DOI: 10.5433/1679-0359.2019v40n6Supl2p3079

The use of fluconazole associated with surgical excision in the treatment of equine cutaneous pythiosis

Uso do flucozanol associado à excisão cirúrgica no tratamento da pitiose cutânea equina

Armando de Mattos Carvalho[1*]; Thayanne Caroline Pereira Munhoz[2]; Hugo Shisei Toma[3]; Jéssica Pamela Velasco dos Santos[2]; Luiz Gustavo Schneider de Oliveira[4]; Fabiana Marques Boabaid[4]; Andressa Batista da Silveira Xavier[5]; Glaucia Denise Kommers[6]

Abstract

Cutaneous pythiosis is an important disease affecting horses raised in regions with a hot climate and water stagnation, occurring throughout all regions of Brazil. The disease progresses rapidly, and treatment for chronic lesions is challenging. The purpose of this study was to evaluate the efficacy of fluconazole after surgical excision and electrocauterization of cutaneous lesions in horses naturally infected with *Pythium insidiosum*. We treated 10 horses with cutaneous pythiosis, whose diagnoses were confirmed by histopathological and immunohistochemical examinations. After surgical debridement of lesion, animals received fluconazole orally for 21 days, in combination with topical treatment for the wounds. After 7 days of therapy, there was decreased serosanguineous secretion and no kunkers in the wounds in all horses. All lesions healed completely after therapy, and there were no recurrences 10 months after discharge. When associated with surgical excision, oral fluconazole therapy was an effective treatment in clinical cases of equine cutaneous pythiosis.
Key words: Therapy. Antifungal medication. Surgery. *Pythium insidiosum.*

Resumo

Pitiose cutânea é uma enfermidade importante que acomete equinos criados em regiões de clima quente e com presença de água estagnada, sendo reportada em todas as regiões do Brasil. Possui evolução rápida e o tratamento de lesões crônicas ainda é considerado um desafio. O objetivo do presente trabalho foi a avaliação da eficácia do fluconazol após a excisão cirúrgica e termocauterização de feridas cutâneas de equinos naturalmente infectados por *Pythium insidiosum*. Para o estudo foram utilizados 10 equinos com pitiose cutânea, com diagnóstico confirmado por meio da avaliação histopatológica e da imuno-

[1] Prof., Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, MG, Brasil. E-mail: armandodvm@gmail.com
[2] Discentes, Faculdade de Medicina Veterinária, Universidade de Cuiabá, UNIC, Cuiabá, MT, Brasil E-mail: taty_caroline_munhoz@hotmail.com; jessicavelasco_santos@hotmail.com
[3] Prof., Departamento de Medicina Veterinária, Universidade Federal de Lavras, UFLA, Lavras, MG, Brasil. E-mail: hugost@ig.com.br
[4] Profs., Faculdade de Medicina Veterinária, Universidade de Cuiabá, UNIC, Cuiabá, MT, Brasil. E-mail: luiz.omicron@gmail.com; fabianaboabaid@hotmail.com
[5] Profª, Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, MG, Brasil. E-mail: asilveiravet@gmail.com
[6] Profª, Universidade Federal de Santa Maria, UFSM, Santa Maria, RS, Brasil. E-mail: glaukommers@yahoo.com
[*] Author for correspondence

Received: Nov. 28, 2018 - Approved: July 09, 2019

histoquímica. Após o desbridamento cirúrgico da lesão, os animais receberam fluconazol por via oral durante 21 dias, associado a tratamento tópico da ferida. Em todos os casos, após sete dias do início da terapia foi possível evidenciar diminuição da secreção serosanguinolenta, assim como ausência de *kunkers* na ferida. Todos os animais tiveram cicatrização completa das lesões após a terapia e não houve recidiva após dez meses da alta médica. A terapia com fluconazol administrado por via oral demonstrou ser um tratamento eficaz quando associado à excisão cirúrgica em casos clínicos de pitiose cutânea equina.
Palavras-chave: Terapia. Antifúngico. Cirurgia. *Pythium insidiosum.*

Introduction

Equine cutaneous pythiosis (ECP) has a significant economic impact on the equine agribusiness due to treatment expense, functional loss, and even death of affected animals (MORAES et al., 2013). The disease occurs throughout all regions of Brazil. However, the Pantanal stands out nationally, especially between October and March, because of water stagnation from floods and high temperatures that are typical of the region (SANTOS et al., 2014).

In horses, cutaneous pythiosis is characterized by granulomatous lesions in the subcutaneous and cutaneous tissues, with serosanguineous secretion, intense pruritus, and edema. Lesions develop when animals come into contact with water contaminated by *Pythium insidiosum* zoospores, which penetrate the skin via chemotaxic mechanisms through pre-existing lesions, thereby initiating infection (GROOTERS, 2014). Presumptive diagnosis is based on epidemiological and clinicopathological data and confirmed by immunohistochemical examination (DÓRIA et al., 2014).

Equine cutaneous pythiosis rapidly progresses, and treatment efficacy is influenced by the size and site of lesions, disease duration, and possibly the animal's age and physiological state (GROOTERS, 2014). Surgical excision is the most commonly applied therapy and is preferably combined with drugs such as potassium iodide or antifungal agents such as amphotericin B (DÓRIA et al., 2012, 2015; CARVALHO et al., 2016). We also found reports of therapeutic success with 'Pitium-Vac' immunotherapy (SAMPAIO et al., 2016) and application of corticosteroid triamcinolone acetonide (CARDONA-ÁLVAREZ et al., 2016).

Of the azole antifungals, fluconazole has the least effect on hepatic microsomal enzymes. The minimal plasma binding and greater therapeutic index allow for higher dosages in the treatment of a variety of fungal infections (WALLEN et al., 2016). In horses, the application of fluconazole has successfully treated conidiobolomycosis (TAINTOR et al., 2004), coccidioidomycosis (HIGGINS et al., 2006), and candidiasis (REILLY; PALMER, 1994). We found no description of therapeutic use in ECP, but there are promising *in vitro* results regarding the combined actions of terbinafine with fluconazole against *Pythium insidiosum* (CAVALHEIRO et al., 2009).

In this study, we aimed to evaluate the efficacy and potential side effects of oral administration of fluconazole as a complementary therapy to surgical excision and electrocauterization for the treatment of cutaneous lesions in horses naturally infected with *Pythium insidiosum*. Our hypothesis was that administration of fluconazole, combined with surgical excision, would enable cutaneous lesions to heal completely without any side effects.

Materials and Methods

This study was approved by the Ethics Committee on the Use of Animals of the University of Cuiabá under no. 03/2015 and was developed with the consent of all those responsible for the horses involved. The study included 10 horses, seven females (four pregnant) and three males,

aged between 1.5 y and 8 y (mean 4.9 ± 2.2 y), weighing between 270 and 430 kg (mean 342.5 ± 56.2 kg). The animals were of the Pantaneiro, Mangalarga, Quarter Horse, and Mestizo breeds. They all received medical attention at the University Veterinary Hospital, between March 2015 and April 2017. Their medical histories revealed that they had come into contact with flooded areas, there was an increase in cutaneous volume with ulcerated surfaces, local pruritus, and serosanguineous exudate, and there were yellowish white necrotic masses called kunkers, varying from 2 to 10 mm in diameter. Lesions were found on the abdomen and thoracic and pelvic limbs.

Animals were kept in stalls and fed alfalfa hay, commercial pellet feed and mineral supplementation, and were given *ad libitum* access to water during the period for the study. All horses underwent a physical examination (heart and respiratory rates, capillary refill time, intestinal motility, and rectal temperature). We also measured cutaneous lesions (length and width) with a tape measure and photographed them, and evaluated the anatomical site, disease evolution, and macroscopic appearance, before initiating experimental procedures. We arbitrarily classified the lesions either as small, when the length was ≤ 25 cm, or large, when the length was > 25 cm (Table 1).

Table 1. Classification and characteristics of cutaneous pythiosis lesions in ten horses relative to length, site, and healing time (epithelialization).

Animal	Length (cm)	Location	Healing time (days)
1	38	RPL	90
2	26	LPL	30
3	41	LPL	90
4	26	VRA	21
5	33	VRA	30
6	42	LPL	60
7	24	RTL	120
8	22/14	RPL/LPL	120
9	16	RTL	45
10	24	VRA	21

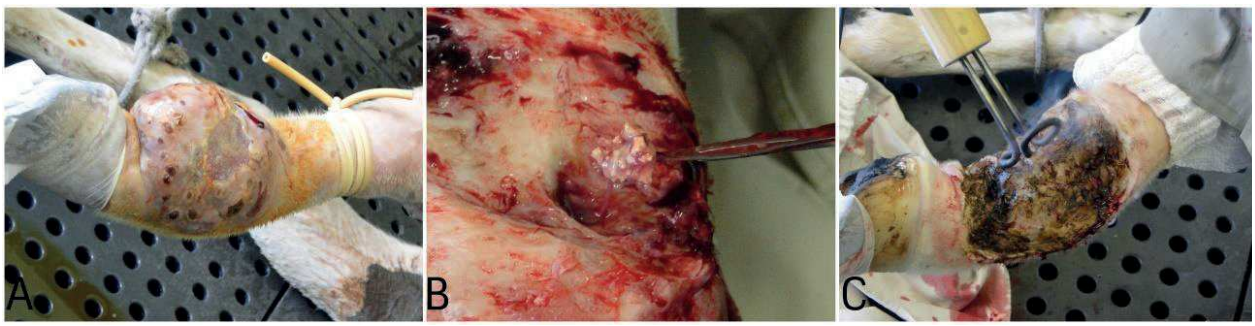
RPL: right pelvic limb; LPL: left pelvic limb; VRA: ventral region of abdomen; RTL: right thoracic limb.

For surgical procedures, animals were fasted for 12 h and premedicated with xylazine (0.5 mg kg^{-1}, IV). After 10 min, we administered ketamine (2 mg kg^{-1}, IV) combined with midazolam (0.1 mg kg^{-1}, IV) in the same syringe, for anesthesia induction. Subsequently, total intravenous anesthesia was induced with continuous infusion of guaiacol glyceryl ether (50 mg ml^{-1}), ketamine (1 mg ml^{-1}), and xylazine (0.5 mg ml^{-1}), at an infusion rate of 1 - 1.5 ml kg^{-1} h^{-1}.

After horses were anesthetized, they were positioned according to the site of the cutaneous lesion and secured with ropes and braces. We washed the lesion and adjacent regions with soap and water and used an antiseptic preparation containing povidone-iodine and tincture of iodine. We utilized a garrote to contain bleeding when the site of the lesion was at the extremity of the limb. Subsequently, exuberant granulation tissue was surgically debrided (Figure 1). We avoided

bone or joint exposure and abdominal penetration during the procedure. Fragments of lesions containing kunkers were collected, stored in 10% formalin solution, and sent for histopathological and immunohistochemical evaluation using anti-*Pythium insidiosum* antibody to confirm diagnosis, based on the technique described by Trost et al. (2009). After surgically removing all of the affected tissue, we applied electrocautery for hemostasis (Figure 1) followed by compression bandages for lesions on the limbs.

Figure 1. Surgical procedure in a horse with pythiosis in the fetlock of right pelvic limb. (A) Use of garrote on the limb to contain bleeding. (B) Presence of kunkers during surgical debridement. (C) Electrocauterization with electric probe after complete excision of the affected tissue.



After recovery from anesthesia, animals received a single oral dose of 10 mg kg^{-1} of fluconazole, diluted in 5 ml of corn glucose syrup to facilitate administration. Once a day for the following 20 days, fluconazole was administered orally in doses of 5 mg kg^{-1}, always diluted in 5 ml of corn glucose syrup. We provided analgesia by administering phenylbutazone for 3 d (2.2 mg kg^{-1}, IV, once daily). We dressed the lesions on the limbs every 3 d with topical 0.1% povidone-iodine and compression bandages, until complete epithelization. Topical 0.1% povidone-iodine were used daily on the abdomen, until they healed completely.

We collected blood samples for complete blood counts and biochemical analyses, to monitor liver and renal functions before treatment and weekly, for a total of 4 weeks. For blood testing, we adopted traditional methods for determining the complete blood count, corpuscular volume, and hemoglobin concentration, using an automatic hematology analyzer (Symex pocH-100iV Diff). We conducted differential white blood cell counts on blood smears stained with a quick Romanowsky-type stain. We determined the actions of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) and urea and creatinine concentrations by kinetic or colorimetric methods, using specific commercial reagents (Bioclin) and spectrophotometer readings (Bioplus 200).

Results and Discussion

All horses presented a history of contact with flooded areas and pruritic cutaneous lesions in the abdomen or thoracic or pelvic limbs, wherein 60% of lesions were classified as large and the rest as small (Table 1). Lesions constituted an increase in volume characterized by exuberant granular tissue with an ulcerated surface, containing serosanguineous secretion and yellowish white necrotic masses, known as kunkers. These findings are similar those previously reported (WATANABE et al., 2015).

Treatment for ECP by surgical debridement of exuberant granulation tissue, associated with oral

administration of fluconazole, for 21 consecutive days was an effective, feasible, and easy method of reducing necrotic areas and controlling the infection process. Although surgical excision is the preferred treatment, lesions often progress to a stage that does not allow for complete resection, and infection recurrence in these cases is common. Thus, surgical excision combined with antifungal therapy increases therapeutic efficacy (DÓRIA et al., 2015), consistent with the therapeutic association observed in the present study.

Equine cutaneous pythiosis is a rapidly progressing disease, and although new therapies have been tested, there is no standard treatment for chronic lesions that have developed over a period of several months, with many clinical cases presenting challenges for veterinarians (GROOTERS, 2014). Therapeutic success is based on the time since disease onset, site and size of the lesion, and speed and intensiveness of therapy (WATANABE et al., 2015). Even with the time between emergence of lesions and beginning of treatment varying from 2 to 5 months (average 3 months), a complete clinical solution was possible for all cases of ECP in this study

Horses accepted oral administration of fluconazole diluted in corn glucose well, which demonstrates the practicality of this method for drug administration. Fluconazole has advantages over other antifungal agents. It has a broad spectrum of activity and a long half-life, shows fewer adverse reactions and only a minor effect on hepatic microsomal enzymes. Bioavailability above the mean inhibitory concentration (8.0 mg ml^{-1}) reported for most fungal pathogens in horses, and a high unbound fraction and associated water solubility, enables immediate distribution throughout the organism (LATIMER et al., 2001).

Fluconazole is easily absorbed in horses after oral administration, 100% bioavailable, and has pharmacokinetic properties similar to those in intravenous administration (LATIMER et al., 2001). These characteristics, along with the therapeutic success, absence of adverse effects, and easy administration of the drug diluted in corn glucose once a day, are factors that support fluconazole application for therapy in EPC. Oral administration of potassium iodide is also practical. Nevertheless, potential complications should be noted, such as iatrogenic hypothyroidism and iodism (DÓRIA et al., 2008), in addition to potentially inducing abortion in mares (WEESE; YU, 2013).

Even though fluconazole has effectively treated fungal infections, such as conidiobolomycosis (TAINTOR et al., 2004), coccidioidomycosis (HIGGINS et al., 2006) and candidiasis (REILLY; PALMER, 1994) in horses, according to Luis-León and Pérez (2011), there are no effective antifungal drugs against *Pythium insidiosum* because there is no ergosterol in the membrane of this agent, which is the main target of antifungal drugs. Contrary to this assertion, synergism has been observed by the combination of the antifungal drugs terbinafine and fluconazole for control of *Pythium insidiosum in vitro* (CAVALHEIRO et al., 2009). Further, in contrast to the non-efficacy of antifungal agents on oomycetes, intravenous regional perfusion (IRP) using miconazole (WORSTER et al., 2000) and amphotericin B is reportedly a successful therapeutic for ECP (CARVALHO et al., 2016; DÓRIA et al., 2012, 2015), which highlights the lack of knowledge regarding the mechanisms of action of these drugs on oomycetes. It is likely that the main mechanism of action of antifungal agents against *Pythium insidiosum* is modulation of the host's immune system against pathogens, either by recruiting phagocytes or releasing cytokines (BEN-AMI et al., 2008; MESA-ARANGO et al., 2012).

Clinical variables of all horses remained within normal physiological values throughout the entire period of study. In laboratory analysis (Table 2), five horses presented with anemia at the beginning of the experiment, a common condition in animals with pythiosis, due to blood loss from ulcerated cutaneous lesions. However, after the 7th day of therapy, three

returned to normal, and the two remaining animals continued to present physiological abnormalities 21 days after beginning treatment. All animals presented with leukocytosis due to neutrophilia and eosinophilia, arising from inflammatory processes, which are also characteristic hematological alterations in chronic cases of ECP (DÓRIA et al., 2015). Nonetheless, after 7 days of treatment, normal total leukocyte numbers returned in half of the horses, and 21 days after initiation of treatment, numbers returned to those considered normal for the species (JAIN, 1993) in the five remaining animals.

Table 2. Hematological and biochemical test values (mean ± standard deviation) for horses affected by cutaneous pythiosis and treated with surgical excision and oral administration of fluconazole. Data collected before (week 0) and after (1-4 weeks) therapy.

Week	0	1	2	3	4	Reference[1]
Erythrocytes ($\times 10^6$ μl^{-1})	4.6±0.7	5.1±0.6	5.9±0.9	6.9±0.8	7.9±0.9	6.8-12.9
Packed cell volume (%)	28.4±6.1	30.0±4.8	31.5±4.5	35.0±4.2	34.6±3.3	32-53
Hemoglobin (g dl^{-1})	10.9±0.5	11.8±0.8	12.9±0.8	14.2±1.7	14.9 ±1.9	11-19
Leukocytes ($\times 10^3$ μl^{-1})	21.7±6.4	13.4±5.2	12.6±5.1	11.1±4.0	9.4±1.5	5.4-14.3
Neutrophils ($\times 10^3$ μl^{-1})	15.6±2.3	10.6±1.7	8.4±2.3	6.3±2.1	4.7±1.1	2.2-8.5
Lymphocytes ($\times 10^3$ μl^{-1})	4.2±1.0	1.6±0.3	2.6±0.8	4.3±1.0	4.5±1.0	1.5-7.7
Monocytes ($\times 10^3$ μl^{-1})	0.6±0.2	0.2±0.4	0.7±0.3	0.2±0.2	0.1±0.3	0-1
Eosinophils ($\times 10^3$ μl^{-1})	1.1±0.1	1.0±0.1	0.7±0.1	0.4±0.1	0.3±0.1	0-1
Platelets ($\times 10^3$ μl^{-1})	277.2±98.6	280.6±117.7	289.9±85.1	288.6±76.9	273.2±59.4	100-350
AST (U l^{-1})	215.7±53	198.6±36	181.3±40	203.5±58.3	202.1±45	0-366
ALP (U l^{-1})	270.8±63	248.2±62	185.3±52	169.8±46	159.8±29	0-395
GGT (U l^{-1})	21.4±10	19.9±9.0	20.5±6.1	21.7±6.6	21.8±5.5	0-62
Urea (mg dl^{-1})	19.5±4.0	21±8.0	19.1±6.3	19.46±3.1	21±4.4	21-51
Creatinine (mg dl^{-1})	1.2±0.3	1.2±0.2	1.0±0.2	1.1±0.3	1.0±0.2	1.2-1.9

AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase.
[1]Reference range for horses: Kaneko et al. (1997) and Jain (1993).

Despite reports that fluconazole therapy in horses may lead to elevated liver enzymes (SPINOSA et al., 2006), the treated animals did not show alterations in biochemical test results (Table 2), indicating that treatment for ECP with fluconazole, at the instituted dosage and administration times, did not impact liver or renal function. Activity values for AST, ALP, and GGT enzymes, and urea and creatinine values remained within physiological ranges for the species (KANEKO et al., 1997).

According to Spinosa et al. (2006), fluconazole is not recommended for treatment of pregnant females, given that it might induce abortion and potentially be teratogenic. However, in the present study, we observed no malformations in foals of pregnant females medicated with the drug, and there were no complications during foaling. These findings corroborate a report by Taintor et al. (2004) who used fluconazole as therapy for conidiobolomycosis in pregnant mares without subsequent birth defects. Although fluconazole was used successfully in therapy for ECP in four pregnant mares without any apparent complications in the present study, future indications should be based on assessment of the benefits and risks, and with the owner's consent to potential fetal harm.

Although IRP with amphotericin B is an effective in therapy for ECP, with a success rate of up to 100%

of cases treated (CARVALHO et al., 2016; DÓRIA et al., 2012, 2015), it may only be applied to lesions on the horse's limbs, not to lesions on the abdomen, such as in some of the cases reported in this study. Compared with the systemic administration of amphotericin B, fluconazole is found to be safe and presents no side effects (liver or renal), while there are reports of renal toxicity associated with amphotericin B (ADAMS, 2003).

Immunotherapy and the administration of triamcinolone are other therapeutic methods reported for ECP. However, confirmation of diagnosis is indicated prior to initiating therapy with these drugs, as they are not effective for diseases considered to be differential diagnoses of pythiosis in horses, such as zygomycosis (GROOTERS, 2014). These fungal diseases would probably have a favorable therapeutic response to the antifungal agent fluconazole, as previously reported (HIGGINS et al., 2006; TAINTOR et al., 2004).

A limiting factor of this study was the absence of a control group. However, this is justified by the use of animals with naturally occurring ECP that were referred to the veterinary hospital. Aside from animal welfare issues, it is difficult to persuade owners to allow animals to undergo experimentation in the control (placebo) group or the group with only surgical debridement of cutaneous lesions, especially for this serious disease that rapidly progresses. It is important to note that in cases of ECP, time from the onset of disease is a determining factor in therapeutic success (GROOTERS, 2014) and that the inclusion of a control group might have compromised the efficacy of conventional therapy in control group animals in the period following the of the study. These data are consistent with the findings of Watanabe et al. (2015) who argue that early institution of aggressive therapy is necessary for total remission of lesions. We have also noted previous studies without a control group (DÓRIA et al., 2012, 2015).

In the present study, fluconazole therapy was initiated after surgical debridement, and we did not detect any impact on anesthesia recovery times. No further surgical interventions were necessary for any of the animals after beginning fluconazole therapy. This observation is important in light of reports of increased anesthesia recovery time in horses that had been previously treated with fluconazole, and had undergone general anesthesia (KREIN et al., 2014). Therefore, special care is required in anesthesia recovery if further surgical intervention is necessary after initiating drug therapy.

Through histopathological examination, we identified extensive proliferation of fibroblasts, conjunctive matrix deposition, and perpendicular neovascularization (granulation tissue) with multifocal eosinophilic necrotic centers, in the dermis (Figure 2), corresponding to kunkers in macroscopy. The necrotic areas contained negative images of tubiform structures that were rarely septate and had irregular ramifications, as visualized by Grocott's methenamine silver stain, which are compatible with pseudohyphae of *Pythium insidiosum*. In the surrounding areas of necrosis, we noted a marked infiltration of eosinophils and neutrophils, with occasional lymphocytes and plasma cells. Immunohistochemical analysis confirmed the diagnosis of cutaneous pythiosis in all horses (Figure 2).

Treatment with surgical excision, associated with the administration of fluconazole, resulted in complete solutions for the lesions in all 10 horses in this study. Seven days after beginning treatment, we found no evidence of local pruritus, and lesions had a pinkish surface and no serosanguinous secretion (Figure 3). After 21 days of therapy, the two lesions on the abdomen showed complete epithelial tissue recovery. Lesions less than 25 cm in length healed by 45 days post-surgery, while lesions longer than 25 cm healed by 90 days, which is similar to results obtained in prior studies (DÓRIA et al., 2012, 2015). Ten months after discharge from the hospital, we asked owners about the recurrence of ECP, and no recurrences were reported for any of the horses treated.

Figure 2. Histopathological and immunohistochemical examination of cutaneous lesions in horses. (A) Dermis, the upper left corner of the image shows a necrotic eosinophilic area containing negative images of tubiform structures with marked peripheral infiltration of eosinophils, neutrophils, and occasional lymphocytes, plasma cells, and macrophages; H&E stain; obj. 40×. (B) Dermis, along the periphery of the areas of necrosis, with predominantly eosinophilic infiltration; H&E stain; obj. 40×. (C) Silver impregnation evidencing tubiform structures with irregular ramifications in necrotic tissue; Grocott's stain; obj. 40×. (D) Positive immunohistochemical marker for *Pythium insidiosum*; obj. 40×.

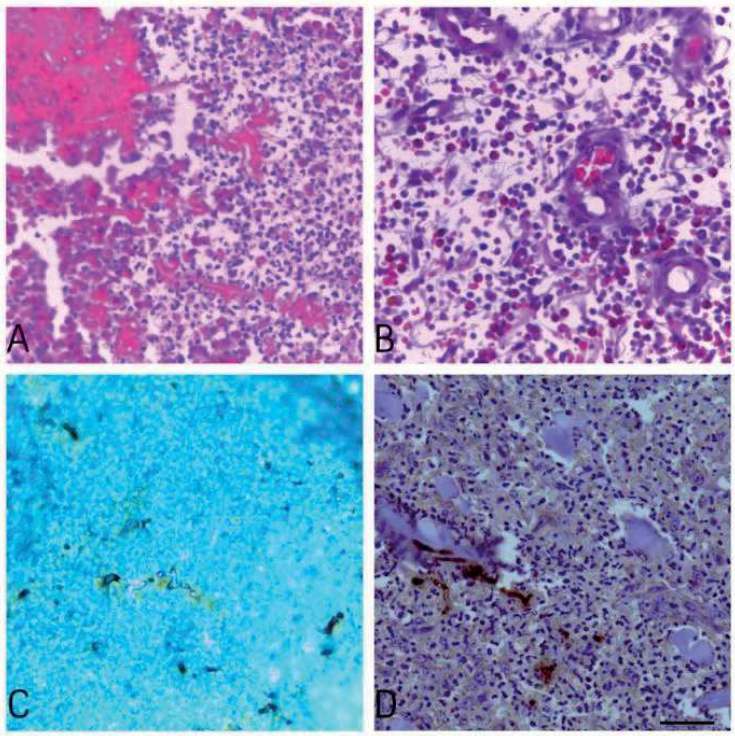
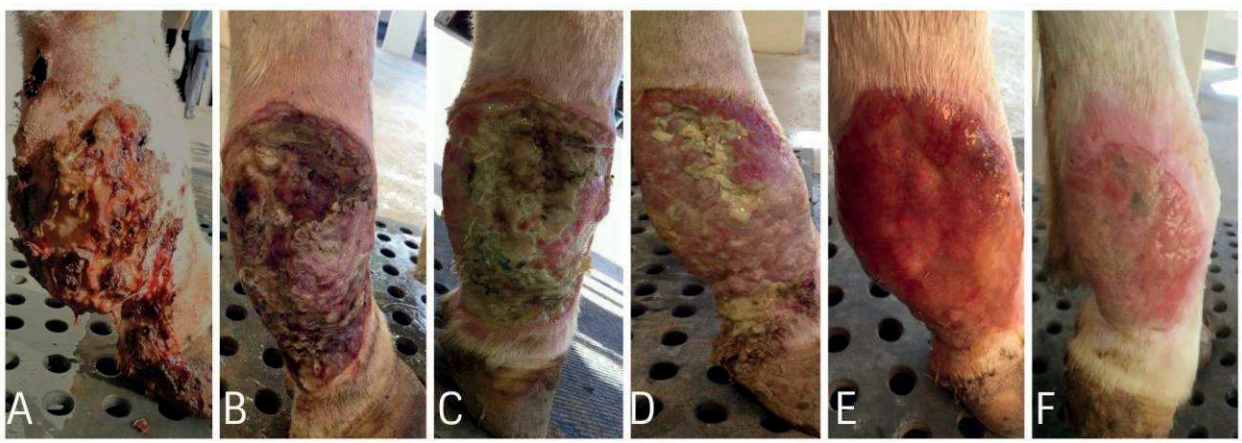


Figure 3. Progression of equine cutaneous pythiosis lesion in the plantar region of the right pelvic limb of one animal treated with surgical excision, electrocauterization, and fluconazole. (A) Before surgical debridement, evidencing a cutaneous lesion with serosanguineous secretion. (B) Seven days after surgery and fluconazole therapy, we observed a transition in the necrotic areas due to electrocauterization and the formation of granulation tissue. (C) 14 days after beginning therapy. (D) 21 days after beginning therapy. (E) 28 days after beginning therapy. (F) 45 days after beginning therapy, with evidence of wound contraction and epithelization of granulation tissue.



Conclusions

These results demonstrate that, in combination with surgical excision of exuberant granulation tissue caused by *Pythium insidiosum* infection, oral administration of fluconazole is effective for the treatment of ECP. Furthermore, there were no adverse reactions in any animals.

Acknowledgments

This study received financial support from Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES), funding code 001.

References

ADAMS, H. R. *Farmacologia e terapêutica em veterinária*. 8. ed. Rio de Janeiro: Guanabara Koogan, 2003. 778 p.

BEN-AMI, R.; LEWIS, R. E.; KONTOYIANNIS, D. P. Immunopharmacology of modern antifungals. *Clinical Infectious Diseases*, Oxford, v. 47, n. 2, p. 226-235, 2008. DOI: 10.1086/589290

CARDONA-ÁLVAREZ, J.; VARGAS-VILÓRIA, M.; PATARROYO-SALCEDO, J. Pythiosis cutánea en equinos tratados con acetonida de triamcinolona. Parte 1. Caracterización clínica. *Revista MVZ Córdoba*, Montería, v. 21, n. 3, p. 5511-5524, 2016. DOI: 10.21897/rmvz.825

CARVALHO, A. M.; GALIZA, G. J. N.; TOMA, H. S.; MUNHOZ, T. C. P.; KOMMERS, G. D. Tratamento de pitiose em égua gestante utilizando perfusão regional intravenosa com anfotericina b e dimetilsulfóxido. *Revista Brasileira de Ciência Veterinária*, Niterói, v. 23, n. 1, p. 28-31, 2016. DOI: 10.4322/rbcv.2016.025

CAVALHEIRO, A. S.; MABONI, G.; AZEVEDO, M. I.; ARGENTA, J. S.; PEREIRA, D. I.; SPADER, T. B.; ALVES, S. H.; SANTURIO, J. M. In vitro activity of terbinafine combined with Caspofungin and Azoles against Pythium insidiosum. *Antimicroial. Agents and Chemotherapy*, Washington, v. 53, n. 5, p. 2136-2138, 2009. DOI: 10.1128/AAC.01506-08

DÓRIA, R. G. S.; CANOLA, P. A.; RIBEIRO, G.; DI FILIPO, P. A.; DIAS, D. P. M.; VALADÃO, C. A. A. Hipotireoidismo iatrôgenico em equino decorrente de excesso de iodo: relato de caso. *Arquivo Brasileiro de Medicina Veterinária e Zootec*nia, Belo Horizonte, v. 60, n. 3, p. 521-524, 2008. DOI: 10.1590/S0102-09352008000300001

DÓRIA, R. G. S.; CARVALHO, B. C.; FREITAS, S. H.; LASKOSKI, L. M.; COLODEL, E. M.; MENDONÇA, F. S.; SILVA, M. A. G.; GRIGOLETTO, R.; NETO FANTINATO, P. Evaluation of intravenous regional perfusion with amphotericin B and dimethylsulfoxide to treat horses for pythiosis of a limb. *BMC Veterinary Research*, London, v. 11, n. 152, p. 152-159, 2015. DOI: 10.1186/s12917-015-0472-z

DÓRIA, R. G. S.; FREITAS, S. H.; LINARDI, R. L.; MENDONÇA, F. S.; ARRUDA, L. P.; BOABAID, F. M.; VALADÃO, C. A. Treatment of pythiosis in equine limbs using intravenous regional perfusion of amphotericin b. *Veterinary Surgery*, Davis, v. 41, n. 6, p. 759-765, 2012. DOI: 10.1111/j.1532-950X.2012.01019.x

DÓRIA, R. G. S.; FREITAS, S. H.; MENDONÇA, F. S.; ARRUDA, L. P.; BOABAID, F. M.; MARTINS FILHO, A.; COLODEL, E. M.; VALADÃO, C. A. A. Utilização da técnica de imuno-histoquímica para confirmar casos de pitiose cutânea equina diagnosticados por meio de caracterização clínica e avaliação histopatológica. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 66, n. 1, p. 27-33, 2014. DOI: 10.1590/S0102-09352014000100005.

GROOTERS, A. M. Pythiosis and Zygomycosis. In: SELLON, D. C.; LONG, M. T. *Equine infectious diseases*. 2th ed. St. Louis, Missouri: Elsevier, 2014. p. 415-421.

HIGGINS, J. C.; LEITH, G. S.; PAPPAGIANS, D.; PUSTERLA, N. Treatment of *Coccidiodes immitis* pneumonia in two horses with fluconazole. *Veterinary Record*, London, v. 159, n. 11, p. 349-351, 2006. DOI: 10.1136/vr.159.11.349

JAIN, N. C. *Essentials of veterinary hematology*. Davis, California: John Wiley Professio, 1993. 417 p.

KANEKO, J. J.; HARVEY, J. W.; BRUSS, M. L. *Clinical biochemistry of domestic animals*. California: Academic, 1997. 932 p.

KREIN, S. R.; LINDSEY, J. C.; BLAZE, C. A.; WETMORE, L. A. Evaluation of risk factors, including fluconazole administration, for prolonged anesthetic recovery times in horses undergoing general anesthesia for ocular surgery: 81 cases (2006-2013). *Journal of the American Veterinary Medical Association*, Schaumburg, v. 244, n. 5, p. 577-581, 2014. DOI: 10.2460/javma.244.5.577

LATIMER, F. G.; COLITZ, C. M. H.; CAMPBELL, N. B.; PAPICH, M. G. Pharmacokinetics of fluconazole following intravenous and oral administration and

bodyfluid concentrations of fluconazole following repeated oral dosing in horses. *American Journal of Veterinary Research*, Chicago, v. 62, n. 10, p. 1606-1611, 2001. DOI: 10.2460/ajvr.2001.62.1606

LUIS-LEÓN, J.; PÉREZ, R. Pythiosis: uma patologia emergente em Venezuela. *Salus online*, Cidade, v. 15, n. 1, p. 79-94, 2011.

MESA-ARANGO, A. C.; SCORZONI, L.; ZARAGOZA, O. It only takes one to do many jobs: Amphotericin B as antifungal and immunomodulatory drug. *Frontiers in Microbiology*, Washington, v. 3, n. 1, p. 1-10, 2012. DOI: 10.3389/fmicb.2012.00286

MORAES, A. S.; SANTURIO, J. M.; TOMICH, T. R.; ABREU, U. G. P.; JULIANO, R. S.; FFERNANDES, F. A. *Impactos econômicos, sociais e ambientais de tecnologias da EMBRAPA Pantanal: Pitium-Vac® - Imunoterápico contra Pitiose Equina*. Corumbá: EMBRAPA, 2013. p. 37-38.

REILLY, L. K.; PALMER, J. E. Systemic candidiasis in four foals. *Journal of the American Veterinary Medical Association*, Illinois, v. 205, n. 3, p. 464-466, 1994.

SAMPAIO, A. J. S. A.; GOMES, R. G.; COSENZA, M. Utilização de imunoterápico no tratamento da pitiose equina. *Arquivos de Ciências Veterinárias e Zoologia da UNIPAR,* Umuarama, v. 19, n. 3, p. 165-169, 2016. DOI: 10.25110/arqvet.v19i3.2016.6090

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: result of 76 cases. *Journal of Equine Veterinary Science*, New York, v. 34, n. 2, p. 270-274, 2014. DOI: 10.1016/j.jevs.2013.06.003

SPINOSA, H. S.; GORNIA, S. L.; BERNARDI, M. M. *Farmacologia aplicada a Medicina Veterinária*. 4. ed. Rio de Janeiro: Guanabara Koogan, 2006. 897 p.

TAINTOR, J.; CROWE, C.; HANCOCK, S.; SCHUMACHER, J.; LIVESEY, L. Treatment of conidiobolomycosis with fluconazole in two pregnant mares. *Journal Veterinary Internal Medicine*, Greenwood Village, v. 18, n. 2, p. 363-364, 2004.

TROST, M.; GABRIEL, A.; MASUDA, E.; FIGHERA, R.; IRIGOYEN, L.; KOMMERS, G. Aspectos clínicos, morfológicos e imunoistoquimicos da pitiose gastrintestinal canina. *Pesquisa Veterinária Brasileira*, Seropédica, v. 29, n. 8, p. 673-679, 2009. DOI: 10.1590/S0100-736X2009000800012

WALLEN, K.; FINKEL, R.; PANAVELIL, T. *Farmacologia ilustrada.* 6. ed. Porto Alegre: Artmed, 2016. 645 p.

WATANABE, M. J.; ALONSO, J. M.; ALVES, A. L. G.; YAMADA, A. L. M.; BOSCO, S. M. G.; RODRIGUES, C. A.; HUSSNI, C. A. Equine pythiosis: Report of 28 cases from São Paulo State, Brazil. *Semina: Ciências Agrárias*, Londrina, v. 36, n. 2, p. 909-916, 2015. DOI: 10.5433/1679-0359.2015v36n2p909

WEESE, J. S.; YU, A. A. Infectious folliculitis and dermatophytosis. *Veterinary Clinics of North America: Equine Practice*, Fort Collins, v. 29, n. 3, p. 559-575, 2013. DOI: 10.1016/j.cveq.2013.09.004

WORSTER, A. A.; LILLICH, J. D.; COX, J. H.; RUSH, B. R. Pythiosis with bone lesions in a pregnant mare. *Journal of the American Veterinary Medical Association*, Illinois, v. 216, n. 11, p. 1795-1798, 2000. DOI: 10.2460/javma.2000.216.1795