

CONTRIBUTION TO THE STUDY OF CUTANEOUS PYTHIOSIS IN EQUIDAE FROM NORTHERN PANTANAL, BRAZIL

CONTRIBUIÇÃO AO ESTUDO DA PITIOSE CUTÂNEA EM EQUIDEOS DO PANTANAL NORTE, BRASIL¹

C. E. P. SANTOS¹, J. M. SANTURIO², E. M. COLODEL³, R. S. JULIANO⁴,
J. A. SILVA⁵, L. C. MARQUES^{6*}

SUMMARY

Clinical and epidemiological study of pythiosis in the Equidae family is reported for three herds from the Northern region of Pantanal, Mato Grosso. The diagnosis of pythiosis was confirmed by pathological examinations and serological tests. Sick animals were submitted to immunotherapy as basis treatment. Clinical and epidemiological data mostly showed similarity with other regions. From a total of ten treated animals, immunotherapy was effective with complete cure in 70% of the cases, and reached 90% when combined with surgery, being a feasible choice of treatment for animals in the field.

KEY-WORDS: Immunotherapy. Pantanal. Pythiosis.

RESUMO

Estudo clínico, epidemiológico e terapêutico da pitiose em equideos é relatado em três rebanhos distribuídos no Pantanal Norte de Mato Grosso. O diagnóstico de pitiose foi confirmado por exames patológicos e sorológicos. Animais enfermos foram submetidos à imunoterapia como tratamento base. O monitoramento dos casos tratados de pitiose foi realizado por um período de três anos. Os dados clínicos e epidemiológicos em sua maioria apresentam similitude com outras regiões. De um total de dez animais tratados, a imunoterapia se mostrou eficaz na cura cabal de 70% dos casos e associando à cirurgia em 90%, sendo uma alternativa de tratamento exequível em animais de campo.

PALAVRAS-CHAVE: Imunoterapia. Pantanal. Pitiose.

¹ Doutorando do Programa de Pós-Graduação em Medicina Veterinária Unesp-Jaboticabal. Universidade Federal de Mato Grosso. Faculdade de Agronomia, Medicina Veterinária e Zootecnia. Departamento de Clínica Médica Veterinária. Av. Fernando Correa da Costa 2367. Bairro Boa Esperança. Cuiabá – MT. CEP 78060-900.

² Universidade Federal de Santa Maria. Laboratório de Pesquisas Micológicas (LAPEMI/UFSM). RS.

³ Universidade Federal de Mato Grosso. Laboratório de Patologia Veterinária (LPV/UFMT). Cuiabá-MT.

⁴ Empresa Brasileira de Pesquisa Agropecuária - Embrapa Pantanal CPAP. Corumbá-MS.

⁵ Empresa Matogrossense de Pesquisa, Assistência e Extensão Rural S/A. Poconé-MT.

⁶ Departamento de Clínica e Cirurgia Veterinária. Faculdade de Ciências Agrárias e Veterinárias (FCAV). UNESP-Jaboticabal. Jaboticabal SP.

*Corresponding author: lmarques@fcav.unesp.br

INTRODUCTION

Pythiosis, “swamp cancer”, caused by the oomycete *Pythium insidiosum* typically results in lesions in horses and other animals, including humans. The zoospores live in water, by the leaves and plant debris. In Colombia, this disease is popularly known as “espúndio equino”, and in Brazil as “ferida brava” and “mal dos pântanos”(LACAZ et al., 2002). Still, in the northern and southern wetlands it is also known as “ferida da moda” (SANTURIO et al., 2004).

The Pantanal region is a floodplain of approximately 140,000 km² located in the Midwest region of Brazil and stands as the largest wetland in the world (SILVA & ABDON, 1998). In this region, disease is one of the bottlenecks that determines major economic losses. Horses are lost if the disease is not diagnosed in time to promote adequate treatment, since it can evolve unfavorably according to Leal et al. (2001) and culminate in the deaths of infected animals.

In horses, *P. insidiosum* causes granulomatous and ulcerative skin lesions with progressive characteristic, most often located in the distal portion of the members and to a lesser extent, in the ventral thoracoabdominal region, chest and head (CHAFFIN et al., 1995; LUVIZARI et al., 2002; FREI Jr. et al., 2007; CESCÓN et al., 2008).

This study aims to describe the clinical, epidemiological and therapeutic aspects of pythiosis in horses from the northern region of Pantanal in Brazil.

MATERIAL AND METHODS

This is a prospective study of pythiosis in horses distributed in three farms in the municipality of Pocone, Northern Pantanal of Mato Grosso, during three cycles of flooding (2007 a 2009).

The monitoring of the animals was performed every 14 days in the first four months, followed by once a month until six months and every six months until 36 months.

The diagnosis of suspected cases was confirmed by immunoenzymatic serological tests and/or incisional biopsies of the lesions. To perform the first biopsies, the animals were sedated using acepromazine 0.1 mg/kg and xylazine 1 mg/kg. The collected tissues were fixed in 10% formalin, embedded in paraffin and cut into five-micrometer thick sections. These sections were stained using hematoxylin and eosin (HE) and methenamine-silver (GMS) (PROPHET et al. 1992) or immunohistochemistry, performed at the Laboratory of Veterinary Pathology (LPV), Federal University of Mato Grosso according to the Labeled Streptavidin Biotin (LSBA) method by Brown et al. (1988) and Gimeno et al. (1999), with polyclonal primary antibody anti-*Pythium insidiosum* produced in rabbits and processed by Mycological Research Laboratory (LAPEMI).

Serum was obtained from the blood samples collected by puncturing the jugular vein, using hypodermic needles 40 x 12 mm. The search for anti-

Pythium specific antibodies in horse serum was performed using the indirect ELISA technique (SANTURIO et al., 2006) at the LAPEMI, Universidade Federal de Santa Maria.

After infection by *Pythium* was confirmed by one or both techniques (ELISA and/or histopathology), ten animals underwent immunotherapy. (Pitium-Vac®), a product patented by LAPEMI/UFMS, was injected subcutaneously at 14-day intervals. If the treatment was not responsive after the fifth dose (approximately two and a half months of treatment), a partial surgical excision of the affected area was performed, followed by three more applications of immunotherapy, at the same time interval. The biopsy was not performed in one mare due to its advanced pregnancy stage, and the presumptive pythiosis diagnosis was based on epidemiological, clinical and macrobiological aspects of the lesion, and the presence of anti-*Pythium* serum antibodies (ELISA). One of the studied animals was not treated due to senility, severe weakness and advanced stage of disease (over 9 months).

RESULTS AND DISCUSSION

Table 1 shows the profiles of the equidae and evolution of the cutaneous pythiosis cases in three properties in Northern Pantanal, Mato Grosso, Brazil from 2007 to 2009. We investigated suspect pythiosis lesions in male and female horses aged between 18 months and 16 years. The affected horses were of non defined (5) and Pantaneira (5) breed. One case was observed in mule. Pythiosis occurred more frequently in horses from the Northern Pantanal, Mato Grosso and of the Pantaneira and mixed breed. Leal et al. (2001) claim that the presence of the disease is not related to breed, age or sex of the horses. Possibly this finding is justified by the adaptation and population dominance of these animals in flooded fields of the region.

Based on the INMET (2009) climate data for the region, three pythiosis cases occurred at the beginning of the rainy season (November- December) and eight cases occurred at height of the rainy season (January-March). This case distribution was similar to that occurred elsewhere (LEAL et al. 2001), that is, more cases at the height of the floods when weather conditions favor the growth of the microorganism in the environment.

The temperature during these periods varied between 28 and 36°C. The production of zoospores requires temperatures between 30 and 40°C and water accumulation in wetlands and ponds (MILLER & CAMPBELL, 1982). These climatic and environmental characteristics are found practically throughout the year in the Northern Pantanal region of Mato Grosso, obviously increasing the number of cases of the susceptible population that inhabits the region.

In the farms, there were several places where slow draining ponds are formed and abundant vegetation where the horses often remained. The evolution of the lesions from the moment of the approach, varied between 7 and 15 months. In two properties, where

Table 1 - Profile of the animals affected by pythiosis and its evolution in three farms in the Northern region of Pantanal (2007 to 2009).

Animal profile			Lesion profile			Therapeutic profile	
Species	Age (years)	Sex	Local	presentation	Evolution time (days)	Treatment - N° doses	Evolution
Equine	2,5	Male	Limb	Single	45	A-3	Healed
Equine	1,5	Male	Limbs	Multiple	60	B-6	Healed
Equine	2	Female	Limb	Single	21	A-6	Healed
Equine	6	Female	Abdomen	Single	15	A-2	Healed
Equine	3,5	Male	Limb	Single	90	B-8	Healed
Equine	3	Female	Limbs	Multiple	30	A-2	Healed
Equine	16	Female	Perineum	Multiple	270	C	Died
Equine	9	Male	Limbs	Single	180	A-6	Healed
Mule	5	Female	Limb	Multiple	↑360	A-5	Died
Equine	2	Female	Thorax	Single	90	A-5	Healed
Equine	2	Female	Limb	Single	30	A-5	Healed
Equine	3	Female	Limb	Multiple	7	A-3	Healed

A - Immunotherapy alone

B - Immunotherapy associated with surgical excision

C - Not treated

most of the cases (9) were found, the herds remained on clay type soil. Although, it is necessary to perform more studies in other soils, the hypothesis that this type of soil favors or increase the incidence of the disease may be related to the fact that these soils are usually poorly permeable to water, thus favoring the accumulation and the increase of aquatic vegetation, ideal substrate for proliferation of the oomycete, producing mobile zoospores that according to Santurio et al. (2006) constitute the infective form of *P. insidiosum*.

Granulation tissue associated with *P. insidiosum* reaches diameters of 15 cm between two to three weeks average, with progressive and rapid growth, but slows down when the lesions become larger than 30 cm. The animals, except one (who died after the clinical approach), were reportedly in satisfactory general health at the beginning of the study, however, as the lesions evolved, weight loss was observed. Itching and self-injury were observed in all horses and two also had claudication. The lesions were predominantly on the limbs, most distally located. These were characterized by rapid evolving skin lesions, ulcerated, with abundant serosanguineous secretion and itching, and sometimes “kunkers” were found in the sinus, sporadically ejected by digital

pressure. The locations of the lesions, as well as morphological features, were in agreement with Leal et al. (2001), Sallis et al. (2003) and Frey Jr. et al. (2007).

Histopathological findings were homogenous, predominantly around necrotic areas, infiltrates of eosinophils, neutrophils and mononuclear cells with proliferation of conjunctive tissue and neovascularization. Hyphae-like structures were visualized within the necrotic foci. The borders of hyphae were stained black by silver impregnation (Grocott method), these structures, slightly septate, were morphologically consistent with *P. insidiosum*. In immunohistochemical marking, a positive finding was crucial to confirm the diagnosis, because, according to Reis Júnior & Nogueira (2002) *P. insidiosum* hyphae has specific epitopes of the Stramenopila kingdom, therefore different from other deep mycoses. Still, the analyzed tissue fragments showed no evidence of lesions suggesting habronema or sarcoid. Sallis et al. (2003) reported that samples received in laboratory routine, had a presumptive diagnosis of cutaneous habronema or sarcoid; however, further laboratory tests confirmed pythiosis diagnosis.

Additionally, the animals were positive for the indirect ELISA test. Studies have shown that ELISA is very effective in diagnosing pythiosis, has greater



Figure 1 - (A) Circumscribed pythiosis lesion in a horse at 45 days of evolution. (B) Same animal 14 days after the first subcutaneous immunotherapy Pitium-Vac®. The edges of the wound became less deep and the wound is drier. (C) 14 days after the second application of Pitium-Vac®. (D) the same horse in complete remission after three applications.



Figure 2 - (A) Elliptical pythiosis lesion with approximately three months evolution. (B) after the 5th subcutaneous application of Pitium-Vac® exhibiting a parched wound; however, refractory and developing extensive granulation tissue. (C) after surgical excision followed by three more doses of the immunotherapy. (D) the same horse healed after 8 more doses applied every 14 days.

sensitivity and specificity similar to immunodiffusion (SANTURIO et al., 2004). Currently, it is considered the most efficient immunological technique for early diagnosis, showing sensitivity, specificity and efficiency of 97.72%, 90.27% and 93.1%, respectively (SANTURIO et al., 2006). This test consolidates the diagnosis at an early stage, it is safe and causes no stress for the animals. We used only this additional test as a support diagnostic for the mare in advanced stage of pregnancy.

Except for the horse that died a week after the initial approach, every other horse underwent immunotherapy (n=10), and satisfactory response was observed in 70% of the cases (Figure 1). Animals that did not respond satisfactorily to immunotherapy were also submitted to surgical excision of granulation tissue (Figure 2). Thus, immunotherapy combined or not with surgical excision, healed 90% of cases. Conservative treatments, surgical and both, clinical and surgical, have been proposed for pythiosis with varying success. According to Hubert & Grooters (2002) the most common treatment consists of total excision of the granuloma, combined with specific immunotherapy. Other drugs such as potassium iodide, amphotericin B and immunotherapy have been used too, with varying results; however, the cure in the latter ranged from 43 to 83% for pythiosis cases (MENDOZA et al., 1996; RODRIGUES & LUVIZOTO, 2000; MONTEIRO 1999 apud SALLIS et al., 2003; SANTURIO et al., 2004; BIAVA et al., 2007 and FREY Jr. et al., 2007). There was no negative influence of immunotherapy in animal pregnancy with respect to parturition and foals. Edema and increased volume of variable length on the local of immunotherapy, were transient adverse reactions that evolved to complete remission without sequels.

Therapeutic efficacy is strongly coupled with disease temporality, therefore, when the disease is old, immunotherapy results are not good (LACAZ et al., 2002). The cases followed a pattern regarding recovery time that was relatively proportional to granuloma development plus the immunotherapy time needed for complete recovery. For example, a horse with pythiosis that had been evolving for 90 days and treated for more than 60 days took about five months for the complete healing of the skin wounds. These results call attention, since the slow response to treatment can be interpreted as refractory and determine withdrawal.

After treatment, the horses were inspected during two more flooding cycles and one horse had reinfection. Initially, the treated lesions were on the limb, but pythiosis reinfection after two years occurred in large ventral lesions on the abdomen and were again responsive to immunotherapy. It seems that even animals that already had the disease did not develop lasting immunity. This fact is reinforced by studies in which immunotherapy was not effective as prevention, thus having only healing effect (SANTURIO et al., 2001; MENDOZA & NEWTON, 2005).

Three deaths occurred during the studies, one in each herd, related to old or multiple wounds. One senile horse died untreated with a chronic injury after

nine months, a mule with two different lesion sites that were treated, and while the wound on the limb went into remission during treatment, the chest wound continued to evolve associated with severe weight loss. The third horse also had multiple lesions that did not respond to immunotherapy alone. Although the wounds were completely healed when surgical technique was combined with immunotherapy, the horse lost weight gradually and had nonspecific infarction of mammary lymph node and it was found dead. Leal et al. (2001), Frey Jr. et al. (2007) and Maciel et al. (2008) claim that animals that are left untreated or treated late, in general die, possibly due to release of cytokines, such as tumor necrosis factor, causing severe systemic and metabolic injuries (KLEIN & HOREJSI, 1997). The exact cause of death could not be determined, since the investigation was impaired due to the advanced state of autolysis and attack from natural predators at the time the carcasses were found.

CONCLUSIONS

The use of immunotherapy as an alternative treatment of pythiosis, associated or not with surgical procedure is promising and applicable on the field. Adequacy of management and observational practices to help spot cutaneous lesions as well as other diseases early are essential for the maintenance of a healthy herd of horses in the Northern region of Pantanal. The temporal evolution and early diagnosis of pythiosis in horses is fundamental for the success of the immunotherapy approach.

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