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**Original Article**

# Evaluating the efficacy of surgical excision and topical dimethyl sulphoxide (DMSO) in the treatment of equine cutaneous pythiosis

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## Abstract

**Background:** Cutaneous pythiosis in horses is a chronic ulcerative granulomatous disease caused by the oomycete *Pythium insidiosum*. **Aims:** The objective of the present study was to evaluate the response of cutaneous pythiosis in horses to surgical excision and topical dimethyl sulphoxide (DMSO). **Methods:** Thirty horses were presented clinically with pruritus, fistulae discharging serosanguineous fluid, and output kunkers in different body areas (limb, abdomen, neck, and face). The clinical diagnosis was confirmed by isolation of the causative agent and histopathology. All animals were treated by surgical excision alone, or surgical excision followed by topical DMSO. The healing process was monitored every week macroscopically to evaluate the response to treatment until complete recovery. **Results:** The existence of *Pythium insidiosum* was confirmed in all cases. Histologically, affected horses were characterized by granulation tissue with abundant eosinophils. The size of wounds and the clinical features of pythiosis lesions decreased more after surgical debridement with DMSO application than surgical excision alone. The cutaneous pythiosis lesions were completely recovered at 35 ± 7 and 60 ± 5 days after the surgical excision with topical DMSO and surgical excision alone, respectively. **Conclusion:** The combination of surgical excision and topical DMSO is found an effective treatment for cutaneous pythiosis in horses.

**Key words:** Dimethyl sulphoxide, Horse, Pythiosis, *Pythium insidiosum*, Surgical excision

## Introduction

Pythiosis is a life-threatening chronic infectious disease of humans and animals, especially horses (Mendoza *et al*., 2003; Loreto *et al*., 2012; Lerksuthirat *et al*., 2017; Chitasombat *et al*., 2020). It has been increasingly reported from tropical, subtropical, and temperate countries, including Brazil, USA, and Egypt (Mosbah *et al*., 2012; Elkhenany *et al*., 2019; Romero *et al*., 2019). Cutaneous pythiosis of horses occurs commonly in Egypt, especially in the north of the Delta region (Mosbah *et al*., 2012; Elkhenany *et al*., 2019). Kafrelsheikh governorate is one of the suitable areas for pythiosis in Egypt due to its warm and wet climate as well as lakes.

Pythiosis is caused by *Pythium insidiosum*, an oomycete organism of the kingdom *Stramenopila*, family *Pythiaceae* (Gaastra *et al*., 2010). The *pythium* genus includes more than 200 species, most of which live in soil as plant pathogens (Rivierre *et al*., 2005). In animals, there are different forms of pythiosis, but equine cutaneous/subcutaneous lesions are the most common forms (Gaastra *et al*., 2010).

Cutaneous pythiosis lesions may look like a tumor and clinically resemble habronemiasis, cutaneous fungal infections caused by *Conidiobolus* and *Basidiobolus* spp., and squamous cell carcinoma (Cafarchia *et al*., 2013). Cutaneous pythiosis lesions are characterized with granulomatous and ulcerative lesions with multiple draining tracts and serosanguinous discharge (Gaastra *et al*., 2010). The presence of coral-like and hard necrotic irregular masses and calcifications with white-yellowish color, containing hyphae and eosinophil infiltrates is called "kunkers" (Cardona-Alvarez *et al*., 2017). Pythiosis is manifested with anemia, and markable leukocytosis, neutrophilia and eosinophilia, leading to huge economic losses in the horse racing industry (Chaffin *et al*., 1995; Carvalho *et al*., 2019).

*Pythium insidiosum* is not a true fungus; it resists most of antifungal agents due to the lack of ergosterol (targeted by antifungal drugs) (Doria *et al*., 2012). Therefore, treatment of pythiosis in equine is a matter of challenge. Various studies have been established to treat cutaneous pythiosis including antifungals such as ketoconazole, potassium or sodium iodide, miconazole, itraconazole, fluconazole, amphotericin B, and triamcinolone acetonide with or without surgical excision of the lesions to improve outcomes (Chaffin *et al*., 1995; Doria *et al*., 2012; Grooters, 2013; Loreto *et al*., 2014; Cardona-Alvarez *et al*., 2017; Carvalho *et al*., 2019; Elkhenany *et al*., 2019; Chitasombat *et al*., 2020).

Dimethyl sulfoxide (DMSO) is a highly polar, stable

substance with remarkable solvent property (Capriotti and Capriotti, 2012). Dimethyl sulfoxide has reported beneficial properties with regard to wound healing (Duimel-Peeters *et al*., 2003; Capriotti and Capriotti, 2012). It is applied to the skin to reduce pain, decrease swelling and promote healing of wounds and burns (Atiba and Ghazy, 2015). It is usually used as a solvent for antifungal drugs (Randhawa, 2008). Recently, it demonstrated a notable inhibitory effect on a wide variety of bacteria, and fungi (Randhawa, 2006; Tarrand *et al*., 2012; Hassan, 2014); for example, it showed the growth inhibition on some species of *Candida* and *dermatophytes* (Randhawa, 2006; Randhawa *et al.*, 2007; Randhawa, 2008). A combination of amphotericin B plus DMSO was recently found to be effective in cutaneous pythiosis in horses (Dias *et al*., 2012; Doria *et al*., 2015). To our knowledge, DMSO alone has not been used in the treatment of equine cutaneous pythiosis. The purpose of this study is to clinically evaluate the efficacy of topical application of DMSO after surgical debridement of the ulcerative granulomatous lesions of cutaneous equine pythiosis.

## Materials and Methods

### Cases

Thirty horses (from Kafrelsheikh governorate) were admitted to Department of Surgery, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh city, Egypt, with ulcerative wounds or tumor-like masses located in ventral abdomen, thorax, limb, neck and face in the period from 2013-2019. The animal information were recorded; it consist of breed (Arabian (n=9) and mixed breeds (n=21)); sex (mare number=18 and stallion number=12); age (ranged from 20 months to 11 years old); weight (350-450 kg); and anatomical location of the lesions. The clinical features of the cases are described in Table 1.

### Diagnosis

Diagnoses of the presented cases were based on clinical, mycological, and histopathological examinations.

*Mycological examination*

According to Grooters (2013), a wet mount examination was performed directly on the tissue and kunker samples by placing them in 20% potassium hydroxide (KOH) and examining them microscopically to detect the presence of hyphae of *P. insidiosum*. Then, a positive diagnosis was performed by culturing the fresh kunkers on the surface of Sabouraud-dextrose agar (SDA) (SDA; Oxoid, UK) and incubation at 37°C for 24 h, which was enough time to observe the hyphal growth of the pathogen. Colonies on SDA were examined for their macro and micromorphological characters.

*Histopathological examination*

Small portions of the clinical specimens (tissue and kunker samples) were fixed in 10% formalin, cut at 5 µm, and stained with haematoxylin and eosin (H&E) and Gomori's methenamine silver (GMS) stain.

### Anesthesia and surgical procedures

All surgical procedures were done either standing deep sedation or general anesthesia. In case of standing procedures, the horses were sedated using xylazine HCl 1 mg/kg (Xyla-Ject, ADWIA Pharmaceuticals Co., Cairo, Egypt); if necessary, it was repeated after 10-15 min. For the general anesthesia, horses were initially fasted for 12 h before surgery and an intravenous (IV) catheter was applied just before surgery. Horses were sedated using xylazine HCl 1 mg/kg and then anesthetized using IV thiopental sodium at a dose of 6.6 mg/kg (Thiopental sodium, Egyptian International Pharmaceutical Industries Co. (EIPICO) 10th of Ramadan city, Egypt). The horses were positioned with

**Table 1:** Responses to surgical excision with topical DMSO, and surgical excision alone in horses with cutaneous pythiosis

| Cases | Surgical excision with topical DMSO group | | | Cases | Surgical excision group | | |
|---|---|---|---|---|---|---|---|
| | Age (year)/sex | Lesions location/size ($mm^2$) | Outcome/duration to cured | | Age (year)/sex | Lesions location/size ($mm^2$) | Outcome/duration to cured |
| 1 | 5/F | Abdomen/170 × 175 | Cured/45 day | 1 | 3/F | Abdomen/180 × 165 | Relapsed*/64 day |
| 2 | 4/F | Abdomen/135 × 115 | Cured/38 day | 2 | 5/M | Abdomen/175 × 190 | Relapsed*/75 day |
| 3 | 4/F | Abdomen/140 × 150 | Cured/40 day | 3 | 4/F | Abdomen/150 × 110 | Relapsed*/65 day |
| 4 | 6/F | Abdomen/70 × 95 | Cured/37 day | 4 | 3/F | Abdomen/110 × 90 | Cured/57 day |
| 5 | 3.5/F | Abdomen/130 × 130 | Cured/42 day | 5 | 8/F | Abdomen/88 × 105 | Cured/55 day |
| 6 | 6/M | Abdomen/80 × 75 | Cured/34 day | 6 | 5.5/M | Abdomen/77 × 95 | Cured/53 day |
| 7 | 5/F | Abdomen/120 × 90 | Cured/39 day | 7 | 7/F | Thorax/120 × 170 | Relapsed*/68 day |
| 8 | 4/F | Abdomen/100 × 100 | Cured/35 day | 8 | 3.5/F | Thorax/150 × 155 | Cured/59 day |
| 9 | 3/M | Thorax/80 × 90 | Cured/28 day | 9 | 1.6/M | Thorax/70 × 75 | Cured/45 day |
| 10 | 3.5/M | Thorax/100 × 145 | Cured/37 day | 10 | 4.5/F | Limb/51 × 57 | Cured/48 day |
| 11 | 5/F | Limb/53 × 65 | Cured/28 day | 11 | 4/M | Limb/47 × 100 | Cured/56 day |
| 12 | 3/M | Limb/45 × 80 | Cured/33 day | 12 | 5/M | face/40 × 30 | Cured/35 day |
| 13 | 4/M | Limb/60 × 70 | Cured/36 day | 13 | 6/F | Neck, face/130 × 130 , 45 × 30 | Relapsed*/no data |
| 14 | 10.5/M | Limb/50 × 65 mm | Cured/28 day | | | | |
| 15 | 3.5/M | Face/60 × 50 mm | Cured/28 day | | | | |
| 16 | 5/F | Neck/250 × 210 mm, 150 × 50 mm | Cured/35 day | | | | |
| 17 | 4/F | Neck/40 × 45 mm | Cured/21 day | | | | |

DMSO: Dimethyl sulfoxide, F: Femal, and M: Male. * Relapsed cases may require second or more surgical removal of pythiosis lesions due to recurrence of lesions

the lesions uppermost. All lesions in the limbs were examined by X ray before surgical removal procedure. Surgical excision of the lesions was performed; while it was not possible for the cases with critical and/or very large deep anatomical lesions. In those cases, the lesions including sinuses and kunkers were removed by surgical debridement as much as possible. Bleeding was controlled using cauterization and pressure bandage. All wounds were allowed to heal by second intention.

## Postsurgical procedures

Postsurgical medical treatment included the use of tetanus toxoid (1500 IU) and IV fluid therapy such as ringer solution and 25% Dextrose solution just after surgery. Systemic antibiotic (Pentomycine®, AM Trading, 1 ml/25 kg body weight (BW)) was given for 5 days to control secondary bacterial infection and anti-inflammatory like flunixin meglumine (Finadyne®, Schering-Plought, 1 ml/45 kg BW) was prescribed for 3 days to control pain and inflammation.

The cases were divided into two groups:

Group 1: Surgical excision in which the wound was cleaned daily with povidone-iodine solution only

Group 2: Surgical excision with topical DMSO in which the wound was cleaned daily with povidone-iodine solution 10-15 min before the topical application of 10-25 ml (according to the size of the wound) DMSO 99% (Oxford Laboratory, Mumbai, India) until complete healing

The size of lesions (length and width) were determined at day of admission using a ruler. All lesions were evaluated weekly by the gross appearance of wounds for secretions, and presence of the sinuses and kunkers. The wound area was determined by NIH Image J analyzer software (downloaded from http://imagej.nih.gov/ij/) (Atiba and Ghazy, 2015). The wounds were compared to the photographic appearance of the same wounds weekly, until complete healing. Changes in the wound size were expressed as percentages of wound reduction from the original wound size at the admission day.

## Results

Clinical history revealed that all horses which had initially small wounds or nodular abscess did not respond to both topical and systemic antibiotic. Gradually, the size of these wounds increased to be a tumor-like mass (ranging from 10-25 cm in diameter) (Table 1, Figs. 1A-F). All cases were presented after 1-3 months from the beginning of the lesions. In the infected horses,the cutaneous lesions were located in the ventral abdomen (n=14) (Figs. 1A-F), thorax (n=5), limb (n=6), neck (n=3) (Figs. 2A-F), and face (n=2) (Fig. 3A-H). The clinical examination of the lesions was single or multiple granulomatous masses with fistulas oozing serosanguineous discharge and/or ulcerative mass that was usually destroyed by self-mutilation of affected horses due to severe pruritus. Larger lesions were commonly associated with the formation of yellowish hard coral-like masses known as kunkers, usually surrounded by purulent exudate and ranged from 3-6 mm in diameter (Figs. 2A-F and Figs. 3A-H). After X ray examination, none of the admitted cases had lesions extended to the underlying bone. All affected horses presented with decreased appetite and weight loss.

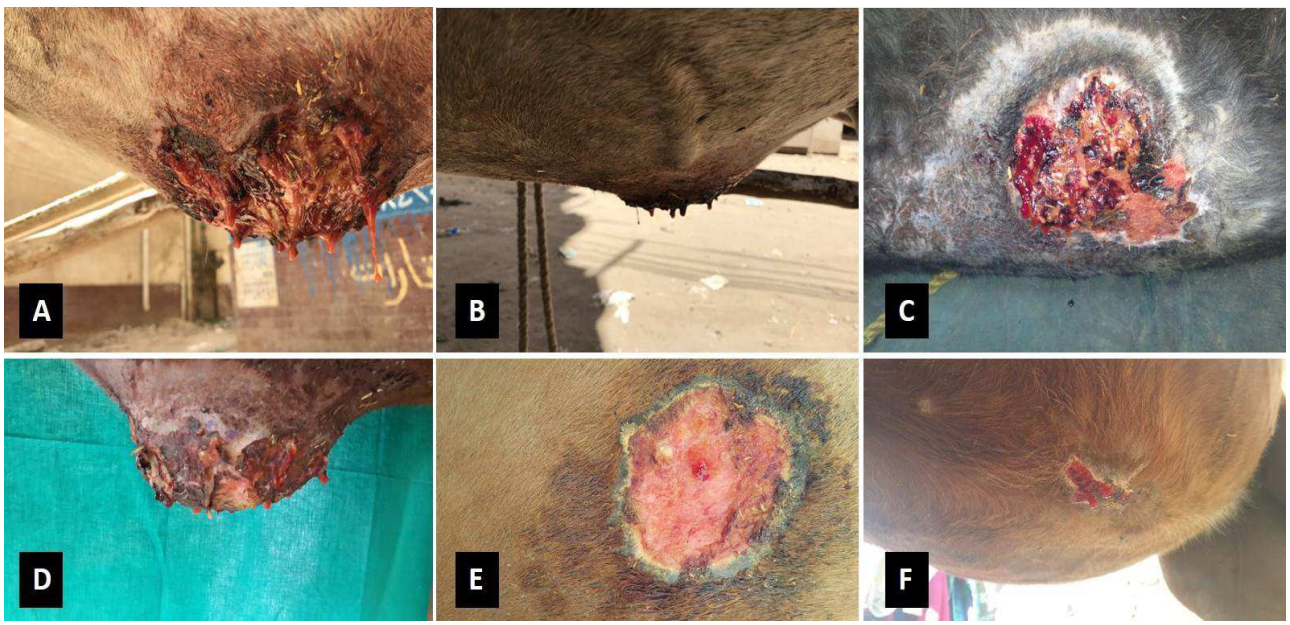


**Fig. 1:** Response to surgical excision alone (**A**, **B**, **C**) and surgical excision with topical DMSO (**D**, **E**, **F**) in horses with proved cutaneous pythiosis in the abdominal wall. (**A**) Ulcerative mass with fistulas oozing serosanguineous discharge; (**B**) Case **A** after 14 days, showing moderate degree of purulent discharge with a large number of sinuses; (**C**) Another case (relapsed case) after 28 days, showing unhealthy granulation tissue with sinuses and kunkers; (**D**) Tumor-like mass in the abdomen with multiple fistulae; (**E**) and (**F**) Case **D** after day 21 and 35 of post-surgical, respectively, note the healthy granulation tissue, epithelization, wound contraction with hair growth


**Fig. 2:** Response to surgical removal of pythiosis lesions and topical DMSO in a horse with cutaneous pythiosis in the neck and under mandible. (**A**, **B**) On day 0, showing fistulae containing kunkers (white arrow), surrounded by purulent exudates; (**C**, **D**) On day 14, showing epithelialization line with healthy granulation tissue without fistulae and kunkers; (**E**) On day 28, showing minimal scar with hair growth; (**F**) On day 42 completely healed


**Fig. 3:** Response to surgical excision alone (**A**, **B**, **C**, **D**), and surgical excision with topical DMSO (**E**, **F**, **G**, **H**) in horses with proved cutaneous pythiosis in the face and/or neck. (**A**) and (**B**) The same case 28 and 35 days after surgical removal of pythiosis lesion; (**C**) and (**D**) The same case (relapsed case) showing recurrence of lesions in the face and neck, respectively, after 21 days of surgical excision, (**E**) Ulcerated, granulomatous and bleeding lesion; (**F**) Collecting biopsied tissues and kunkers; (**G**) Surgical removal of necrotic tissues and Kunkers; and (**H**) On day 28, showing complete healing

## Mycological and histopathological findings

The collected necrotic tissue and kunkers specimen with direct KOH examination under light microscopy showed the presence of large numbers of sparsely septated hyaline hyphae of *P. insidiosum* and numerous vesicles within the hyphae (Fig. 4A). Macroscopically, the cultured specimen of kunkers showed white colonies without aerial mycelium after about 24 h of incubation (Fig. 4B), while the microscopic examination of the colonies showed *P. insidiosum* hyphae with broad branches at an acute angle and sparsely septate (Fig. 4C). The histopathological examination of the specimen showed *P. insidiosum* hyphae after staining with silver in Grocott methenamine stain (Fig. 4D).

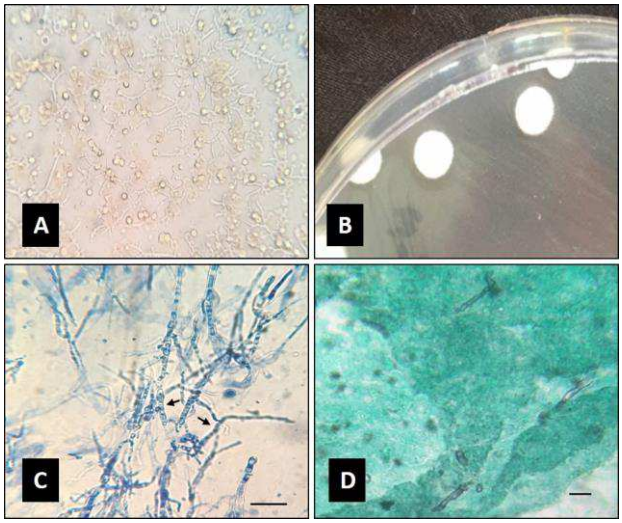


**Fig. 4:** Mycological and histopathological examination of necrotic tissue and kunkers specimen. (**A**) A large numbers of sparsely septated hyphae of *P. insidiosum* and numerous vesicles within the hyphae (direct KOH examination under light microscopy, ×400); (**B**) Macroscopic aspect of *P. insidiosum* showing a white colony surface without aerial mycelium; (**C**) Microscopic aspect of *P. insidiosum* hyphae with broad branches at acute angle and sparsely septate (arrow) (LPCB staining at ×400, scale bar = 50 µm); (**D**) Histopathologic demonstration of tissue biopsy, non-septate intra-lesional hyphae of *P. insidiosum* stained with silver in Grocott methenamine stain, (×400, scale bar = 40 µm)

## Response of cutaneous pythiosis to surgical excision alone or surgical excision with topical DMSO

After surgical debridement in the surgical excision alone group, the animals showed different degrees of clinical manifestation according to the admission size and the degree of surgical excision. It was observed that the small size lesions (less than 120 × 120 $mm^2$) with a higher degree of surgical removal procedure, were better in lesion improvement. It was found that on day 7 after surgical excision, the lesions of incomplete surgical excision showed a moderate degree of purulent discharge with large number of sinus, kunkers, and pruritus. On day 14, those lesions showed light to moderate degree of purulent discharge with evidence of sinus, kunkers. In the surgical excision alone group, 5 cases (3 cases in the abdomen, one case in the thorax, and one case in the neck and face) were recorded recurrence after 14-28 days from the admission time. They required repeated surgical debridement with a recurrence rate about 38.5% (Table 1).

After surgical debridement and topical DMSO, all lesions on day 7 became smaller with appearance of healthy granulation tissue and less purulent discharge with no evidence of kunkers and sinuses. On day 14, lesions became smaller with normal tissue and hair growth around the remaining lesion. In the surgical excision with topical DMSO group, 100% of the cases were cured (Table 1). The mean percentage change in wound area after surgical debridement with topical DMSO, and surgical excision only to the original wound area was 61 ± 8% and 85 ± 5% on day 7, 33 ± 9% and 69 ± 7% on day 14, 21 ± 9% and 53 ± 5% on day 21, respectively (Fig. 5). The cutaneous pythiosis lesions were completely recovered at 35 ± 7 and 60 ± 5 days after the surgical excision with topical DMSO and surgical excision alone, respectively; a complete epithelialization with hair growth was detected depending on the lesion size (Figs. 1A-F, 2A-F and 3A-H).

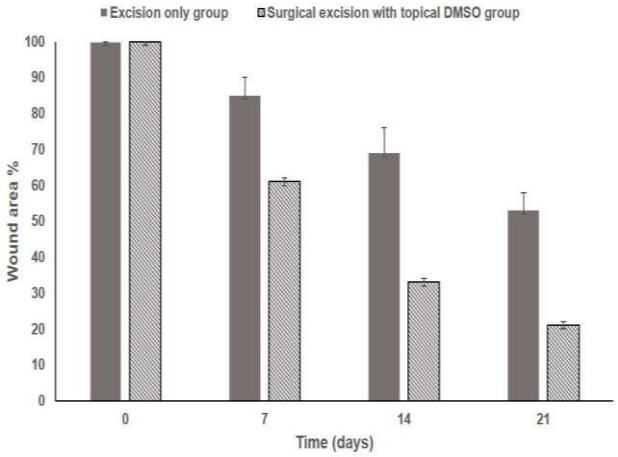


**Fig. 5:** Comparison of the mean percentage change in wound area in excision only group and surgical excision with topical dimethyl sulfoxide (DMSO) group to the original wound area at the day of admission (day 0). Data were reported as mean±SD

## Discussion

Earlier studies have shown that clinical diagnosis of cutaneous pythiosis may be confused. Therefore, the confirmed diagnosis was based on isolation of *P. insidiosum* hyphae in the necrotic tissues (Mosbah *et al*., 2012). In the present study, the diagnosis was confirmed by mycological isolation of *P. insidiosum*.

Equine pythiosis treatment is still as a matter of challenge to veterinarians (Grooters, 2013). There are many factors affecting the results of successful treatment of cutaneous pythiosis such as the site, size and duration of the lesion, the method of treatment, and the animal health condition (Mosbah *et al*., 2012). The surgical excision was considered one of the important managements of the cutaneous pythiosis (Doria *et al*., 2015; Carvalho *et al.*, 2019). However, in some cases, entire surgical excision was not possible in lesions involving critical and/or very large, deep anatomical areas, which generates risks to the affected animals (Gaastra *et al*., 2010). Moreover, pythiosis has a high rate of recurrence after surgical excision only in both humans and animals, because the surgical margin of the lesions may not be free of infection. (Gaastra *et al*., 2010; Chitasombat *et al*., 2020). In the current study, the excision only group showed long time of recovery and recurrence of lesions (38.5%) in some cases. Recurrent cases in this study characterized by large size lesions, not entirely surgicaly removed at the admission time.

Therefore adjunct therapy was recommended in such cases to reduce the risk of recurrence.

Previous studies have determined that surgical excision has been combined with immunotherapy, and/or systemic, intra-lesional, and topical administration of antifungal drugs to improve therapeutic efficacy (Leal *et al*., 2001; Loreto *et al*., 2014; Carvalho *et al*., 2019). Use of immunotherapeutic vaccine in horse exhibits various efficacies (60 to 85%) (Santos *et al*., 2014). *Pythium insidiosum* is not a fungus. Thus, most antifungal drugs have shown divergent results against this pathogen (Doria *et al*., 2012; Grooters, 2013; Loreto *et al*., 2014; Carvalho *et al*., 2019; Romero *et al*., 2019).

Only a few studies have investigated whether DMSO affects the growth of pathogenic fungi, but none was conducted for the treatment of cutaneous pythiosis. Doria *et al*. (2015) reported that intravenous regional limb perfusion with Amphotericin B in a 10% solution of DMSO was effective for treating horses with the cutaneous lesion of pythiosis in limbs but not to cutaneous lesions on the face, neck, and abdomen. In the present study, topical application of DMSO showed no exudation in all cases and no evidence of kunkers and sinuses within 7 days after surgical debridement, with no evidence of recurrence in all cases. In the present study, growth inhibition of *P. insidiosum* and complete recovery of pythiosis lesions were observed with a high concentration of topical DMSO (99%). In a study by Lerksuthirat *et al*. (2017) demonstrated that *in vitro* susceptibility testing of the *P. insidiosum* isolates were sensitive to the antifungal agents, but only at high concentrations. Doria *et al*. (2012) speculate that increased concentrations of amphotericin B impair or inhibit *P. insidiosum*. Similarly, our finding suggests that *P. insidiosum* can be inhibited using higher concentrations of DMSO. Our preliminary study showed that the higher concentration of DMSO was more effective in treating cutaneous pythiosis (data not shown). Additionally, our previous study demonstrated that high concentration of DMSO (99%) has no harmful effects on the skin, and it improved the skin wound healing after burn in dogs (Atiba and Ghazy, 2015). Inhibitory mechanism of DMSO against *P. insidiosum* is unclear. Therefore, additional studies *in vitro* susceptibility of *P. insidiosum* to DMSO are crucial for a better understanding of the mechanism of action.

In this study, the daily use of DMSO after surgical excision of pythiosis lesions was shown to be more efficient in the healing process in comparition with surgical excision only. Therefore, the present data suggest that the combination of surgical excision and the daily usage of high concentration of topical DMSO can be considered as a new candidate for the treatment of equine cutaneous pythiosis infection.

## References

**Atiba, A and Ghazy, A** (2015). The effects of topical dimethyle sulfoxide on second-degree burn wound healing in dogs. A. J. Vet. Sci., 45: 6-12.

**Cafarchia, C; Figueredo, LA and Otranto, D** (2013). Fungal diseases of horses. Vet. Microbiol., 167: 215-234.

**Capriotti, K and Capriotti, JA** (2012). Dimethyl sulfoxide: History, chemistry, and clinical utility in dermatology. J. Clin. Aesthet. Dermatol., 5: 24-26.

**Cardona-Alvarez, J; Vargas-Vilória, M and Patarroyo-Salcedo, J** (2017). Pythiosis cutaneous in horses treated with triamcinolone acetonide. Part 2. Histological and histochemical description. Rev. MVZ. Cordoba. 22: 5638-5652.

**Carvalho, ADM; Munhoz, TCP; Toma, HS; dos Santos, JPV; de Oliveira, LGS; Boabaid, FM; Xavier, AB and Kommers, GD** (2019). The use of fluconazole associated with surgical excision in the treatment of equine cutaneous pythiosis. Semin. Agrar., 40: 3079-3088.

**Chaffin, MK; Schumacher, J and McMullan, WC** (1995). Cutaneous pythiosis in the horse. Vet. Clin. North Am. Equine Pract., 11: 91-103.

**Chitasombat, MN; Jongkhajornpong, P; Lekhanont, K and Krajaejun, T** (2020). Recent update in diagnosis and treatment of human pythiosis. Peer J., 8: 1-30.

**Dias, DPM; Doria, RGS; Pereira, RN; Canola; PRA and Di Filippo, PA** (2012). Topical treatment using Amphotericin B and DMSO for an atypically located equine cutaneous pythiosis. Acta Sci. Vet., 40: 75-78.

**Doria, RGS; Carvalho, MB; Freitas, SH; Laskoski, LM; Colodel, EM; Mendonca, FS; Silva, MAG; Grigoletto, R and Neto, PF** (2015). Evaluation of intravenous regional perfusion with amphotericin B and dimethylsulfoxide to treat horses for pythiosis of a limb. BMC Vet. Res., 11: 1-7.

**Doria, RGS; Freitas, SH; Linardi, RL; Mendonca, FD; Arruda, LP; Boabaid, FM and Valadao, CA** (2012). Treatment of pythiosis in equine limbs using intravenous regional perfusion of Amphotericin B. Vet. Surg., 41: 759-765.

**Duimel-Peeters, IGP; Houwing, RH; Teunissen, CP; Berger, MPF; Snoeckx, LHEH and Halfens, RJG** (2003). A systematic review of the efficacy of topical skin application of dimethyl sulfoxide on wound healing and as an anti-inflammatory drug. Wounds. 15: 361-370.

**Elkhenany, H; Nabil, S; Abu-Ahmed, H; Mahmoud, H; Korritum, A and Khalifa, H** (2019). Treatment and outcome of horses with cutaneous pythiosis, and meta-analysis of similar reports. Slov. Vet. Res., 56: 281-291.

**Gaastra, W; Lipman, LJA; De Cock, AWAM; Exel, TK; Pegge, RBG; Scheuwater, J; Vilela, R and Mendoza, L** (2010). *Pythium insidiosum*: An overview. Vet. Microbiol., 146: 1-16.

**Grooters, AM** (2013). Pythiosis and zygomycosis. In: Sellon, DC and Long, MT (Eds.), *Equine infectious diseases*. (2nd Edn.), St Louis, Saunders Elsevier. PP: 415-421.

**Hassan, AS** (2014). The antibacterial activity of dimethyl sulfoxide (dmso) with and without of some ligand complexes of the transitional metal ions of ethyl coumarin against bacteria isolate from burn and wound infection. J. Nat. Sci. Res., 4: 106-111.

**Leal, ABM; Leal, AT; Santurio, JM; Kommers, GD and Catto, JB** (2001). Equine pythiosis in the Brazilian Pantanal region: Clinical and pathological findings of typical and atypical cases. Pesqui. Vet. Bras., 21: 151-156.

**Lerksuthirat, T; Sangcakul, A; Lohnoo, T; Yingyong, W; Rujirawat, T and Krajaejun, T** (2017). Evolution of the sterol biosynthetic pathway of *Pythium insidiosum* and related oomycetes contributes to antifungal drug resistance. Antimicrob. Agents Chemother., 61: 1-14.

**Loreto, ES; Alves, SH; Santurio, JM; Nogueira, CW and Zeni, G** (2012). Diphenyl diselenide *in vitro* and *in vivo*

activity against the oomycete *Pythium insidiosum*. Vet. Microbiol., 156: 222-226.

**Loreto, ES; Tondolo, JS; Pilotto, MB; Alves, SH and Santurio, JM** (2014). New insights into the *in vitro* susceptibility of *Pythium insidiosum*. Antimicrob. Agents Chemother., 58: 7534-7537.

**Mendoza, L; Mandy, W and Glass, R** (2003). An improved *Pythium insidiosum*-vaccine formulation with enhanced immunotherapeutic properties in horses and dogs with pythiosis. Vaccine. 21: 2797-2804.

**Mosbah, E; Karrouf, GIA; Younis, EA; Saad, HS; Ahdy, A and Zaghloul, AE** (2012). Diagnosis and surgical management of pythiosis in draft horses: report of 33 cases in Egypt. J. Equine Vet. Sci., 32: 164-169.

**Randhawa, MA** (2006). The effect of dimethyl sulfoxide (DMSO) on the growth of dermatophytes. Japanese J. Med. Mycol., 47: 313-318.

**Randhawa, MA** (2008). Dimethyl sulfoxide (DMSO) inhibits the germination of Candida albicans and the arthrospores of Trichophyton mentagrophytes. Japanese J. Med. Mycol., 49: 125-128.

**Randhawa, MA; Hamad, S and Aljabre, M** (2007). Dimethyl sulfoxide (DMSO) has an additive effect and alters minimal inhibitory concentrations of antifungal drugs. J. Rawalpindi. Med. Coll., 11: 54-60.

**Rivierre, C; Laprie, C; Guiard-Marigny, O; Bergeaud, P; Berthelemy, M and Guillot, J** (2005). Pythiosis in Africa. Emerg. Infect. Dis., 11: 479-481.

**Romero, A; Garcia, J; Balestie, S; Malfatto, F; Vicentino, A; Sallis, ESV; Schild, AL and Dutra, FQ** (2019). Equine pythiosis in the Eastern wetlands of Uruguay1. Pesqui. Vet. Bras., 39: 469-475.

**Santos, CEP; Ubiali, DG; Pescador, CA; Zanette, RA; Santurio, JM and Marques, LC** (2014). Epidemiological survey of equine pythiosis in the Brazilian pantanal and nearby areas: Results of 76 cases. J. Equine Vet. Sci., 34: 270-274.

**Tarrand, JJ; LaSala, PR; Han, XY; Rolston, KV and Kontoyiannis, DP** (2012). Dimethyl sulfoxide enhances effectiveness of skin antiseptics and reduces contamination rates of blood cultures. J. Clin. Microbiol., 50: 1552-1557.