

## SCIENTIFIC COMMUNICATION

## CUTANEOUS PYTHIOSIS IN A SLAUGHTERED HORSE: A CASE REPORT

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## ABSTRACT

Cutaneous pythiosis in a horse submitted for slaughter is described in Northern Paraná, Brazil. The animal presented an ulcerative, granulomatous lesion on the lateral surface of the left forelimb just above the elbow joint. Histopathological evaluation revealed an eosinophilic granulomatous reaction associated with intralésional hyphae characteristic of *Pythium insidiosum* visualized by Gomori's methenamine silver stain. Other cutaneous equine lesions easily confused with cutaneous pythiosis are discussed and differentiated. Suggestion for gross identification of these lesions and the final destination of horsemeat infected by *P. insidiosum* during Federal Inspection are given.

KEY WORDS: Pythiosis, *Pythium insidiosum*, cutaneous equine mycoses, public health.

## RESUMO

PITIOSE CUTÂNEA NUM CAVALO ABATIDO: UM RELATO DE CASO. Pitiose cutânea é descrita num cavalo abatido no norte de Paraná, Brasil. O animal apresentou uma lesão ulcerativa, granulomatosa na superfície lateral do membro esquerdo logo acima do cotovelo. Avaliação histopatológica revelou uma reação granulomatosa eosinofílica associada a hifas intralésionais característica de *Pythium insidiosum* visualizadas na coloração especial de prata de Gomori. Outras lesões cutâneas de eqüinos são discutidas e diferenciadas. Sugestões para a identificação grosseira destas lesões e a destinação final de carne eqüina infectada pelo *P. insidiosum* durante a Inspeção Federal são fornecidas.

PALAVRAS-CHAVE: Pitiose, *Pythium insidiosum*, micoses cutâneas de eqüinos, saúde pública.

Equine cutaneous pythiosis (ECP) is an invasive, ulcerative, granulomatous lesion caused by the aquatic fungus-like pathogen *Pythium insidiosum* (CHAFFIN et al., 1995; FOIL, 1996; MENDOZA et al., 1996). Equine pythiosis occurs in tropical, semitropical, and temperate countries (MENDOZA et al., 1996; PIER, et al., 2000), and has been described in several Brazilian States (LEAL et al., 2001a). Pythiosis occurs in cats (THOMAS & LEWIS, 1998), dogs (BENTINCK-SMITH et al., 1989; THOMAS & LEWIS, 1998), cattle (SANTURIO et al., 1998), and humans (SHNEEP et al., 1998; THITTHANYANONT et al., 1998).

Although pythiosis has been described in humans, there are no published reports of transmission between animals and from animals to humans (MENDOZA et al., 1996). Transmission of ECP has been directly related to the attraction of aquatic zoospores of *P. insidiosum* to cutaneous lesions, after which there is encystation

of zoospores in the new habitat (MENDOZA et al., 1996; PIER, et al., 2000). However, hyphae, resting spores, and oogonia of *P. insidiosum* have also been associated with transmission (MENDOZA et al., 1996). Therefore, exudate from contaminated material could be considered as a likely source of zoonotic infection (CHAFFIN et al., 1995). This may indicate that the exact zoonotic potential of this disease has yet to be elucidated.

In the horse, *P. insidiosum*-associated lesions are more frequently described in the skin and subcutaneous tissues than in other anatomic locations (CHAFFIN et al., 1995; PIER, et al., 2000). The development of cutaneous lesions is directly related to the parts of the body that are in direct contact with aquatic zoospores of *P. insidiosum* (MENDOZA et al., 1996), which explains the frequent occurrence of lesions on the distal extremities, and the ventral regions of the

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thorax and the abdomen (FOIL, 1996). Intestinal equine pythiosis has also been described (CHAFFIN et al., 1995; FOIL, 1996). Recently, atypical cutaneous pythiosis lesions have been related in horses (LEAL et al., 2001b).

Equine pythiosis is not well known by practicing veterinarians in Northern Paraná and no recorded documentation of this disease in this State has been found. The State of Paraná houses two of the national Federal Inspection Services for the inspection and approval of horsemeat for exportation. Most horses obtained for slaughter in Paraná are from pythiosis endemic neighboring states. Therefore, adequate identification of cutaneous equine lesions is fundamental during routine Federal Inspection. The importance of this article is to describe a case of cutaneous pythiosis diagnosed in a horse that was slaughtered by the Federal Inspection Service in Northern Paraná. This article also offers suggestions for the identification, differentiation, and the destination of *P. insidiosum*-associated lesions in horses submitted for routine Federal Inspection.

The horse in question was a mixed-breed five-year-old female from the city of Itapeva, São Paulo. The animal was in poor body condition during the external pre-sacrifice examination. She presented a large (12 x 15 cm) ulcerated elevated granulomatous mass, located on the lateral surface of the left forelimb just above the elbow joint; a serous-sanguineous secretion was observed oozing from the mass. Cutaneous pythiosis was suspected and the animal was isolated for routine necropsy evaluation. At necropsy, the affected local was well demarcated and restricted to the subcutaneous conjunctive tissue. Deep muscular tissues were not affected. Several layers of yellowish material (kunker) were observed in necrotic sinuses. A sample from this area was fixed in 10% formalin solution and submitted for routine histopathological evaluation. Selected sections were observed with Gomori's methenamine silver (GMS) stain. The tissue received at the Laboratório de Patologia Veterinária do Centro Universitário de Maringá was an extensive section (3 x 10 x 15 cm) of connective tissue partially covered by ulcerated (5 x 8 cm) skin. Transverse sections of the tissue revealed irregular, necrotic areas (0.3 – 1.5 cm de diameter) forming various sinuses, with a yellowish, material (kunkers) in the centre. Most necrotic centers were surrounded by fibrous tissue and presented more than one kunker (Fig. 1). In other areas, kunkers were observed without the corresponding necrotic tissue.

Histopathological evaluation revealed extensive necrosis of superficial epithelial tissues associated with a marked inflammatory influx of neutrophils and perivascular edema. Various extremely eosinophilic areas were observed in the underlying dermis. These eosinophilic areas, which corresponded

microscopically to the kunkers observed grossly, consisted mainly of dead or degenerated eosinophils admixed with cellular debris, collagen, and fungal hyphae. Hyphae with thick and almost parallel walls that measured between 3.5 – 5.5mm in diameter were negatively stained by Hematoxylin and Eosin. However, these hyphae, peripheral in location, were more easily identified by GMS staining (Fig. 2). Few hyphae were septate and some were branched; branching hyphae formed right angles. Most eosinophilic areas were surrounded by an extensive layer of inflammatory influx, principally neutrophils with a more reduced proportion of macrophages. Between adjacent eosinophilic masses there was marked proliferation of connective tissue, moderate to severe proliferation of newly formed vessels, and an intense influx of eosinophils.

The gross and microscopic alterations associated with the anatomic location described in this study are characteristic of granulomatous lesions induced by *P. insidiosum* in horses (MILLER & CAMPBELL, 1984; CHAFFIN et al., 1995; MENDOZA et al., 1996). Although isolation or other more sensitive identification methods were not utilized in this study to definitely characterize the pathogen, the finding of kunkers, and right angled, thick walled, 2.5–8.5µm diameter hyphae associated with eosinophilic granulomatous reaction observed by GMS staining are sufficient to diagnose ECP by histopathology (MILLER & CAMPBELL, 1984; FOIL, 1996; MENDOZA et al., 1996). Most hyphae observed were located at the margins of the eosinophilic mass (Fig. 2); this location is considered characteristic of *P. insidiosum* (MILLER & CAMPBELL, 1984). We believe that this is the first description of equine pythiosis in the State of Paraná. According to the Federal Inspection Service, similar granulomatous lesions have been observed in four prior cases, but these were not totally diagnosed. This may indicate that the occurrence of equine pythiosis in Paraná is relatively frequent, but probably underdiagnosed.

In this study, various kunkers were observed within necrotic sinuses or surrounded by fibrous connective tissue (Fig. 1). Kunkers are the hallmark of equine pythiosis and represent an immunological reaction (the Splendore-Hoeppli phenomenon) to the invading agent (MENDOZA et al., 1996). Kunkers are seen only in equine pythiosis, being absent in the canine, feline, and bovine variant of the disease (FOIL, 1996; MENDOZA et al., 1996). These coral-like structures were also described in atypical equine pythiosis (LEAL et al., 2001b). Grossly, atypical lesions are huge, dangling, non-ulcerated granulomatous masses with epidermal pseudoepitheliomatous hyperplasia, hyperplasia of sebaceous glands associated with an influx of neutrophils, eosinophils, and marked proliferation of granulation tissue microscopically (LEAL et al., 2001b).



Fig. 1 - Transverse section of submitted tissue (after fixation in formalin solution). There are various kunkers within necrotic sinuses; more than one kunker are seen in some sinuses.

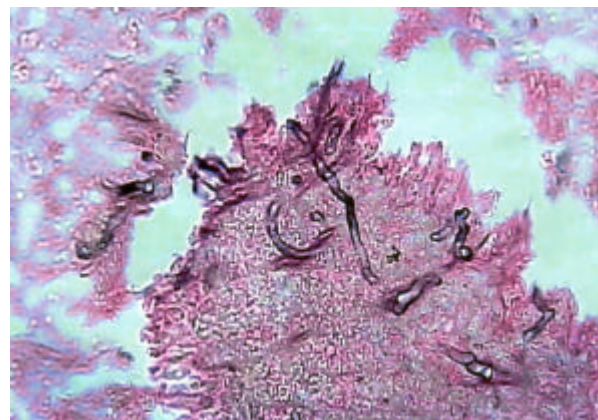


Fig. 2 - Photomicrograph of submitted tissue. Note the marginal location of various cross and longitudinal section of *Pythium insidiosum* hyphae (Gomori's methenamine silver (GMS) stain; 500 X).

ECP must be differentiated from squamous cell carcinoma, exuberant granulation tissue, cutaneous habronemiasis, and other mycotic infections (MILLER & CAMPBELL, 1984; EVANS, 1990; MENDOZA et al., 1996). Although these diseases could be inadvertently confused with ECP grossly, each disease presents different microscopical features which facilitate differentiation.

Squamous cell carcinomas are malignant, ulcerative, epithelial tumors induced by solar radiation, trauma, or carcinogens that are microscopically characterized by keratin pearls and intracellular bridges (HARGIS, 1995). These cutaneous tumors are more frequently diagnosed in areas that are poorly pigmented and do not have any definite anatomical location. Granulation tissue is characterized by neovascularization, abundant proliferation of fibrous connective tissue associated with an influx of mononuclear inflammatory cells. In some cases the development of this granulation tissue turns exuberant (keloid), resulting in whitish, elevated tumor-like cutaneous lesions grossly (RINGLE, 1997).

Cutaneous habronemiasis is caused by larvae of *Habronema* sp. or *Draschia* sp. deposited on the skin by flies, and is more frequently observed in either traumatized areas of the legs, or on soft locations such as the medial canthus of the eye and the prepuce. Grossly there are single or multiple, proliferated, ulcerative lesions that reveal small yellow to white foci on section. Microscopically, this lesion is characterized as nodular dermatitis associated with marked influx of eosinophils, epithelioid macrophages around entrapped larvae (HARGIS, 1995). In some cases of cutaneous habronemiasis, kunkers of *P. insidiosum* have been observed, making this diagnosis more difficult grossly (FOIL, 1996; MENDOZA et al., 1996).

Basidiobolomycosis and conidiobolomycosis are cutaneous granulomatous mycotic infections caused by *Basidiobolus ranarum* and *Conidiobolus coronatus*, respectively, which may be easily confused with ECP (MILLER & CAMPBELL, 1984). Of these two diseases, basidiobolomycosis is more similar to pythiosis clinically, occurring in almost the same anatomic locations (MILLER & CAMPBELL, 1984; MENDOZA et al., 1996). However, microscopically, basidiobolomycosis is more edematous, while pythiosis is highly fibrotic (MILLER & CAMPBELL, 1984). Alternatively, lesions of conidiobolomycosis are restricted almost exclusively to the nasal region (MILLER & CAMPBELL, 1984; MENDOZA et al., 1996). The size of the fungal hyphae is of particular importance in histopathological diagnosis, and is fundamental to differentiate between these three granulomatous cutaneous equine infections (MILLER & CAMPBELL, 1984; FOIL, 1996; MENDOZA et al., 1996). Comparatively, hyphae of *P. insidiosum* are relatively small (2.6 - 6.4  $\mu\text{m}$  in diameter), *C. coronatus* are intermediate (5.1 - 12.8  $\mu\text{m}$ ), while *B. ranarum* are very large (5.1 - 20.5  $\mu\text{m}$ ), thereby facilitating histopathological diagnosis (MILLER & CAMPBELL, 1984).

We recommend that horses submitted for slaughter with large, ulcerative, or non-ulcerative, cutaneous, granulomatous lesions on the extremities, or/and the ventral abdominal or thoracic regions be isolated for complete routine necropsy. These routine procedures must be realized with extreme caution, using long-sleeved overalls or similar uniform to prevent contact of exudates with exposed non-intact skin, since the exact zoonotic potential of this disease has yet to be elucidated. Additionally, we recommend that meat from infected horses be totally condemned for human consumption and should be subjected to elevated temperatures (over 70°C) before being released for animal utilization. This is because cases of intestinal

and osseous equine pythiosis have been considered as metastasis of primary cutaneous lesions (MENDOZA et al., 1996), so the entire carcass must be condemned as a preventative health measure. It must be remembered that even though lesions in lymph nodes are not always evident, lymphatic dissemination is relatively common in equine pythiosis (MENDOZA et al., 1996; LEAL, 2001a; 2001b), so enlarged lymph nodes adjacent to these lesions must be examined and removed.

#### REFERENCE

- BENTINCK-SMITH, J.; PADHYE, A.A.; MASLIN, W.R.; MASLIN, W.R.; HAMILTON, C.; McDONALD, R.K.; WOODY, B.J. Canine pythiosis – isolation and identification of *Pythium insidiosum*. *J. Vet. Diagn. Invest.*, v.1, p.295-298, 1989.
- CHAFFIN, M.K.; SCHUMACHER, J.; McMULLAN, W. C. Cutaneous pythiosis in the horse. *Vet. Clin. North Am. Equine Pract.*, v.11, n.1, p.91-103, 1995.
- EVANS, A.G. Diseases of the skin. In: SMITH, B.P. (Ed) *Large animal internal medicine*. St. Louis: Mosby, 1990. p.1258-1295.
- FOIL, C.S. Update on Pythiosis (Oomycosis). In: ANNUAL MEETING OF THE AMERICAN VETERINARY MEDICAL ASSOCIATION 133., 1996, Schaumburg, IL. *Proceedings*. Schaumburg: 1996. p. 57-63.
- HARGIS, A. M. Integumentary system. In: CARLTON, W.W. & McGAVIN, M.D. (Eds.) *Thomson's Special Veterinary Pathology*. St. Louis: Mosby, 1995. p.1258-1295.
- LEAL, A.B.M.; LEAL, A.T.; SANTURIO, J.M.; KOMMERS, G.D.; CATTO, J.B. Pitiose equina no Pantanal brasileiro: aspectos clínico-patológicos de casos típicos e atípicos. *Pesqu. Vet. Brás.*, v.21, n.4, p.151-156, 2001a.
- LEAL, A.T.; LEAL, A.B.M.; FLORES, E.F.; SANTURIO, J.M. Pitiose. *Ciênc. Rural*, v.31, n.4, p.735-743, 2001b.
- MENDOZA, L.; AJELLO, L.; MCGINNIS, M.R. Infections caused by the oomycetous pathogen *Pythium insidiosum*. *J. Mycol. Méd.*, v.6, p.151-164, 1996.
- MILLER, R.I. & CAMPBELL, R.S.F. The comparative pathology of equine cutaneous phycomycosis. *Vet. Pathol.*, v.21, p.325-332, 1984.
- PIER, A.C.; CABANES, F.J.; CHERMETTE, R.; FERREIROS, L.; GUILLOT, J.; JENSEN, H.E.; SANTURIO, J.M. Prominent animal mycoses from various regions of the world. *Med. Mycol.*, v.38, suppl. 1, p.47-58, 2000.
- RINGLER, D.J. Inflammation and repair. In: JONES, T.C., HUNT, R.D., KING, N.W. (Eds.). *Veterinary Pathology*, 6. ed. St. Louis: Mosby, 1997. p.113-157.
- SANTURIO, J.M.; MONTEIRO, A.B.; LEAL, A.T.; KOMMERS, G.D.; DE SOUSA, R.S.; CATTO, J.B. Cutaneous *Pythiosis insidiosum* in calves from the Pantanal region of Brazil. *Mycopathology*. v.141, p.123-125, 1998.
- SHENEP, J.L.; ENGLISH, B.K.; KAUFMAN, L.; REARSON, T.A.; THOMSON, J.W.; KAUFMAN, R.A.; FRISCH, G.; RINALDI, G. Successful medical therapy for deeply invasive facial infection due to *Pythium insidiosum* in a child. *Clin. Infect. Dis.*, v.27, p.1388-1393, 1998.
- THITITHANYANONT, A.; MENDOZA, L.; CHUANSUMRIT, A.; PRACHARTAM, R.; LAOTHAMATAS, J.; SATHAPATAYAVONGS, B.; LOLEKHA, S.; AJELLO, L. Use of immunotherapeutic vaccine to treat life-threatening human arteritic infection caused by *Pythium insidiosum*. *Clin. Infect. Dis.*, v.27, p.1394-1400, 1998.
- THOMAS, R.C. & LEWIS, D.T. Pythiosis in dogs and cats. *Comp. Cont. Educ.*, v.20. n.1, p.63-74, 1998.

Received on 8/8/02

Accepted on 18/11/02