

Four cases of equine bone lesions caused by *Pythium insidiosum*

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Introduction

PYTHIOSIS is usually a granulomatous disease of skin and subcutis in horses (Austwick and Copland 1974; Miller and Campbell 1982; Mendoza and Alfaro 1986), and cattle (Miller, Bruce and Archer 1985), of skin and intestinal tract in dogs (O'Neill-Foil, Short, Fadek and Kunkle 1984; Miller 1985), and of skin and blood vessels in man (Sathapatayavongs *et al* 1989). The disease is caused by *Pythium insidiosum*, a microorganism in the class Oomycetes, kingdom Protista (de Cock *et al* 1987). *P. insidiosum* was previously known under the names *Hyphomyces destruens* (Bridges and Emmons 1961), *Pythium* sp (Austwick and Copland 1974) and *P. gracile* (Ichitani and Amemiya 1980). It is also known as swamp cancer, leeches, bursate, summer sores, espundia and others (de Cock *et al* 1987).

Pythiosis in horses is usually associated with the end of the rainy season in tropical and subtropical countries. *P. insidiosum* develops its life cycle in stagnant water producing biflagellated zoospores that penetrate into the horse through open skin (Miller 1983). After two days, successful zoospore skin penetration, one may observe a swelling area of 5 mm in diameter that increasing rapidly until it reaches 100 to 200 mm in diameter within about two weeks. Lesions two to five months old are commonly reported in Costa Rica (Mendoza and Alfaro 1986), usually due to fruitless treatments.

Surgical therapy is used widely to treat this disease (Miller 1981). However, it is not practical on limbs because critical anatomical structures are located in these areas (McMullan *et al* 1977). Furthermore, lesions can reappear if the necrotic masses called 'leeches' or 'kunkers' which contain the hyphae of this oomycete microorganism, are not removed completely. Immunotherapy (vaccination with products derived from *P. insidiosum*) have been successful in Australia and Costa Rica to treat horses in early pythiosis, but not in chronic stages (Miller 1981; Mendoza and Alfaro 1986). In addition, treatment with Amphotericin B and iodine has also been reported (McMullan 1977), but Amphotericin B is expensive and time consuming; iodine treatment requires many hours of attention daily, and both have toxic side effects.

Lesions are usually located in the subcutaneous tissues. However, there have been reports in which lymphatic vessels (Murray, Ladds, Johnston and Pott 1978), lungs (Goat 1984), the intestinal tract (Brown and Roberts 1988), and bones (Mendoza, Alfaro and Villalobos 1988) were also affected. This report deals with four cases of pythiosis with bone involvement in horses.

Case 1

Case History

An eight-year-old Costa Rican criollo horse from a farm in the Pacific zone of Costa Rica was presented with an extensive granulomatous lesion three months old, measuring 80 to 100 mm in diameter. The lesion was on the metatarsal phalangeal region of the right hind leg. Pruritus, oedema, lameness and secondary bacterial contamination were observed.

Clinical and pathological findings

A bone biopsy taken from the lesion showed an eosinophilic inflammatory reaction around sequestered necrotic masses ('leeches', 'kunkers'). Giant cells, lymphocytes, macrophages, neutrophils and plasma cells were also found. Gomori's methenamine silver stained tissue section revealed narrow aseptate hyphae 4.5 to 10 µm in diameter. The necrotic masses from the skin lesion were washed twice with antibiotic solution (streptomycin sulphate plus cryptopen penicillin, Glaxo UK Ltd.), and then cut in 3 mm diameter pieces which were washed with distilled water, cultured onto Sabouraud dextrose agar, and incubated at 37°C for 48 h. A white flat strain was recovered in pure culture. When the cultures were transferred to 2 per cent water agar plus grass blades, sporangia and biflagellate zoospores were noticed in the induction media (Mendoza and Prendas 1988). The strain was identified as *P. insidiosum* using De Cock *et al* (1987) methodology.

A serum sample taken for evaluation in an immunodiffusion test (ID) for pythiosis (Mendoza, Kaufman and Standard 1986) showed a positive result. Briefly, *P. insidiosum* (ATCC-58643) strain from a horse with pythiosis in Costa Rica, was cultured in 1.0 litre flasks containing 500 ml of Sabouraud dextrose broth, and incubated statically at 37°C for 10 days. The cultures were then filtered and the resulting mass cells discarded. The supernatant obtained was concentrated 20X in a stir cell (Amicon Corp. Massachusetts) and was used as an antigen against sera from rabbits inoculated with *P. insidiosum*, or sera from a horse with proven pythiosis as a control.

Outcome

The horse died from unknown causes five days later. No post mortem examination was performed.

Case 2

Case History

A six-year-old Costa Rican criollo mare was referred to the School

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of Veterinary Medicine, Universidad Nacional, for evaluation of an extensive ulcerative granulomatous lesion. The lesion was five months old, 90-200 mm in diameter and located on the metatarsal and phalangeal regions of the left hind leg. Lameness, oedema and automutilation due to the pruritus were evident.

Clinical and pathological findings

Radiography revealed extensive disorganised bone proliferation (exostosis) and osteolysis of the distal third metatarsal bone, both proximal sesamoid bones and the first and second phalanges. The bone proliferation also compromised the articular margins of the metatarso-phalangeal joint (Fig 1). A diagnosis of osteomyelitis was made and a bone biopsy performed.

Histopathologically, we observed an eosinophilic granuloma with numerous sequestered necrotic areas, a few giant cells as well as lymphocytes, neutrophils and bone proliferation. Tissue sections stained with Gomori's methenamine silver revealed aseptate hyphae 4.5 to 10.5 μm in diameter. *P. insidiosum* was isolated in pure culture on Sabouraud dextrose agar using the methodology described in the previous case. A serum sample was positive against *P. insidiosum* antigen in an ID test.

Outcome

Due to the animal's poor physical condition, euthanasia was carried out. No lesions caused by this microorganism were observed in the internal organs. Histopathologically, the same inflammatory reaction already described for the biopsy sample was found in the tissue from the affected limb. After soft tissue digestion of the affected leg, the bone lesions observed radiologically were confirmed (Fig 2).



Figure 1: The radiograph shows disorganised bone proliferation of the distal third metatarsal bone, first and second phalanges and the articular margins of the metacarpo-phalangeal joints

Case 3

Case History

A five-year-old Costa Rican saddle horse was presented to the School of Veterinary Medicine for evaluation of two granulomatous lesions located on the metatarsal and phalangeal region on both posterior limbs. The lesions had developed over four months and were 75 to 180 mm in diameter. Lameness of both affected legs was severe and the horse could not stand. Contamination with gram-negative bacteria was also observed.

Clinical and pathological findings

X-rays of both extremities revealed bone proliferation only on the left hind limb. A biopsy showed identical findings to those described in the two previous cases. Narrow, aseptate hyphae 3.8 to 10.5 μm in diameter along with the bone proliferation were noticed in Gomori's methenamine silver stain. Cultures were contaminated with bacteria and *P. insidiosum* was not isolated. A serum sample for evaluation by ID test showed at least six bands in common with the positive anti-*P. insidiosum* serum control.

Outcome

The horse died from endotoxic shock seven days after admission. The necropsy showed no pythiosis lesions in the internal organs.

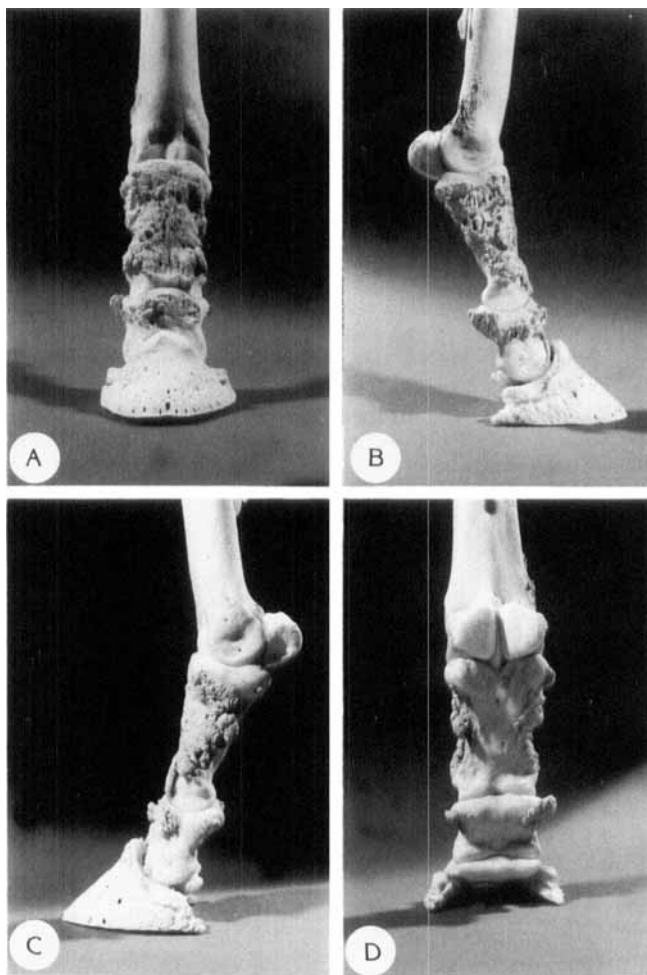


Fig 2: Case 2 after soft tissue digestion showing exostosis of distal third metatarsal bone, the proximal sesamoid bone, first and second phalanges (A, B, C, D) and the articular margins of the metacarpo-phalangeal joints (B and C)

Case 4

Case History

A Costa Rican criollo horse was presented in poor physical condition with a granulomatous lesion of unknown evolution on the right fore leg. The lesion involved the metacarpo-phalangeal region and the coronary band.

Clinical and pathological findings

A radiograph showed bone involvement of PI, II and III with osteoarthritic changes of the coffin joint. Pockets of decreased density, consistent with abscesses within the hoof laminae, were also observed. Histopathological studies showed necrotic masses surrounded by an eosinophilic inflammatory reaction along with bone proliferation. A Gomori's methenamine silver stain revealed narrow aseptate hyphae inside the necrotic masses (Fig 3). *P. insidiosum* was isolated on Sabouraud dextrose agar after 48 h of incubation at 37° C from the bone lesions. The ID test showed at least five precipitin bands of identity with the positive control serum.

Outcome

The horse died of emaciation two weeks later.

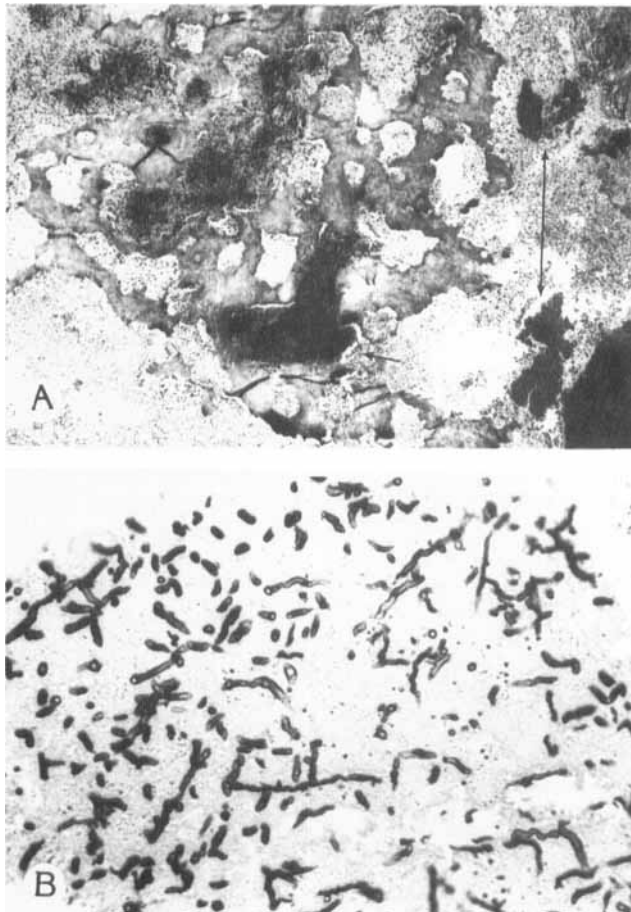


Fig 3: Microphotograph A shows a haematoxylin-eosin stained section of a bone biopsy. Eosinophilic inflammatory reaction, bone proliferation and a necrotic mass (Leeche, Kunker) (arrows) X 200. Microphotograph B shows broad, irregular non-septate hyphae of *P. insidiosum* inside the necrotic masses in a methenamine silver stain of the same tissue section on microphotograph A, X 400

Discussion

Radical surgery to treat pythiosis in horses consists of removing large portions of affected tissue. Although the technique is used widely, it is not practical in lesions on limbs, because of the critical anatomical structures involved (McMullan, Joyce, Hamselka and Heitman 1977). Thus, most cases become chronic. Because limbs are affected by *P. insidiosum* more frequently than other parts of the horse's anatomy (Miller and Campbell 1982; Mendoza and Alfaro 1986), the number of horses with bone lesions may be higher than previously suspected (Mendoza *et al* 1988).

In a recent report, Mendoza *et al* 1988, determined that the age of the lesions was an important factor in the development of bone lesions in horses with active pythiosis. These authors believe that the bone is involved mostly in the chronic phase rather than the early stages of pythiosis. This is supported by the fact that, among 45 Costa Rican horses afflicted with pythiosis of the limbs with lesions one month or less in evolution, no bone involvement was observed.

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