Scientific Electronic Archives
Issue ID: Sci. Elec. Arch. 8:3 (2015)
October 2015
Article link:
http://www.seasinop.com.br/revista/index.php?journal=SEA&page=article&op=view&path%5B%5D=193
Included in DOAJ, AGRIS, Latindex, Journal TOCs, CORE, Discoursio Open Science, Science Gate, GFAR, CIARDRING, Academic Journals Database and NTHRYS Technologies, Portal de Periódicos CAPES.

ISSN 2316-9281

Pythiosis: a therapeutic approach

C. M. C. Falcão[1], R. A. Zanetti[2], C. E. P. dos Santos[1]

[1] Universidade Federal de Mato Grosso – Campus Cuiabá
[2] Universidade Federal do Rio Grande do Sul, Porto Alegre

*__Author for correspondence:__ carloseduardo@ufmt.br

Abstract. Pythiosis, a disease caused by the oomycete *Pythium insidiosum*, often presents inefficient response to chemotherapy. It is a consensus that, in spite the several therapeutic protocols, a combination of surgery, chemotherapy and immunotherapy should be used. Surgical excision requires the removal of the entire affected area, with a wide margin of safety. The use of antifungal drugs has resulted in variable results, both in vitro and in vivo, and presents low therapeutic efficiency due to differences in the agent characteristics, which differ from true fungi. Immunotherapy is a non-invasive alternative for the treatment of pythiosis, which aims at modifying the immune response of the host, thereby producing an effective response to the agent. Photodynamic therapy has emerged as a promising technique, with good activity against *P. insidiosum* in vitro and in vivo. However, more studies are necessary to increase the efficiency of the current treatment protocols and consequently improve the cure rates. This paper aims to conduct a review covering the conventional and recent therapeutic methods against *P. insidiosum* infections.
Keywords: *Pythium insidiosum*, review, chemotherapy, immunotherapy, photodynamic therapy.

Contextualization

Infections caused by the oomycetous pathogen *Pythium insidiosum* often poorly respond to chemotherapeutic treatment. Favorable diagnosis is usually obtained with early treatment, including the association of chemotherapy, immunotherapy and radical surgery (Gaastra et al., 2010, Santos et al., 2011b). According to Álvarez et al. (2013), the size and local of the lesions, early start of the treatment and the physiological and nutritional state of the host are important drivers in the cure of the disease.

Numerous experimental therapies are currently been researched worldwide. Many of them were only studied in vitro, and deserve further investigation in vivo. Nonetheless, many of the in vivo studies have not reproduced the laboratory results (Pires et al., 2013). We conducted a review of the current treatment options and future perspectives for the management of pythiosis in animals.

Surgery

Surgical excision is still the traditional treatment option for pythiosis. The procedure is common in horses and humans and involves the removal of all the affected area with a safety margin. This is particularly complicated in horses due to the extensive granulation tissue. Moreover, lesions in members are even more complicated to treat surgically due to the presence of ligaments, tendons, large vessels and nerves. If not completely removed, a single fragment of hypha can regrowth. Indeed, a high rate of reoccurrence (45%) of the disease is seen in this procedure (Santurio et al., 2006, Gaastra et al., 2010, Dias et al., 2012, Pires et al., 2014). In humans, most of the surgical procedures involve amputation of limbs and enucleation of the eye in the cases of vascular and ocular pythiosis, respectively (Krajaejun et al., 2008).

Favorable results are often obtained when the surgery involves small cutaneous/subcutaneous areas where all the affected area can be removed (Leal et al., 2001, Santurio et al., 2006). Nonetheless, most of the cases treated successfully in the literature involve a combination of actions such as surgical therapy, immunotherapy or administration of antimycotic agents.

Antimycotic treatment

Chemotherapy in humans and animals affected by pythiosis is complicated due to the characteristics of the infectious agent. *Pythium* spp. cell wall is composed of cellulose and β-glucan,

whereas true fungi have chitin. The cytoplasmic membrane lacks sterols, such as the ergosterol, the target of action of most antifungal agents commonly used in animals (Álvarez et al., 2013). Such characteristics explain the lack or low efficacy of the use of antifungal drugs to treat pythiosis. Conversely, there are reports where the antimycotic therapy was successful (Gaastra et al., 2010, Loreto et al., 2014). The drugs commonly used, alone or in combination are amphotericin B, ketoconazole, miconazole, fluconazole and itraconazole. Iodine derivatives such as 10% potassium and sodium iodide are also employed with variable efficacy (Álvarez et al., 2013).

The use of antifungal drugs against *P. insidiosum* has obtained inconsistent results in vitro and in vivo. According to Sekhon et al. (1992), the polienes amphotericin B and hamycin did not show satisfactory activity, whereas the azoles fluconazole, ketoconazole and miconazole inhibited the growth of *P. insidiosum* in vitro. In another study, Shenep et al. (1998) reported that amphotericin B, fluocytosine, miconazole and griseofulvin did not show activity against *P. insidiosum*, whereas itraconazole had moderate activity and terbinafine was effective against the strain tested. Indeed, the combination of terbinafine and itraconazole was synergic in vitro and was employed with success in the treatment of a young patient with ocular pythiosis. Triscott et al. (1993) described two cases of human patients with subcutaneous infection in the periorbital area that responded well to the treatment with amphotericin B. This is contradictory to the results observed in vitro with this drug (Leal et al., 2001, Santurio et al., 2006).

Iodine agents have been used in the treatment of pythiosis, mainly in horses, associated to surgical procedure. Notwithstanding, there are controversies on the use of iodine compounds. According to Gonzales et al. (1979), the use of potassium iodide alone or in combination with surgery is effective in the cases of subcutaneous infections, mainly after the surgical removal of the infected area. Chaffin et al. (1992) also reported success of the surgery and posterior treatment with sodium iodide in horses with cutaneous pythiosis. Conversely, the intravenous administration of potassium iodide did not show satisfactory results in two horses, even after the surgical procedure (Meireles et al. 1993). According to Rodrigues et al. (2004), the oral administration of potassium iodide in combination to surgery is more efficient in the cases of subcutaneous pythiosis, However, the use of iodine products should be handle with care due to the side effects caused by excess iodine intake, such as iatrogenic hypothyroidism (Dória et al., 2008).

Caspofungin, a drug of the echinocandin class that inhibits the synthesis of β-glucans, which are present in the cell wall of oomycetes, has also been tested against *P. insidiosum.* Pereira et al. (2008) compared two protocols, caspofungin and immunotherapy with PitiumVac®, in the treatment of rabbits experimentally infected. The lesions were significantly reduced in both groups when compared to the control, untreated group. Histologically, a reduction in the quantity of hyphae and in the areas of necrosis was observed in both groups. The results obtained with the caspofungin treatment were even better than the immunotherapeutic treatment, notwithstanding, lesions resumed growth after the end of the drug administration. The in vivo results corroborate the in vitro results, indicating that caspofungin action against *P. insidiosum* is merely fungistatic (Pereira et al., 2007). Another echinocandin, micafungin, showed better in vitro results when in comparison to caspofungin, especially when associated to an iron chelator (Zanette et al., 2015). However, the high cost of echinocandins designates immunotherapy as still the best option for pythiosis treatment, especially in horses.

The combination therapy using antifungal agents with different mechanisms of action has emerged as a suitable treatment option for *P. insidiosum* infections. Argenta et al. (2008) investigated the in vitro activity of itraconazole, voriconazole and terbinafine, alone and in combination, against *P. insidiosum* isolates. The authors highlighted the good activity of terbinafine. Other combinations that demonstrated synergism in vitro were amphotericin B and terbinafine, terbinafine and azoles and terbinafine and caspofungin (Loreto et al., 2014). Argenta et al. (2012) studied the activity of terbinafine, itraconazole, caspofungin, fluvastatin, and ibuprofen against 15 *P. insidiosum* strains, using two-drug and three-drug combinations in vitro and in rabbits with pythiosis. For the in vivo study, 20 rabbits were divided in four groups: group 1, terbinafine and itraconazole, group 2, terbinafine, itraconazole and fluvastatin, group 3, terbinafine and caspofungin, and group 4, untreated control. Two-drug combinations were synergistic against 47% of the strains, and antagonism was not observed. Three-drug combinations were mainly indifferent, although synergism and sometimes antagonism were also observed. Histologically, the number of hyphae was statistically decreased in Group 2 in comparison with the other three groups, and can be an alternative for the clinical treatment of pythiosis.

Antibiotics that act by inhibiting the protein synthesis, such as the macrolide and tetracycline classes, have been demonstrated to also inhibit the growth of *P. insidiosum* in vitro. Nonetheless, in vivo studies are still needed to confirm the efficacy of these drugs (Loreto et al., 2014). In a similar study, Mahl et al. (2012) evaluated the in vitro susceptibility of *P. insidiosum* strains against the aminoglycosides gentamycin, neomycin, paromomycin and streptomycin and against the minocycline-derived tigecycline. Among them, the best results were observed for tigecycline, highlighting its potential for the use in animal trials and in the clinic.

The oral administration of an antifungal agent simultaneously with topical application

enhances the efficacy of the treatment. Against pythiosis, the use of oral antifungals has been demonstrated inconsistent once the infections are characterized by the difficulty of the drug to reach the intravascular compartment and act locally on the lesion. Dias et al. (2012) reported a case of cutaneous pythiosis with atypical location, where therapeutic success was obtained by the combination of surgical excision of the granulomatous tissue and the topical application of amphotericin B associated to dymethylsulphoxide (DMSO), which enhances the diffusion of the drug at the lesion site.

Dória et al. (2012) studied the effects of the intravenous regional perfusion of amphotericin B after surgical excision and thermocautery of exuberant granulation in limbs of horses with pythiosis. Ninety-two percent of the treated horses had complete resolution of the lesions after one or two treatments with amphotericin B. Adverse drug reactions such as inflammation of the injection site, edema and pain during regional palpation of the limb, including transitory lameness, were observed but resolved in two weeks. This protocol proved to be an effective technique for treatment of pythiosis in equine limbs with minimal side effects.

Immunotherapy

The aim of immunotherapy is to modify the immune response of the host to produce a more efficient response against an installed disease. It has been shown to be an alternative, non-invasive method for the control of pythiosis in humans and animals (Loreto et al., 2014). The initial experiments were conducted by Miller (1981), who obtained an immunobiologic product from killed ultrasonicated *P. insidiosum* mycelium. Currently, other researchers have made modifications to the original formula to increase the efficacy and safety of immunotherapy (Mendoza et al., 2003, Santurio et al., 2003).

In Brazil, a immunotherapeutic product named PitiumVac®, patented and produced by the Laboratório de Pesquisas Micológicas of the Federal University of Santa Maria and by the Brazilian Corporation of Agricultural Research (EMBRAPA-PANTANAL), has been used for more than two decades against equine pythiosis. The efficacy of the product ranges from 50 to 83% in equines, whereas 100% of efficacy has been observed in bovines. Nonetheless, the results in dogs and cats are disappointing (Santurio et al., 2006). This variation in success rate between species may be related to the late diagnosis of the disease in companion animals, which impairs a strong response of the immune system to the antigens contained in the immunotherapy (Gaastra et al., 2010). Nonetheless, there are cases of successful treatment when associated to surgical procedure and/or chemotherapy (Pereira et al., 2013).

In humans, immunotherapy was first successfully used in 1998 in a thalassemic, 14 year-old, Thai boy, after failure of surgery and antimycotic therapy (Thitithanyanont et al., 1998). In a clinical trial of patients with vascular pythiosis, Wanachiwanawin et al. (2004) reported the use of immunotherapy as a salvage therapy.

The chances of cure using immunotherapy increase with the early diagnosis and beginning of the treatment. It is a consensus that lesions with more than 60 days of evolution, i.e., chronic, have a change of cure from 20 to 40%, whereas in lesions with less than 20 days the chances of cure are around 100% (Loreto et al., 2014). The response to immunotherapy is also dependent of the type of antigen used, as demonstrated by Santurio et al. (2003), who evaluated three different protocols of antigen obtaining, vortexed, sonicated and a mixed product. Rabbits receiving the vortexed immunotherapic were most effectively protected, showing a decrease in the area of coastal nodules due to *P. insidiosum* infection by 71.8% after 26 weeks of evaluation. The inefficacy observed in the sonicated antigens was attributed to the denaturation of proteins.

The protocol of treatment using PitiumVac® consists of subcutaneous injections at 14-day intervals until a complete healing of the lesion is observed. A small reaction at the injection site is often observed, tending to disappear in two weeks. The number of doses is variable, and in some horses an increase of the efficacy is observed in weekly applications (Loreto et al., 2014). The treatment period of immunotherapy depends upon the size, localization, time from beginning of the disease and individual response of each animal. In an epidemiology study of equine pythiosis in the Pantanal region, Santos et al. (2014) reported efficacy of 79.41 and 84.62% in the use of immunotherapy alone and immunotherapy associated to surgery, respectively. Other interesting report involving a facial case of equine pythiosis with 60 days of evolution was reported by Santos et al. (2011a). The treatment with five doses at 14-day intervals was effective, and after 70 days of treatment only a small scar was observed. It should be noted that the slow response to the treatment should not be treated as refractory.

The immunological mechanisms involved in the immunotherapy are not completely understood. According to Miller (1981), the progressive characteristic of the disease in immunocompetent horses suggests an inadequate or blocked immune response. The authors suggest that even though the hyphae are antigenic, they are not recognized by the host due to the exacerbated immune response (Santurio et al., 2006).

A likely explanation for the mechanism of action of immunotherapy is related to the change in the immune response of the host. During the infection, the dendritic cells of the host recognize the exoantigens present in the outer surface of *P. insidiosum* hyphae. These cells release interleukin-4 (IL-4) which stimulates naïve T-helper lymphocytes (Th0) to trigger a Th2-driven immune response, releasing more IL-4 and also IL-5. The presence of IL-4 blocks the Th1 response and stimulates the

production of IgE, IgM and IgG by the B lymphocytes. The presence of IL-5 and IgE mobilizes eosinophils and mast cells to the lesion site that later degranulate over *P. insidiosum* hyphae, a phenomenon known as "Splendore-Hoeppli". This mechanism results in the production of structures named "kunkers", which are observed only in horses. Hyphae of the oomycete are kept viable within the "kunkers", releasing great quantities of exoantigens and locking the immune response in a Th2 fashion. This seems to be an evolutionary strategy of the microorganism. The antigens present in immunotherapy differ from those of the natural infection. Upon injection of immunotherapy, dendritic cells recognize the intracellular antigens and release interferon-γ (IFN-γ), which stimulates Th0 lymphocytes to trigger a proinflammatory immune response (Th1). This response is characterized by the presence of IFN-γ and IL-2, which stimulates the production of cytotoxic lymphocytes and macrophages that could eliminate the pathogen from the infected tissues. It is hypothesized that immunotherapy also releases B cells and protective IgG classes that could protect the host for short period of times (Mendoza & Newton, 2005, Santurio et al., 2006, Gaastra et al., 2010, Loreto et al., 2014). It is important to note that the increase in the levels of anti-*P. insidiosum* antibodies are associated with the positive response to treatment (Loreto et al., 2014).

Santos et al. (2011d) described a case of reinfection in a foal after two years of the successful immunotherapeutic treatment. The lesion appeared in a different anatomical region and it was bigger than the first. The response to the second treatment was better in comparison to the first approach, using less number of doses. This could have been due to the shorter period until the beginning of treatment or to an immunological memory that was insufficient to prevent the reinfection but able to aid in the new treatment. This case highlights that the protective antibodies decrease after the treatment, and the animal can be infected again if kept in the same environment (Loreto et al., 2013). Notwithstanding, the use of adjuvants could enhance the preventive action of immunotherapy, as demonstrated in rabbits with pythiosis (Leal et al., 2002).

Immunotherapy against *P. insidiosum* infections has been used with relative success, mainly in horses. Indeed, it is a consensus that an effective treatment of pythiosis should include immunotherapy, surgery and chemotherapy using a combination of antifungals and/or antibiotics, as discussed before.

Photodynamic therapy

The photodynamic therapy (PDT) is a therapeutic approach that has been recently introduced against pythiosis with favorable results (Pires et al., 2014). The technique is based on the interaction between a dye and light, at a specific wavelength, in the presence of oxygen to cause cell death. Under irradiation, the dye, called photosensitizer, reacts with the molecular oxygen producing reactive oxygen species (ROS) that are highly toxic for cells. The PDT has been used with success against bacteria, fungi, virus and parasitic infections. One of the advantages of the technique is the low probability of selection of resistant microorganisms, once that resistance against ROS is almost impossible to occur (Pires, 2012).

In humans, the PDT is used in a number of therapies, as in the treatment of neoplasms, non-neoplastic dermatitis such as photoaging, basal cell carcinoma and Bowen's disease. This technique also permits the simultaneous treatment of multiple tumors, with fast recover and good aesthetic result (Issa & Manela-Azulay, 2010). The use of PDT has been described to be effective in inactivating *P. insidiosum* both in vitro and in vivo, and may represent a new possibility for the treatment of pythiosis (Pires et al., 2013). The photosensitizer agent was applied at the lesion site in four horses, in two sessions. The authors reported reduction of the lesion size, appearance of healing areas and recover of the limb movement. Pires et al. (2013) evaluated the PDT in vitro and in rabbits with experimental pythiosis. The photosensitizers Photogem and Photodithazine were studied in vitro using laser irradiation. The inhibition rate ranged from 60 to 100% and showed better results in comparison to the use of amphotericin B. In the in vivo study, six rabbits with pythiosis were treated with the same photosensitizers. The results were also very encouraging.

A more detailed study on the mechanisms of action of PDT against *P. insidiosum* was conducted by Pires et al. (2014). The authors exposed *P. insidiosum* mycelium against three photosensitizers, methylene blue, Photogem and Photodithazine. Among the agents, Photodithazine showed more homogenous distribution inside the cell and an inhibition of the growing rate over 98%, which makes this photosensitizer an interesting option for clinical treatment of pythiosis. The PDT also has the advantage to be used as many times as necessary, including in debilitated animals. On the other hand, it is necessary to surgically remove extreme granulation tissue because of limited light penetration (Pires, 2012). Further studies involving naturally infected animals are needed to establish an effective PDT protocol for the treatment of *P. insidiosum* infections.

Conclusion

Pythiosis is a devastating disease with high indexes of morbidity and mortality. The disease deserves attention in the human and animal medicine because of the need of incipient diagnosis and treatment to obtain favorable prognosis. The protocols used in the management of pythiosis are characterized by the variation in the success rates. Therefore, more studies are needed to increase the effectiveness of the current treatment options. A bottleneck for these experiments is that the disease is only reproducible in rabbits, which are expensive,

time-consuming and require staff with expertise in the disease.

References

ÁLVAREZ, J.C., VILORIA, M.V., AVOLA, S.P. Pitiose cutânea em equinos: uma revisão. **Revista CES Medicina Veterinaria y Zootecnia**, Colômbia, v.8, n.1, p. 58-67, 2013.

ÁLVAREZ, J.A.C., VILORIA, M.V., PERDOMO, S.C. Frecuencia de presentación de Pythiosis cutânea bovina (*Pythium insidiosum*) em três explotaciones ganaderas de Córdoba, Colombia. **Revista CES Medicina Veterinaria y Zootecnia**, Colômbia, v.7, n.2, p.47-54, 2012.

AMARAL, L.A., MEIRELLES, M., CURCIO, B., MARTINS, C.F., NOGUEIRA, C.E.W. Relato de pitiose equina com envolvimento visceral. **A Hora Veterinária**, Porto Alegre, ano 32, n.192, p.32-35, 2013.

ARGENTA, J.S., ALVES, S.H., SILVEIRA, F., MABONI, G., ZANETTE, R.A., CAVALHEIRO, A.S., PEREIRA, P.L., PEREIRA, D.I.B., SALLIS, E.S.V., POTTER, L., SANTURIO, J.M., FERREIRO, L. In vitro and in vivo susceptibility of two-drug and three-drug combinations of terbinafine, itraconazole, caspofungin, ibuprofen and fluvastatin against *Pythium insidiosum***. Veterinary Microbiology**, v.157, p.137-142, 2012.

ARGENTA, J.S., SANTURIO, J.M., ALVES, S.H., PEREIRA, D.I.B., CAVALHEIRO, A.S., SPANAMBERG, A., FERREIRO, L. In vitro activities of voriconazole, itraconazole, and terbinafine alone or in combination against *Pythium insidiosum* isolates from Brazil. Antimicrobial **Agents and Chemotherapy**, Washington, v.52, n.2, p. 767-769, 2008.

DIAS, D.P.M., DÓRIA, R.G.S., PEREIRA, R.N., CANOLA, P.A., DI FILIPPO, P.A. Pitiose cutânea equina de localização atípica tratada topicamente com solução de anfotericina B e DMSO. **Acta Scientiae Veterinariae**, v.40, n.4, p.1-8, 2012.

DÓRIA, R.G.S., CANOLA, P.A., RIBEIRO, G., DI FILIPO, P.A., DIAS, D.P.M., VALADÃO, C.A.A. Hipotireoidismo iatrogênico em equino decorrente de excesso de iodo: relato de caso. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.60, n.3, p.521-524, 2008.

DÓRIA, R. G. S., FREITAS, S. H., LINARDI, R. L., MENDONÇA, F. S., ARRUDA, L. P., BOABAID, F. M., VALADAO, C. A. A. Treatment of pythiosis in equine limbs using intravenous regional perfusion of amphotericin B. **Veterinary Surgery**, v.41, p.759-765, 2012.

GAASTRA, W., LIPMAN, L.J.A., DE COCK, A.W.A.M., EXEL, T.K., PEGGE, R.B.G., SCHEURWATER,J., VILELA, R., MENDOZA, L. *Pythium insidiosum*: An overview. **Veterinary Microbiology.** Amsterdam, v.146, n.1-2, p.1-16, 2010.

ISSA, M.C.A., MANELA-AZULAY, M. Terapia fotodinâmica: revisão da literatura e documentação iconográfica. **Anais Brasileiros de Dermatologia**, v.85, n.4, p.501-511, 2010.

KRAJAEJUN, T., SATHAPATAYAVONGS, B., CHAIPRASERT, A., SRIMUANG, S. Do you know human pythiosis? **Journal of Infectious Disease and Antimicrobial Agents**, v.25, n.1, p.45-51, 2008.

LEAL, A.T., LEAL, A.B.M., FLORES, E.F., SANTURIO, J.M. Pitiose. **Ciência Rural**, Santa Maria, v.31, n.4, p. 735-743, 2001.

LEAL, A.T., SANTURIO, J.M., LEAL, A.B.M., PINTO, A.M., GRIEBELER, J, FLORES, E.F., FERREIRO, L., CATTO, J.B. Resposta sorológica de coelhos imunizados com antígenos de *Pythium insidiosum* associados a diferentes adjuvantes. **Ciência Rural**, Santa Maria, v.32, n.6, p.1027-1032, 2002.

LORETO, E.S., TONDOLO, J.S.M., ALVES, S.H., SANTOS, C.E.P., JULIANO, R.S., ROCHA, J.P.S., SANTURIO, S.M. Pitiose equina: aspectos clínico-epidemiológicos, diagnósticos e imunoterápicos. **Revista CFMV**, Brasília, DF, v.19, n.58, p.58-65, 2013.

LORETO, É.S., TONDOLO, J.S.M., ZANETTE, R.A., ALVES, S.H., SANTURIO, J.M. Update on pythiosis immunobiology and immunotherapy. **World Journal of Immunology,** v.4, n.2, p.88-97, 2014.

MAHL, D.L., JESUS, F.P.K, LORETO, É.S., ZANETTE, R.A., FERREIRO, L., PILOTTO, M.B., ALVES, S.H., SANTURIO, J.M. In vitro susceptibility of *Pythium insidiosum* isolates to aminoglycoside antibiotics and tigecycline. **Antimicrobial Agents and Chemotherapy**, v.56, p.4021-4023, 2012.

MENDOZA, L., MANDY, W., GLASS, R. An improved *Pythium insidiosum*-vaccine formulation with enhanced immunotherapeutic properties in horses and dogs with pythiosis. **Vaccine**, v.21, p.2797-2804, 2003.

MENDOZA, L., NEWTON, J.C. Immunology and immunotherapy of the infections caused by *Pythium insidiosum*. **Medical Mycology**, v.43, n. 6, p.477-86, 2005.

PEREIRA, D.I.B., SANTURIO, J.M., ALVES, S.H., ARGENTA, J.S., PÖTTER, L., SPANAMBERG, A., FERREIRO, L. Caspofungin in vitro and in vivo activity against Brazilian *Pythium insidiosum* strains

isolated from animals. **Journal of Antimicrobial Chemotherapy**, v.60, p. 1168-1171, 2007.

PEREIRA, D.I.B., SANTURIO, J.M., ALVES, S.H., AZEVEDO, M.I., SILVEIRA, F., COSTA, F.F., SALLIS, E.S.V., POTTER, L., FERREIRO, L. Comparison between immunotherapy and caspofungin as agents to treat experimental pythiosis in rabbits. **Journal de Mycology Médicale**, v.18, p.129-133, 2008.

PEREIRA, D.I.B., BOTTON, S.A., AZEVEDO, M.I., MOTTA, M.A.A., LOBO, R.R., SOARES, M.P., FONSECA, A.O.S., JESUS, F.P.K., ALVES, S.H., SANTURIO, J.M. Canine gastrointestinal pythiosis treatment by combined antifungal and immunotherapy and review of published studies. **Mycopathologia**, v.176, p.1-7, 2013.

PIRES, L. **Terapia fotodinâmica para inativação do *Pythium insidiosum* – estudo in vitro e in vivo**. 148f. (Dissertação de mestrado em Ciências) – Universidade de São Paulo, São Carlos, Brasil, 2012.

PIRES, L., BOSCO, S.M.G., BAPTISTA, M.S., KURACHI, C. Photodynamic Therapy in *Pythium insidiosum* – An in vitro study of the correlation of sensitizer localization and cell death. **Plos One**, v.9, n.1, p.1-8, 2014.

PIRES, L., BOSCO, S.M.G., SILVA J.R., N.G., KURACHI, C. Photodynamic therapy for pythiosis. **Veterinary Dermatology**, v.24, p. 130-e30, 2013.

RODRIGUES, C.A., LUVIZZOTO, M.C.R., ANHESINI, C.R., WIENEN, I.P., NOGUEIRA, G.M., LOUREIRO, M.G., AGUIAR, A.J.A. Avaliação da excisão cirúrgica ampla, associada à administração oral de iodeto de potássio no tratamento de equinos acometidos de pitiose cutânea severa nas extremidades dos membros. **Brazilian Journal of Veterinary Research and Animal Science**, v.41, p.229, 2004.

SANTOS, C.E.P., JULIANO, R.S., SANTURIO, J.M., MARQUES, L.C. Eficácia da imunoterapia no tratamento de pitiose fácil em equino. **Acta Scientiae Veterinariae**, v.39, n.1, p. 1-5, 2011a.

SANTOS, C.E.P., SANTURIO, J.M., COLODEL, E.M., JULIANO, R.S., SILVA, J.A., MARQUES, L.C. Contribuição ao estudo da pitiose cutânea em equídeos do Pantanal norte, Brasil. **ARS VETERINARIA**, Jaboticabal, SP, v. 27, n.3, p. 134-140, 2011b.

SANTOS, C.E.P., SANTURIO, J.M., MARQUES, L.C. Pitiose em animais de produção no Pantanal Matogrossense. **Pesquisa Veterinária Brasileira**, v.31, n.12, p. 1083-1089, 2011c.

SANTOS, C.E.P., MARQUES, L.C., ZANETTE, R.A., JESUS, F.P.K., SANTURIO, J.M. Does immunoterapy protect equines from the reinfection by oomycete *Pythium insidiosum*? **Clinical and Vaccine Immunology**, Washington, v.18, n.8, p. 1397-1399, 2011d.

SANTOS, C.E.P., UBIALI, D.G., PESCADOR, C.A., ZANETTE, R.A., SANTURIO, J.M., MARQUES, L.C. Epidemiological Survey of equine pythiosis in the brazilian Pantanal and nearby areas: results of 76 cases**. Journal of Equine Veterinary Science**, v.34, p. 270-274, 2014.

SANTURIO, J.M., ALVES, S.H., PEREIRA, D.B., ARGENTA, J.S. Pitiose: uma micose emergente. **Acta Scientiae Veterinariae**, Porto Alegre, v. 34, n.1, p.1-14, 2006.

SANTURIO, J.M., LEAL, A.T., LEAL, A.B.M., FESTUGATTO, R., LUBECK, I., SALLIS, E.S.V., COPETTI, M.V., ALVES, A.H., FERREIRO, L. Three types of immunotherapics against pythiosis insidiosi developed and evaluated. Vaccine, v.21, p. 2535-2548, 2003.

ZANETTE, R.A, JESUS, F.P.K., PILOTTO, M.B., WEIBLEN, C., PÖTTER, L., FERREIRO, L., ALVES, S.H., SANTURIO, J.M. Micafungin alone and in combination therapy with deferasirox against *Pythium insidiosum*. **Journal de Mycologie Médicale**. Online. Doi: 10.1016/j.mycmed.2014.09.002, 2015.