
Mansoura Veterinary Medical Journal

A CASE OF PYTHIOSIS IN A DONKEY

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ABSTRACT

A case of crater-shaped granulomatous ulcer was observed in skin covering knee joint of right forelimb in 8 years old male donkey referred to Mansoura veterinary teaching hospital. Clinical examination of the lesion showed necrotic tissue with tumor appearance, abundant fibrinous-bloody exudation and yellowish-white caseous material (kunkers). Mycological isolation revealed white to off-white colonies at 37°C but not at 25°C. The colonies did not present aerial mycelium, and the mycelial growth penetrated the medium in all cultures. Histopathologically, the lesion with the hematoxylin-eosin (H&E) stain showed severe pyogranulomatous dermatitis, with abundant eosinophilic and neutrophilic inflammatory infiltrate and poorly stained thin septated hyphae. Immunohistochemistry showed positively stained hyphae inside the lesion using rabbit polyclonal antibody. The definitive diagnosis of the disease was based on the clinical features, mycological isolation, histopathological findings and immunohistochemical staining. This report described a case of pythiosis in a donkey.

INTRODUCTION

Equine cutaneous pythiosis (ECP) has been directly related to the attraction of aquatic zoospores of *Pythium insidiosum* (*P. insidiosum*) to the cutaneous lesions, followed by encystation of zoospores in the new habitat (Pier et al., 2000). *P. insidiosum* requires an aquatic environment and organic substrate (e.g., moist, decaying vegetation) for maintenance of its normal life cycle (Chaffin et al., 1995). The lesions were restricted to the skin and subcutaneous tissues in horses (Chaffin et al., 1995; Pier et al., 2000). However, the organism can invade the external genitalia, neck, trunk, dorsal midline, intestinal tract, lymphatic, arteries, lung, trachea, bone, joints, and tendon sheaths (Alfaro & Mendoza, 1990; Chaffin et al., 1992;

Thitithanyanont et al., 1998; Worster et al., 2000). ECP is an invasive, ulcerative, granulomatous, tumor-like lesion caused by aquatic fungus-like pathogen *P. insidiosum*. Equine pythiosis may be misdiagnosed as equine fibroblastic sarcoid (Mosbah et al., 2012). This is a rare case described pythiosis in donkeys in Egypt. The diagnosis was based on clinical signs, mycological, histopathological and immunohistochemistry examination using the polyclonal antibody against *P. insidiosum*.

Case Presentation

An eight-year-old donkey suffering from ulcerative granulomatous lesions in skin covering knee joint of right forelimb was presented to Mansoura Veterinary Teaching Hospital, Mansoura. Clinical examination of the lesion showed necrotic tissue with tumor

appearance, abundant fibrinous-bloody exudation and yellowish-white caseous material (*kunkers*). The donkey was daily washed in a small, stagnant water pond. The patient was pre-medicated with intravenous injection of acepromazine (Vetranquil 1%) at a dose of 0.05 mg/kg body weight (BW). Anesthesia was induced and maintained by infusion of a freshly prepared mixture of 500 mg xylazine HCl, 40 mg midazolam, and 2 g ketamine HCl dissolved in 1 L of 5% dextrose. Before surgery, the animal received slow intravenous injection of warm physiological normal saline (0.9% NaCl) solution with 5% dextrose, penicillin G sodium (20,000 i.u/kg BW), and flunixin meglumine (1.1 mg/kg BW). The affected animal was approached in left lateral recumbency. Total surgical excision of the lesion was performed.

After the surgical excision; tissue and *kunker* samples were taken for mycological examination. Tissues were rinsed in sterile saline solution and transported in ampicillin solution (100 mg/mL in sterile saline) (Grooters et al., 2002). Samples were plated on Sabouraud dextrose agar and then incubated at 25°C and 37°C and examined daily for 3 days post-inoculation.

Other tissue samples were taken from the mass then immediately fixed in 10% neutral buffered formalin and processed according to standard technique. Five-micron-thick paraffin sections were prepared and stained with hematoxylin and eosin (H&E) (Suvarna et al., 2013).

Selected sections of the lesion were immunostained with a horse polyclonal antibody against *P. insidiosum* by using a labeled streptavidin-biotin peroxidase technique as previously described (Miller et al., 1985). The antibodies were produced in New Zealand rabbits from a previous study at the Universidade Federal de Santa Maria (LAPEMI-UFSM)-Brazil (Santurio et al.,

1998). Histological sections of previously diagnosed equine scrotal pythiosis were used as positive control. As negative control, the primary antibody was replaced by antibody diluent in the sections tested.

RESULTS

Transverse sections of the excised mass revealed the presence of characteristic yellow-gray, coral-like materials (*kunkers* or leeches) within the sinus tracts and the granulomatous tissue (Fig. 1). *Kunkers* were firm and gritty in consistency with a diameter ranging from 1 mm to 12 mm. Mycological examinations identified white to off-white colonies around the mixture at 37°C but not at 25°C. The colonies did not present aerial mycelium, and the mycelial growth penetrated the medium in all cultures (Fig. 2A). Microscopically, by lactophenol cotton blue wet mount, the hyphae were broad, 4-10 mm in diameter, hyaline, sparsely septae, and branched, with branches often perpendicular to main hyphae.

Histopathological examination revealed severe pyogranulomatous dermatitis with abundant eosinophilic and neutrophilic inflammatory infiltrate and poorly stained thin septated hyphae (Fig. 2B). Hyphae were rarely septate, irregularly branched, with wide non-parallel walls, surrounded by numerous eosinophils, fewer neutrophils and macrophages, and fibrosis. The walls of the hyphae-like structures stained positively for antibodies against *P. insidiosum* (Fig. 2C) in lesion samples and positive controls, whereas negative control samples were not stained for *P. insidiosum*.

The patient tolerated the operation well and was discharged from the hospital in a good condition.



Fig.1: A, An eight-year-old donkey suffering from ulcerative granulomatous lesions. B, Surgical removal of tumor like mass (white arrow). C, Surgical interference of the lesion.

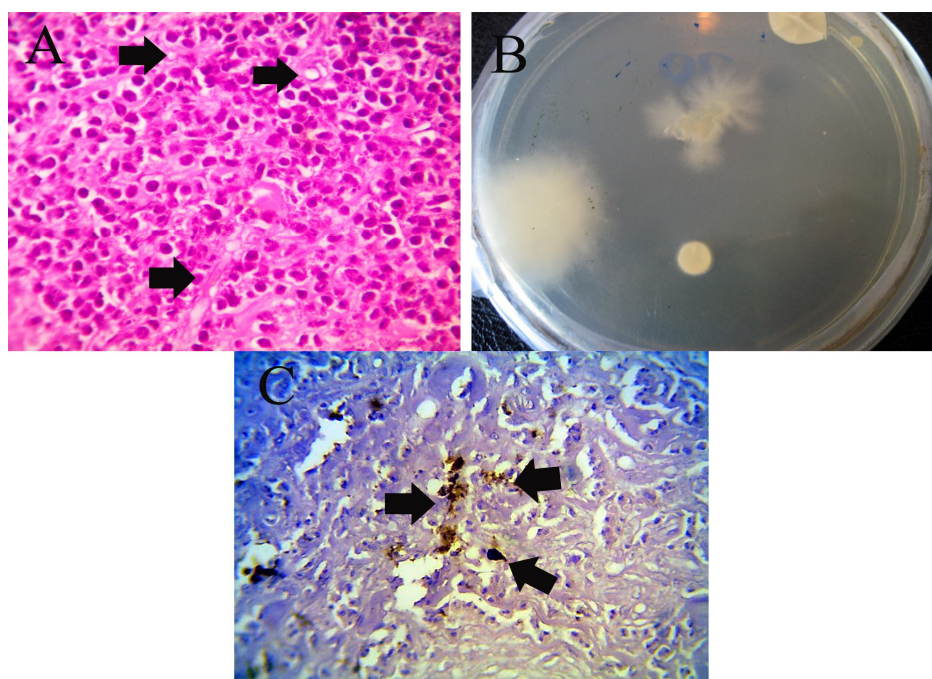


Fig.2: A, Mycological isolation. White colony of *P. insidiosum* without aerial mycelium. B, Histopathological examination of lesion shows the presence of fragment of *P. insidiosum* surrounded by polymorph nucleated cells mainly eosinophils (arrow) which suggests a Splendore-Hoeppli phenomenon (H&E, x 200). C, immunohistochemistry showed positive bright brown immunolabelling inside the lesion using horse polyclonal antibody against *P. insidiosum* (arrows) x 200.

DISCUSSION

The occurrence and high prevalence of pythiosis in equine seem to have been strongly related to the ecology of the aquatic organism and the requirements of high environmental temperatures for rapid growth and asexual reproduction (Tabosa et al., 2004). At the borders of these ponds, animals frequently stand in these swampy waters for long periods. To overcome the long dry period, swimming in water ponds for water reservation is very common (Tabosa et al., 2004). In Egypt, temperatures are high throughout the year (mean of 28°C), and rains usually only occur from January or February. The majority of pythiosis cases were discovered in Egypt between June and August (summer months), which is the time of summer heat and when the rice fields are. The affected horses were used in rice plantation or daily washed in small, stagnant water ponds; a habit for most farmers in Egypt planted (Mosbah et al., 2012). This may have actively drawn the fungus like *P. insidiosum* to the sites of tissue injury, producing the condition. Commonly affected horses with cutaneous pythiosis have had prolonged contact with water in lakes, ponds, swamps, and flooded areas (Vivrette et al., 2000). The annual flooding of the rice fields may have increased the exposure to infectious zoospores by enhancing oospore germination, increasing zoospore production by bringing plant substrates in contact with water, or by enhancing zoospore dispersal by water movement. There is no known age, breed, or sex predilection for the disease (Chaffin et al., 1995). Equine pythiosis was prominent in young animals, 1.5-6 years (Behery, 2007), which could be due to management practice of younger horses preferentially in Egypt. Similar ecologic conditions are associated with the high

prevalence of pythiosis in horses and cattle in tropic and subtropic regions in other countries (Alfaro & Mendoza, 1990). 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 (Mosbah et al., 2012). Total surgical excision of the lesions is most successful provided that all diseased tissue is removed. Reconstructive surgery of the resulting wounds played an important role in their healing, as reported previously (Behery, 2007).

Acknowledgment

The antibody was kindly provided by Prof. Dr. Janio M Santurio Universidade Federal de Santa Maria (LAPEMI-UFSM)-Brazil.

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الملخص العربي حالة بيسيوزيس فى رجل حمار

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تم استقبال حمار يبلغ من العمر ثماني سنوات ويعاني من التقرح الفطري (بزيوزيس) فى الجلد المغطى لمفصل الركبة الرجل الامامية اليمنى بالمستشفى البيطري التعليمي التابع لكلية الطب البيطري جامعة المنصورة فى شهر مارس ٢٠١٣ . وقد أظهر الجلد فى هذه المنطقة سماكة مع وجود ورم حبيبي قيحي متقرح. وقد تكونت الكتلة السمكية من أنسجة بيضاء تميل الى الصفرة مع وجود العديد من القنوات لخروج أو لتفريغ الإفرازات الدموية. واستند التشخيص الأولي على موقع الإصابة، الفحص بالعين المجردة بالإضافة الى وجود kunkers. كما انه تم عزل الفطر على اطباق فى المعمل. وكشف الفحص الهستوباثولوجى عن رد الفعل الحبيبي اليوزيني eosinophilic (granulomatous reaction) يرتبط بوجود خيوط داخل الآفة المميزة لمسبب المرض (*Pythium insidiosum*) والتي صورت بالصبغة المناعية لأجسام الأرنب المضادة متعددة النسخ .