1-year-old and, which are less susceptible to pythiosis) because of the low iron levels in the milk. In contrast, iron deficiency is uncommon in adult horses, and they may have increased levels of circulating iron, especially in the Brazilian Pantanal, which contains high levels of this mineral in the soil, plants and water[70], and where it is observed a high incidence of pythiosis.

Loreto *et al*[71] reported an increase in the unsaturated iron binding capacity (UIBC) in rabbits experimentally infected with *P. insidiosum*, suggesting that there was an increase in the transferrin concentration and/or an increase in the number of transferrin iron receptors, which is compatible with a physiological decrease in the iron availability. Similar results were observed by Zanette *et al*[72], who noted that rabbits experimentally infected with pythiosis presented decreased serum iron levels, increased transferrin levels with low saturations (increased UIBC) and markedly decreased levels of stainable iron in the hepatocytes, which suggests an affinity for iron by *P. insidiosum*.

DIAGNOSIS AND HUMORAL RESPONSE

A classical pythiosis diagnosis is accomplished through clinical and pathological features, in addition to cultural, morphological and reproductive characteristics *in vitro*. A differential diagnosis includes habronemiasis, neoplasms, exuberant granulation tissue, and fungal or bacterial granulomas[73,74]. Microscopic evaluations using 10% KOH (direct examination) can reveal *P. insidiosum* hyaline hyphae and eventually septate-morphology, depending on the clinical material evaluated. This material can easily be confused with filamentous fungi, particularly those of the orders Entomoftorales and Mucorales[11]. A culture from *kunkers* or biopsies can usually be performed on V8 agar, corn meal agar and Sabouraud dextrose agar.

Hyphal growth can be observed after 24 h of incubation at 37 ℃ when submerged in culture medium, and it exhibits a hyaline or white color[75]. Because *P. insidiosum* does not produce reproductive structures in traditional culture media, the induction of zoosporogenesis (asexual zoospore formation) can be obtained by cultivating *P. insidiosum* in sterile blades of grass that are then transferred to a mineral solution[76]. However, the correct identification of this species should be confirmed by molecular methods[77-81].

The production of anti-*P. insidiosum* antibodies was one of the first immunological features described for pythiosis, and these antibodies were easily detected by immunodiffusion and complement fixation tests with antigens that were extracted from the pathogen[82,83]. Studies then confirmed that humans and animals suffering from pythiosis exhibited a humoral immune response upon host-pathogen interaction[3,36,77,84-86], but this response was not sufficient to clear the infection[19,50,87,88]. However, the serological tests developed for detecting antibodies, such as agar gel immunodiffusion, enzyme-linked immunosorbent assay (ELISA), Western blot, latex agglutination and immunochromatographic tests[77-80,89-93], are highly useful for the early diagnosis of pythiosis. In equine pythiosis cases in which the animal is far from reference laboratories, sending serum for ELISA and collecting *kunkers* and tissues for microbiological culture and histopathological analysis are among the primary forms of diagnosis. An early pythiosis diagnosis can also be performed through immunohistochemical[16,94] and molecular methods[80,95].

TREATMENT

Antimicrobial and surgical treatment

Because primary antifungal drugs act directly or indirectly on ergosterol and *Pythium* spp. are unable to synthesize any sterols, it is understandable that pythiosis cases do not respond satisfactorily to antifungal treatments. However, contradictory results have been reported in the use of antifungal agents to treat pythiosis[8,10,49,96].

P. insidiosum isolates have varying *in vitro* susceptibility to antifungal compounds[97,98]. Reviews of antifungal drug associations show that *in vitro* synergism occurs in AmB + terbinafine[99], terbinafine + azole antifungals and terbinafine + caspofungin associations[100]. Additionally, some antibacterial drugs that act as protein synthesis inhibitors (macrolides, tetracyclines and glycylcycline) have been shown to inhibit the *in vitro* growth of *P. insidiosum*[101,102]; nonetheless, experimental *in vivo* tests have not been con-

ducted to demonstrate the clinical effectiveness of these antibiotics.

Successes and failures of pythiosis treatment cases have been reported with combinations of antifungal therapies. The surgical removal of the lesion, the amputation of the affected limb or the enucleation of the affected eye represents the last resort in human pythiosis treatment. However, recurrence rates of 40% have been observed, which illustrates the difficulty of controlling this disease[11]. The implementation of surgical treatment with antifungal drugs or potassium iodide was described in cases of therapeutic healing[73].

Surgically removing all affected tissue is the traditional and most commonly used method for equine pythiosis treatment. The surgery yields good results for small and superficial lesions. However, removing the lesion with a safety margin to avoid recurrences is often hampered by the anatomical regions that are typically involved (distal extremities and the ventral portion of the thoracoabdominal wall)[73].

Immunotherapy

Although the antigens used in vaccine preparation (usually from the infectious agent itself) are intended to trigger a protective response in the host immune system (antibody production), the aim of immunotherapy (antibodies or antigens from the infectious agent) is the objective modification of the host immune response to mount an effective response against a disease that is already present. Despite the fact that a protective vaccine against pythiosis does not currently exist, the immunotherapy developed from protein extracts of *P. insidiosum* cultures is a noninvasive alternative for treating this disease in humans and animals.

Immunotherapy was discovered by serendipity when investigators were working on a skin test for pythiosis in horses, and they found that almost half the animals were cured upon inoculation with *P. insidiosum* immunogen[88,103,104]. The first investigator to use a culture-derived antigen for a skin test was Witkamp[83], but he did not report cure rates in his experiments. Miller[103] was the first researcher to report the use of *P. insidiosum* antigens (sonicated hyphae) with therapeutic potential when injected into horses (n = 30), resulting in 53% healing in the animals with pythiosis (Table 1). During the following year, the same author observed an immunotherapeutic efficiency ratio of 75% when associated with surgical removal[105]. Subsequent studies showed that lesions presenting with more than two months of progress in cases of chronic pythiosis had cure rates of approximately 20%-40% with immunotherapy, and cure rates of 100% were obtained when the lesions had less than 20 d of evolution[17,88,104].

In addition to the lesion evolution time, the manner by which the *P. insidiosum* mycelium is broken to obtain the antigens is also associated with immunotherapy efficacy. In this context, modifications to the original technique as described by Miller[103] have been developed with the aim of increasing the effectiveness and safety of immunotherapy.

Mendoza *et al*[104] tested two immunotherapies by using the cell mass or a concentrated soluble antigen as an antigen, and they observed efficacies of 60% and 70%, respectively, when treating 71 horses. Mendoza *et al*[17] reported that immunotherapy derived from the soluble antigen and sonicated hyphae of *P. insidiosum* cured 72% of the horses (n = 18) with pythiosis.

Santurio *et al*[106] compared the immunotherapy obtained from sonication, maceration (or liquidification) or the combination of these two techniques in experimental pythiosis cases in rabbits and observed that the macerated immunotherapy had a higher efficiency, with a reduction of 71.8% in the lesion sizes and the clinical cure of two rabbits (n = 5). This macerated immunotherapy was lyophilized, and it was valid for more than one year without refrigeration[106]. This treatment exhibited a cure rate of 50% to 83% (n = 19)[107], or 75% (n = 8)[7] and 90% when combined with surgical excision (n = 11)[24] in horses in the Brazilian Pantanal. The best results are typically observed when the disease is in its early stages.

Despite the good immunotherapy performance in equines, immunotherapy in cats and dogs has been disappointing[17,27,31]. One explanation for this failure might be that most dogs and cats with pythiosis are diagnosed several months after the initial onset of infection, resulting in animals with weakened immune systems that respond poorly to immunotherapy[3]. However, the healing of a dog was demonstrated by the combination of immunotherapy and antifungal therapy[108].

The immunotherapy treatment period (no. of doses) is related to the size, location, time of lesion development, and individual patient response. Santos *et al*[24] reported that a horse with 90 d of disease evolution required five months of treatment (eight doses) for complete lesion healing, and they noted that the slowness in the immunotherapy response cannot be interpreted as refractory and in turn end in the premature withdrawal of treatment. Conversely, only two to three doses promoted the effective healing of four horses bearing lesions with seven and 45 d of development.

Field tests with macerated immunotherapy have demonstrated that the efficacy of this treatment is directly associated with early diagnosis. The borderline between a clinical cure and an unsatisfactory response or even nonresponsive cases seems to be 60 d from the appearance of lesions in horses[3]. The treatment consists of subcutaneous applications at 14-d intervals until the complete healing of the granulomatous ulcerative tissue. A mild reaction at the injection site is often observed, and in most cases, it subsides in a few weeks. The number of doses is variable, and some animals respond better to weekly applications. In fact, the only disadvantage of this treatment is the production of protective IgG classes, which impairs serodiagnostic tests such as ELISA and immunochromatography. In this context, blood collection for serological diagnosis of pythiosis should be performed

Table 1 Review of animal pythiosis cases reported in the literature when treated with immunotherapy

Species/*n*	Lesions	Adjunctive therapy	Immunotherapy type[3], doses	Outcome	Ref.
Horses					
40	Various[4]	No or surgery	UF, 3[1] doses at 7-d intervals	C (53%), I (33%)	[103]
5	Limbs	ATM, surgery	UF, 3 doses at 7-d intervals	C (20%), 60(E), 20 (D)	[60]
5	Various[4]	No	SA, 2 doses at 15-d intervals	C (60%)	[88]
1	Limb, bones	No	SA, 2 doses at 7-d intervals	E	[109]
71	Various[4]	Nr	FH or SA, 1 or 2 doses at 7-d intervals	C (66%)	[104]
1	Limb, bones	Surgery, ATM	Nr, 3 doses postsurgical	D	[110]
2	Abdomen	Surgery, ATM	SH, 3 doses at 7-d intervals	C (50%), E (50%)	[111]
19	Various[4]	No	LMH, 3 to 9 doses at 14-d intervals	C (50%-83%)	[107]
18	Various[4]	Surgery, ATM	SA + SH, 2[1] doses at 15-d intervals	C (72%)	[17]
1	Limbs, sub-maxillary	Surgery	Nr	E	[23]
1	Limb	ATM	LMH, 7 doses at 14-d intervals	D	[112]
1	Hind pastern, fetlock	ATB	SA, 3 doses at 1, 7 and 21 d	E	[113]
1	Face	Surgery, ATM	LMH, 5 doses at 14-d intervals	E	[114]
1	Face	ATM	LMH, 5 doses at 14-d intervals	C	[64]
1[2]	Limb, abdomen	No	LMH, 4-5 doses at 14-d intervals	C	[115]
11	Limbs, abdomen	No or surgery	LMH, 2-5 doses at 14-d intervals	C (70%-90%)	[24]
8	Limbs, abdomen	No or surgery	LMH, Nr	C (75%)	[7]
47	Various[4]	No or surgery	LMH, Nr	C (79%-84%)	[9]
Dogs					
1	Cutaneous	AMB, surgery	UF, 1 dose	C	[38]
6	Cutaneous, intestinal	ATM, surgery	SA + SH, 2[1] doses at 15-d intervals	C (33%)	[17]
2	Cutaneous	Itraconazole	SA, 1 or 2 doses at 7-d intervals	E	[31]
1	Cutaneous	No	SA, 2 doses at 14-d intervals	C	[35]
1	Gastrointestinal	ATF, surgery	Nr, 3 doses at 1, 7 and 21 d	C	[32]
1	Gastrointestinal	ATF	SA, 6 doses at 15-d intervals	C	[108]
Camels					
1	Face, stomach	Surgery, ATM	SA + SH, 2[1] doses at 14-d intervals	D	[68]
2	Vulvar	Surgery, ATM	SA, 3 doses at 1, 10, 17 d	C (50%)	[21]
Sheep					
6	Oronasal	No	LMH, 1-5 doses at 14-d intervals	C (16.7%)	[41]

[1]At least; [2]Same animal, cured twice with immunotherapy with reinfection within an interval of two years; [3]Manufacturing process for immunotherapy; [4]Not reported individually (subcutaneous). Nr: Not reported; AMB: Amphotericin B; UF: Ultrasonication of hyphae; SA: Soluble antigens; SH: Sonicated hyphae; FH: Fragmented hyphae; LMH: Lyophilized macerated hyphae; C: Cured; I: Clinically improved; D: Died; E: Euthanized; ATM: Antimicrobials; ATB: Antibacterials; ATF: Antifungals.

Table 2 Review of human pythiosis cases reported in the literature when treated with immunotherapy

n	Lesions	Adjunctive therapy	Immunotherapy type[2], doses	Outcome	Ref.
1	Vascular	ATM, surgery	SA, 2 doses at 14-d intervals	C	[19]
8	Vascular	Surgery/amputation, ATF	SA, 2[1] doses at 14-d intervals	C (50%)	[18]
1	Vascular	Above-knee amputation	SA, Nr	C	[116]
1	Vascular	ATM, limb amputation	SA, Nr	D	[117]
1	Ocular	ATM, enucleation	Nr	D	[118]
1	Vascular	Above-knee amputation	Nr	C	[119]
1	Vascular	ATM, above-knee amputations	Nr	C	[120]
1	Vascular	ATM, above-the-knee-amputation	Nr, 4 doses at 7-d intervals	C	[95]
1	Vascular/disseminated	ATM	SA, 2 doses at 7-d intervals	D	[121]
3	Ocular	ATM, surgery	Nr, 3 doses	C (66%)	[122]

[1]At least; [2]Manufacturing process for immunotherapy. Nr: Not reported; SA: Soluble antigens; C: Cured; D: Died; ATM: Antimicrobials; ATF: Antifungals.

before the application of immunotherapy, thus preventing false-positive results.

Because of the higher incidence of pythiosis in horses, most data on the efficacy of immunotherapy are described in this animal species[7,9,17,23,24,60,64,88,103,104,107,109-115]. However, there are also descriptions of its use in dogs[17,31,32,35,38,108], camels[21,68] and sheep[41] (Table 1). Human immunotherapy was described for both successful and failed treatments in association with surgical procedures and the use of various antimicrobials[18,19,95,116-122] (Table 2). These studies suggest that the injection of *P. insidiosum* immunogens in the form of immunotherapy make antigens available to the host immune system that are not produced during active infection, stimulating a healing response and the formation of immune responses with the presence of mononuclear cells and the disappearance of the eosinophilic reaction around the hyphae.

The proposed mechanism for immunotherapy success is based on a change in the type of cellular response. The immune response observed during pythiosis involves eosinophilic inflammation and the expression of T helper lymphocyte type 2 (Th2) with the release of interleukins 4 and 5 and the mobilization of eosinophils and mast cells. However, the expression of T helper lymphocyte type 1 (Th1) occurs after the immunotherapeutic treatment with the release of interleukin 2 and INF-γ and the mobilization of T lymphocytes and macrophages, which destroy the *P. insidiosum* cells[3]. This approach was observed for the immune response to human pythiosis when interleukin 4 and 5 production was detected in association with high IgE titers; a large amount of inflammatory cells (eosinophils and mast cells) was identified, which indicated a Th2 response during the infection. After immunotherapy, the patients presented high blood levels of interleukin 2 and INF-γ with a mononuclear immune response, which is typical of a Th1 response[18,19]. Additionally, an increase in the enzyme activity of ecto-adenosine deaminase (E-ADA) was observed in a rabbit model of experimental pythiosis, which is also associated with the switch from a Th2 to a Th1 response[123].

Despite the curative properties of this type of immunotherapy, the antibodies that are produced do not prevent host reinfection[2,115]. Santos *et al*[115] described a case of reinfection that occurred two years after the end of a successful immunotherapy treatment against pythiosis. Reinfection occurred at a different anatomical site than the initial infection (abdomen versus left pelvic limb), and although the new lesion was larger (60 cm perilesional edema and ulcerated lesions with approximately 20 cm in diameter), a cure was achieved with four immunotherapy doses (versus the five doses needed in the primary treatment). It is important to note that the levels of antibody's anti-*P. insidiosum* are associated with the response to treatment. Antibody titers are stable or increase in cases of unsuccessful treatment or when there is a persistent or recurrent infection. In cases of healing, substantial reductions of antibody's titers are seen during the subsequent months after the resolution of the infection[35].

Given the above information, we can conclude that effective immunotherapy treatment can be obtained in association with a rapid and accurate diagnosis, and it may or may not be associated with surgical excision.

CONCLUSION

In summary, although the current immunotherapies used for treating pythiosis make use of crude *P. insidiosum* antigens, some studies have described the identification of immunodominant antigens[124,125], and the best aspects of these immunotherapeutic elements could lead to a new vaccination strategy that is more effective and protective. A recent description of the *P. insidiosum* transcriptome[57] uncovered many putative virulence proteins, and it provided a set of candidate targets for the development of better pythiosis diagnosis and treatment modalities. Because the production of IgG by stimulated B cells is known to protect the host for short periods of time[2,115], the development of effective adjuvants and new diagnostic techniques for early disease diagnosis are of utmost importance, primarily for animal and human use in endemic areas.

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