

Clinical Observations And Management Of Equine Pythiosis

Ahmed El-Sayed Behery

Dept of surgery, Faculty of Veterinary medicine Zagazig University

ABSTRACT

Twelve animals (11 horses and a donkey) of both sexes were admitted to the surgery clinic, department of surgery faculty of veterinary medicine, Zagazig University from Mars 2003 – May 2007 with an invasive, ulcerated solitary proliferative, pyogranulomatous lesion on the ventral abdominal wall in horses and on the palmer aspect of the carpus in a donkey. The history, clinical findings and hematology of cases in addition to microbiological and histopathological examination were used in diagnosis of the lesions. This disease commonly affected young animals ranged from 1.5-6 years old. The lesions had a history of extremely pruritic, rapid expansion over a short period and recurrence after more than one of surgical treatment out the clinic. The lesions have characteristic numerous sinuses continuously draining sticky, stringy serosanguinous discharge and branching gritty, yellowish coral-like masses (Kunkers or leeches) within the tissue. The lesions were diagnosed as one of the subcutaneous fungal diseases (pythiosis) caused by *hyphomyces destruens*. Treatment was attempted by surgical excision of the whole lesion with the surrounding normal skin 2cm apart from the junction of the ulcerated and healthy tissues in combination with the use of local and systemic antifungal drugs. The large wounds resulted after lesion excisions were closed by different methods of reconstructive surgery: undermining of the surrounding healthy tissues and multiple punctuate relaxing incisions, single pedicle advancement flap and H-plasty. The outcome of treatment revealed complete cured in eight cases without recurrence in four months postoperative. Recurrence was noticed in two horses within one month and they responded to the second trial of treatment. A case of extended lesion to whole thickness of abdominal wall, was deteriorated, emaciated and died one week postoperative. Septic arthritis was manifested in the case of donkey.

INTRODUCTION

Infectious and non-infectious dermatoses affecting horses were previously described by (1-3). Other than the neoplastic diseases of the horses, some authors studied and classified equine granulomas (2-6). In the horse subcutaneous phycomycosis is characterized by an ulcerative granulomatous lesion containing cores of necrotic tissue called 'Kunker' (2,3,7-9). Phycomycosis is an aggressive granulomatous response to infection of wound with fungus (10,11). Three fungal diseases, namely; pythiosis caused by *Hyphomyces destruens*, basidiobolomycosis caused by *Basidiobolus heptosporus* and conidiobolomycosis caused by *Conidiobolus coronatus* are collectively known as equine phycomycosis (3,7,12,13). Pythiosis is commonly occurred as solitary granulomatous mass on limbs and ventral abdomen while

basidiobolomycosis is confined to the head, neck and chest. Conidiobolomycosis is typified by single or multiple nodules involving the nares and nasal cavity.

In a survey of equine phycomycosis in Australia, pythiosis was the most common disease than other types (5). Equine pythiosis named also phycomycosis, hyphomycosis, bursatti, swamp cancer, Florida horse leeches, Gulf coast fungus, oomycosis (2,10,14). Although the organism is found around the world, it is more concentrated in warm, wet climates (2,10,11,15). The lesions are most often single and may expand dramatically over a short period reaching a very large size in addition to lymphadenopathy may occur if the fungal elements gain access to the draining nodes (2-4,8,9,16,17). Different methods of treatment were used with variable amount of success (1,3,10,12,18-22).

The present study aimed to diagnose the ulcerated growing ghastry irresponsive to treatment lesions by aids of the clinical, hematological, mycological and pathological examination. In addition to treatment of that, lesions and management of wounds produced after surgery were evaluated.

MATERIAL AND METHODS

The study was conducted on 12 animals (11 horse and a donkey) referred to the surgery clinic, department of surgery faculty of veterinary medicine, Zagazig university within four years period (2003-2007). The animals come with an ulcerated non-healed granulomatous lesion (Fig.1,2,3). The history and clinical signs of the infected animals including date of infection, size and location of lesions, age, breed and sex were recorded (Table 1,2). Additional information was obtained regarding the progress of lesions with the type and number of surgeries undertaken by veterinarian before admitting to the clinic. Blood was collected for hematological examination.

Biopsy of lesions were taken under tranquilizations of animals by 0.15 mg/kg I/V (Combelen, Bayer) for mycological and histopathological examinations, two weeks before the treatment. The tissues used for histopathological examinations were fixed in neutral buffered formalin 10%. Blocks were embedded in paraffin sections of 6µm and stained with hematoxylin and eosin in addition to GMS stain. For mycological examination, samples of the irregularly shaped necrotic tissue removed from the cut surface of the lesions were implanted into vegetable extract agar and incubated at 37°C. After growth of the fungus, it was sub cultured on modified Sabouraud's dextrose agar.

Systemic non-steroidal anti-inflammatory drug, phenylbutazone (Butaphenyl, Univet LTD, Ireland) 10ml I/V for three days and daily dressing of the lesion by povidone iodine. (Betadine 10% Nile Co.) were used until surgical treatment after the diagnosis was confirmed.

Restraint and anesthesia

The animals were premedicated by 0.15mg/kg Combelen and anesthetized by Choral hydrate in a dose of 6gm/50kg I/V. The animals having ventral abdominal lesion were recumbent in a dorsal position while the animal has affected in the limb was positioned in a lateral recumbence.

Treatment

The abdominal lesion were covered by sterile gauze and the surrounding area 25cm apart from the lesion border was aseptically prepared. The lesions were removed by incising the normal skin about 2cm from the junction of the ulcerated and healthy tissues. The surrounding healthy skin lip was dissected deeply while the fibrous border of the lesion was observed. The fibrous circular lesion was removed by blunt dissection to its approximate base and separated from the deeply healthy tissues. The lesion was then removed and its base was observed for further evidence of infected tissues. Most of the abdominal layers in site were included and removed resting on the internal sheath of rectus abdominals and peritoneum (Fig 4). All the abdominal layers in site was included and removed in one case. The excessive hemorrhage resulted was controlled. Hosing the area of wound by normal saline and pot. Iodide 1%. Thinning or opening of the abdominal muscles in area was managed by multiple interrupted mattress sutures with non-absorbable material (Dacrofil) as done in herniorrhaphy (Fig 5). The widely produced skin wound after removal of the lesions was closed by different methods as follow:

*For wounds of 10-12 cm diameters, undermining of the surrounding healthy tissues and multiple punctuate relaxing incisions were used for extending the wound lips and relieving tension associated with closure (23). Interrupted horizontal mattress by silk no.3 was used for closing the wound.

*For wounds of 12-20cm diameter, a single pedicle advancement flap of skin was mobilized by undermining and advanced into a wound defect (24). Multiple punctuate

relaxing incisions was used also for relieving tension associated with wound closure.

*For wounds of 20-25cm diameter, an H-plasty was made up of two apposing single pedicle advancement flaps where the skin is available for closure on two sides of the wounds (25) (Fig 4). Multiple punctuate relaxing incisions were used for more extending the two apposing flaps and relieving tension associated with closure of the wound. The skin flaps were sutured to cover the surgical field. Interrupted stitches of silk no.3 were used to tack the skin deep to the abdominal wall to obliterate space and reduce pocket formation (Fig 6).

*For wounds of more than 25cm diameter, an H-plasty, undermining and multiple punctuate relaxing incisions were used to diminish the size of resulted wound where part of wound in the center allowed to heal by granulation tissues.

In the lesion of limb, it was removed from the surrounding border of healthy tissue. The widely wound produced was allowed to heal by second intension.

Postoperative management

Systemic antibiotic penicillin & streptomycin, (Pen-strept, Norbrook) 4ml/kg IM for 10 days and systemic non-steroidal anti-inflammatory phenylbutazone (Butaphenyl, Univet LTD, Ireland) 10ml I/V were injected for three days. Potassium iodide (1gm/30kg/day orally) was used for two weeks. Two times daily dressing of wound by povidone iodine (Betadine 10% Nile Co.) and gauze soaked by 2% of potassium iodide in addition to Alamycin Aerosol, Norbrook were used for two weeks where the sutures were removed. The treated animals were followed up 4 months postoperative.

RESULTS

The clinical finding

The affected animals were cross breed of working animals ranged from 1.5-6 years old. Males and females were manifested. Most of cases admitted to the clinic between Mars and

August. Eleven horses with an ulcerated solitary granulomatous lesion on the ventral abdominal wall and a donkey with a lesion on the palmer aspect of the carpus were observed. The lesions expanded rapidly in a short period, reaching a very large size and had a history of recurrence after more than one of surgical treatment out the clinic. They were extremely pruritic and ranged from 8-25cm in diameter and 2-5cm thickness (Fig 7). Characteristic numerous sinuses continuously draining sticky, stringy serosanguinous discharge which either mats into hair or hangs from the lesions in thick mucopurulent strands dropping on the ground (Fig 1,2,3,7). Branching gritty, yellowish coral-like masses (Kunkers or leeches) were detected within the tissue (Fig 8). Lymphangitis with swelling and edema in the ventral abdomen were observed in chronic cases of larger lesions.

Hematological studies revealed microcytic hypochromic anemia with neutrophilia and eosinophilia.

Mycological examination identified the colonies and the characteristic branching hyphae of *hyphomyces destruens*.

Histopathological examination revealed ulcerated necrotic epidermis with numerous neutrophils infiltration because of secondary bacterial invaders (Fig. 9). Numerous thrombi were seen in the lumens of the underlying blood vessels (Fig. 10). The dermis and subcutaneous tissue of affected skin showed intensely eosinophilic foci of degenerated collagen, surrounded by eosinophils, lymphocytes, histiocytes and occasionally multinucleated giant cells (Fig. 11). The number of eosinophils was highly variable depending on the duration of the lesions. Older lesions generally contained a lesser number of eosinophils. Thick, branched and septated hyphae were only seen by GMS stain, where they stained black on the degenerated collagen and surrounding tissue (Fig. 12). Edematous granulation tissue, congestion and hemorrhages were also detected.

After removal of the lesions, the large wounds that were completely sutured by

different methods of reconstructive surgery, healed by first intension in two weeks. Wound dehiscence was observed in two cases; where their wounds, healed by mixed intension in five weeks. Eight cases responded to the combination of treatment without recurrence in four months postoperative. The horse of

extended lesion to whole thickness of abdominal wall was deteriorated, emaciated and died one a week postoperative. Recurrence was noticed in two horses within one month and they responded to the second trial of treatment. Septic arthritis was manifested in the case of donkey (Table 2).

Table 1. The signalments of animals affected by pythiosis

Animal No.	Age (Yr)	Sex	Month of referred	Duration of infection by month
1	2	M	June	2
2	1.5	M	May	3
3	3	F	June	2
4	5	M	Mar	4
5	4	F	July	3
6	6	F	July	2
7	4	F	May	5
8	3	M	Aug	2
9	2.5	F	July	4
10	2.5	F	July	3
11	3	F	Aug	3
12	5	M	Aug	2

M: Male, F: Female

Table 2 The size of lesion in relation to the wound closure and outcome of treatment

Animal No.	Site of lesion	Size of lesion	Wound closure after	Outcome
1	Ventral abdomen	8x9x2 cm	Undermining & multiple punctuate relaxing incisions	Cured
2	Ventral abdomen	15x17x4 cm	single pedicle advancement flap	Cured
3	Ventral abdomen	8x8x2 cm	Undermining & multiple punctuate relaxing incisions	Cured
4	Ventral abdomen	20x22x5 cm	H-plasty	Recurrence
5	Ventral abdomen	12x14x3 cm	single pedicle advancement flap	Cured
6	Ventral abdomen	12x13x3 cm	single pedicle advancement flap	cured
7	Ventral abdomen	23x25x5 cm	H-plasty and partial closure	Died after a week
8	Ventral abdomen	11x12x3 cm	Undermining & multiple punctuate relaxing incisions	Cured
9	Ventral abdomen	20x21x4 cm	H-plasty	Recurrence
10	Ventral abdomen	18x19x4 cm	single pedicle advancement flap	Cured
11	Ventral abdomen	17x18x4 cm	single pedicle advancement flap	Cured
12	Palmer fore limb	9x7x2 cm	Open healing	arthritis

Fig. 1: Equine pythiosis on the ventral abdomen of a mare, note the circular flat spreading pyogranulomatous lesions

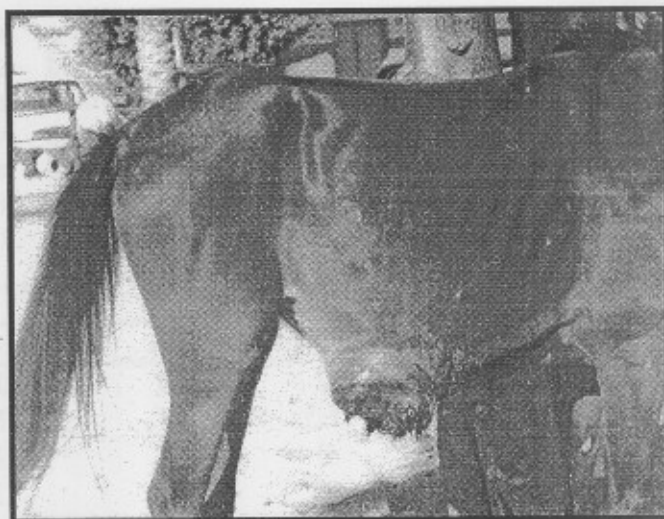


Fig. 2: Equine pythiosis on the ventral abdomen of a mare note the characteristic serosanguinous strands hangs from the lesions



Fig. 3: Equine pythiosis on the palmar aspect of the carpus of a donkey (Solitary and unilateral lesion).



Fig. 4: showing the lesion and most of the abdominal layers in site were removed resting on the internal sheath of rectus abdominis and peritoneum

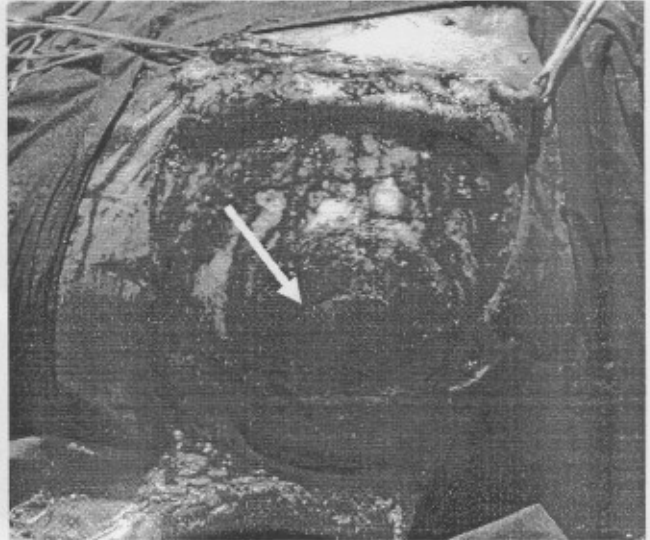


Fig. 5: Thinning or opening of the abdominal muscles in area was managed by multiple interrupted mattress sutures with nonabsorbable material (Dacrofil) as herniorrhaphy

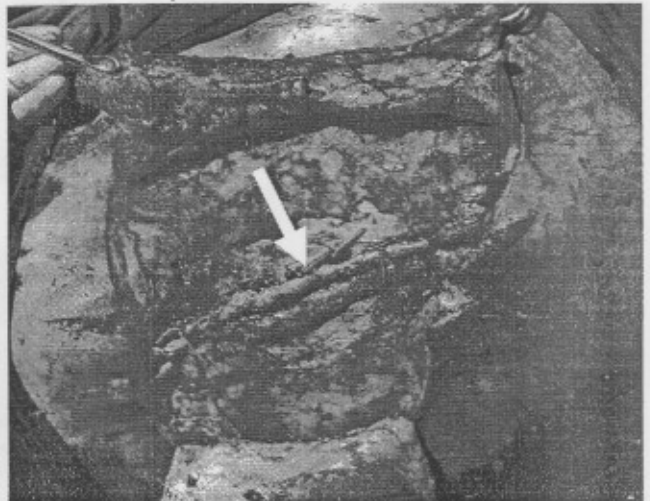


Fig. 6: showing the wound closed by aids of H-plasty made up of two apposing single pedicle advancement flaps and multiple punctuate relaxing incisions (black arrow). Interrupted stitches of silk no.3 were used to tack the skin deep to the abdominal wall to obliterate space and reduce pocket formation (white arrow).

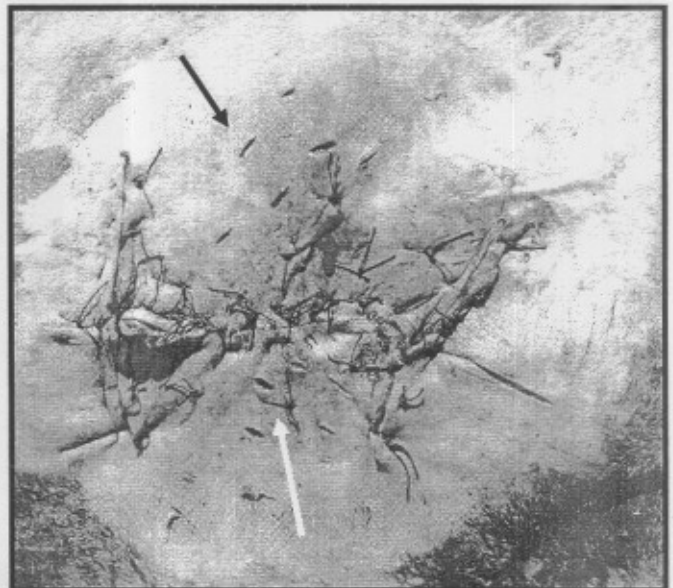


Fig. 7: showing pythiosis of wide lesion ranged from 20 cm diameter and 5cm thickness having characteristic numerous sinuses continuously draining sticky, stringy serosanguinous discharge.

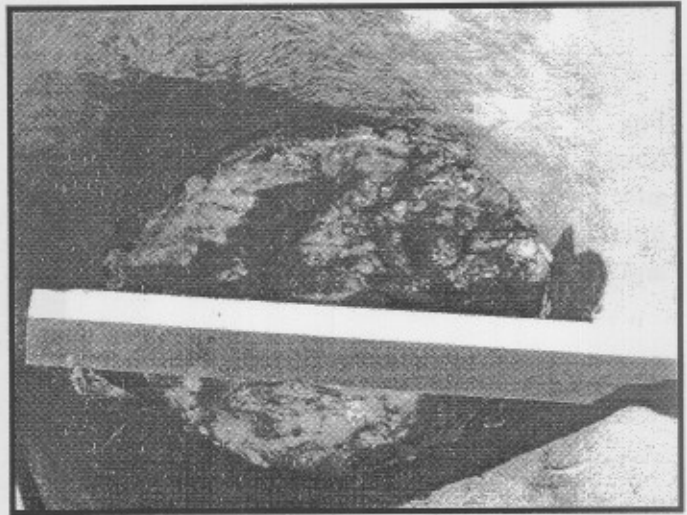


Fig. 8: Branching gritty, yellowish coral-like masses (Kunkers or leeches) were detected within the infected tissue with pythiosis

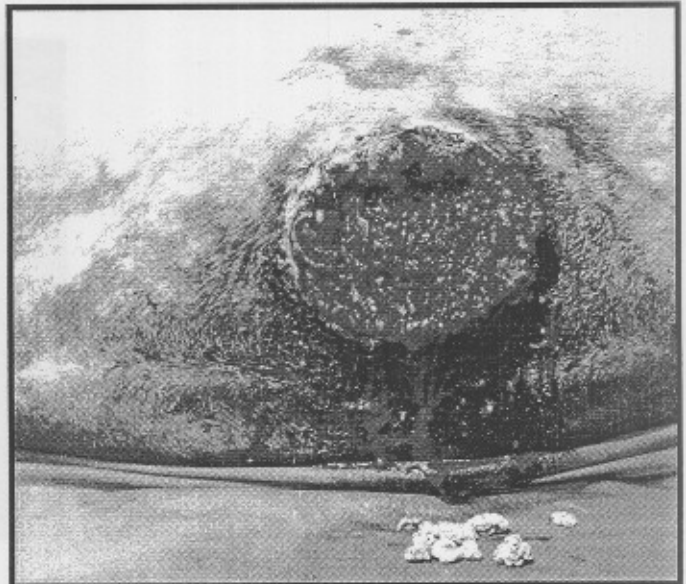


Fig. 9: showing ulcerated necrotic epidermis with numerous neutrophils infiltrations, H&E.X300.

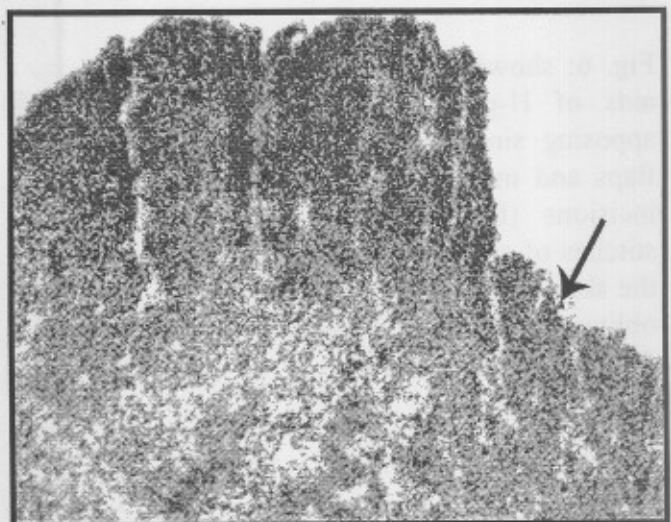


Fig. 10: showing small areas of eosinophilic degenerated collagen surrounded by eosinophils, lymphocytes, histocytes and giant cells. H&E.X1200.

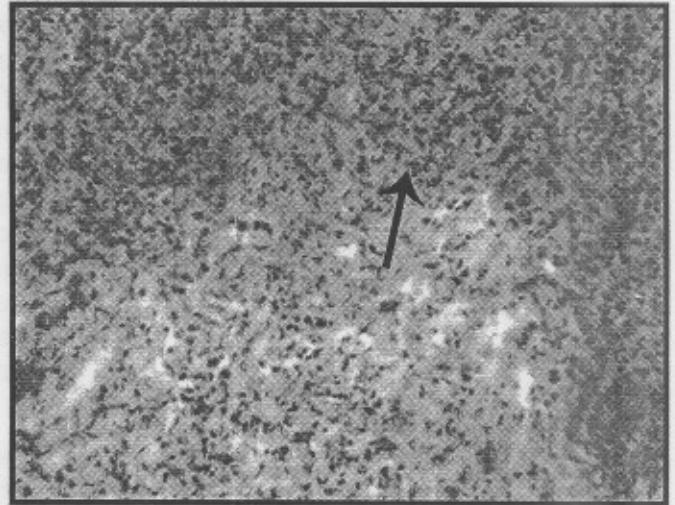


Fig. 11: thrombi in the underlying tissues, H&E.X300.

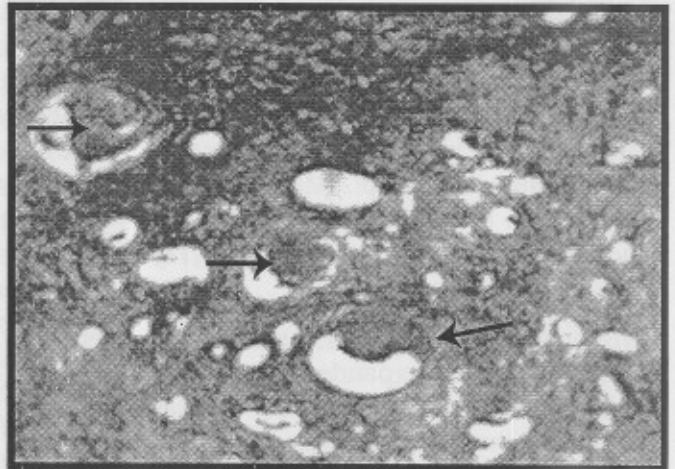
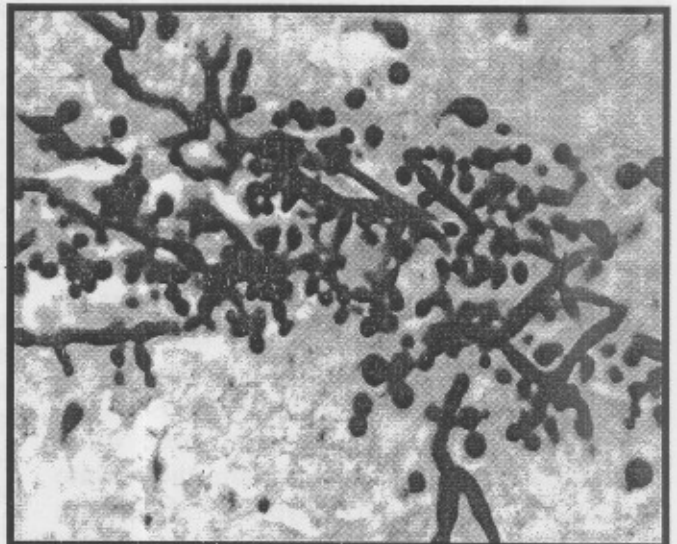


Fig. 12: showing thick branched and septated hyphae stained black by GMS X1200



DISCUSSION

This study deal with one of the granulomatous lesions affecting the skin and subcutaneous of equine species (pythiosis or hyphomycosis). The condition was observed in summer season and commonly occurrence in the tropical and temperate areas (1,5) The fungus is usually associated with swampy conditions where horses either habitually graze in water, or are flood-bound standing in water for long periods (2,8,9). Therefore, the history of using these animals in rice planting may be actively drawn the fungus to sites of tissue damage producing the condition. Equine pythiosis was prominent in young animals (1.5-6 years). Similar observation was recorded (4). On the other hand, other investigators recorded that the lesions did not influenced by age, breed or sex (2,5). The increase the incidence of affection of young age is of interest and to some extent could be due to management practice of younger horses preferentially in Egypt. Most of lesions were detected in the abdomen. Other sites as head and chest were also recorded (2,3,4,18).

The characteristic clinical features of pythiosis were circular, ulcerated, intense pruritic, rapidly expanded pyogranulomatous lesions continuously draining serosanguinous discharge hangs in streaks dropping on the ground and containing branching gritty, yellowish coral-like masses (Kunkers or leeches) within the tissue. These observations are consistent with that reported previously (2-5,7-9). However, like a malignancy, the lesions rapidly destroy surrounding tissue and may spread through the lymphatic vessels. Differential diagnosis of fungal diseases from the other granulomatous lesions as bacterial granuloma, habronema lesions and exuberant granulation tissue and neoplastic lesions is based on the clinical findings, culture and histopathological examination. Habronema lesions tended to be smaller and grew less quickly. Sarcoids and fibromas were often pedunculated and on cross section, their white fibrous texture is fairly typical. Microcytic hypochromic anemia with moderate leukocytosis and eosinophilia and neutrophilia

were also cited (26) Identification of fungus after culturing is based on hyphal morphology (10). The large focal eosinophilic areas of necrotic eosinophils and neutrophils, collagenolysis and fungal observed within the granulomatous inflammation and corresponding to the leeches seen grossly were in agreement with the microscopic findings observed of this condition (2,27). In addition to each neoplastic lesion has its characteristic microscopic picture.

Treatment of equine pythiosis should be instituted as soon as possible after diagnosis because chronic lesions have a poorer prognosis (2,10,18). Surgical excision of the whole lesion is the most common and successful treatment (7,20,21). Systemic administration of antifungal drugs has been used with varying success. Immunotherapy plays a major role in the eventual success of secondary surgery in 30% of affected animals with many complications (12,18). In this study, complete cure in eight cases after treatment and another two cases after the second trial of treatment was due to the wide surgical removal of lesions through the healthy surrounding tissues and the adjunctive; locally and systemic treatment of antimycotic drugs. Similar results were discussed by (10,20,21,28). Deterioration and dying of one animal and septic arthritis in another animal may be due to the behavior of rapid and wide extension of this lesion in the surrounding. In addition, pythiosis resulted in many complications (8,9,17,29). It was noted and confirmed that the results of successful treatment of pythiosis appears to depend on a number of factors such as the type of treatment, size, site and duration of the lesion in addition to the physiological status of the animal (3,9,12). Closure of the large wounds resulted after lesion excision, by different methods of reconstructive surgery played the important role in healing of the wounds in short time by first intension and prevent delayed healing by second intension and prevent production of exuberant granulation tissue, the common complication of wound healing in equine.

ACKNOWLEDGEMENT

The author is highly grateful to Prof. Dr. Mohamed Hamed professor of pathology, Faculty of Vet. Med. Zagazig University for histopathological examination.

REFERENCES

- 1-Pascoe RR (1973): The nature and treatment of skin conditions observed in horses in Queensland. Aust. Vet. J. 49:35.
- 2-Scott SW (1988): Large animal dermatology, W.B. Saunders Company, Philadelphia.
- 3-Shappell KK & Little CB (1992): Special surgical procedures for equine skin. In Equine surgery by Auer JA, W.B. Saunders company, Philadelphia.
- 4-Murray DR, Ladds PW & Campbell (1978): Granulomatous and neoplastic diseases of the skin of horses. Aust. Vet. J. 54, July, 338-341.
- 5-Miller RI & Campbell SF (1982): Clinical observations on equine phycomycosis. Aust. Vet. J., 58, June, 221-226.
- 6-Quinn G (2003): Skin tumors in the horse : clinical presentation and management. Practce, 25:476-483.
- 7-Miller RI & Pott B (1980): Phycomycosis of the horse caused by Basidiobolus heptosporus. Aust. Vet. J. 56, May, 224-227.
- 8-Knottenbelt DC & Pascoe RR (1994): Color atlas of diseases and disorders of the horse, Wolfe, Mosby. London.
- 9-Pascoe RR & Knottenbelt DC (1999): Manual of equine dermatology, WB, Saunders, London.
- 10-Miller RI (1983): Investigations into the biology of three phycomycotic fungi pathogenic for horses, Mycopathologia, 81:23.
- 11-White NA & Moore JN (1998): Current techniques in equine surgery and lameness 2nd Ed., W.B. Saunders Company, Philadelphia, 535-537.
- 12-Miller RI (1981): Treatment of equine phycomycosis by immunotherapy and surgery, Aust. Vet. J. 57, August, 377-382.
- 13-Chaffin MK, Schumacher J and McMullan WC (1995): Cutaneous pythiosis in the horse, Vet Clin. North Am Equine Pract, Apr; 11(1):91-103.
- 14-Ichitani T & Ameniya J (1980): Pythium gracile isolated from the foci of granular dermatitis in the horse, Trans. Mycol. Soc. J., 21:263.
- 15-Malvy D, Riviere JP, Gasnier O, Mienniel B, Roussin C, Receveur MC & Lortholary O (2005): Rhinofacial entomophthoromycosis. About two new cases in Mayotte., Dec., 98(5):350-3.
- 16-Mendoza L, Alfaro AA & Villalobos J. (1988): Bone lesions caused by pythium insidiosum in a horse. J Med. Vet. Mycol 26:5.
- 17-Reis JL, Carvalho EC, Nogueira RH, Lemos LS and Mendoza L (2003): disseminated pythiosis in three horses. Vet Microbilo, Oct 30; 96(3):289-95.
- 18-McMullen, WC (1983): Phycomycosis, In Robinson, NE.,: Current Therapy in Equine Medicine, Philadelphia, W.B. Saunders Co, p.550.
- 19-Sedrish SA, Moore RM, Valdes-Vasquez MA, Haynes PF & Vicek T (1997): Adjunctive use of a neodymium:yttrium-aluminum garnet laser for treatment of pythiosis granulomas in two horses. J Am Vet Med Assoc, Aug 15; 211(4):464-5.
- 20-Dowling BA, Dart AJ, Kessel AE, Pascoe RR & Hodgson DR (1999): Cutaneous phycomycosis in two horses. Aust. Vet. J., 77:780-783.
- 21-Fisher EM (2000): Cutaneous phycomycosis in two horses. Aust. Vet. J. 78:4, 267.
- 22-Livesey L, Schaumacher J, Hancock S, Crowe C, & Taintor J (2004): Treatment of conoidiobolomycosis with fluconazole

- in two pregnant mares. J Vet Intern Med, May-Jun; 18(3):363-4.
- 23-Bailey JV & Jacobs KA (1983):** The mesh expansion method of suturing wounds on the legs of horses, Vet Surg 12:78.
- 24-Swaim SF, Henderson RA and Pidgeon RS (1990):** Small animal wound management, Lea & Febiger, Philadelphia, London.
- 25-Bailey JV (1992):** Principles of reconstructive and plastic surgery. In Equine surgery by Auer JA, W.B. Saunders company Harcourt Brace Jovanovich, Inc Philadelphia.
- 26-Miller RI & Campbell SF(1983):** Hematology of horses with phycomycosis. Aust Vet. J. 60:28.
- 27-Miller RI & Campbell SF(1984):** The comparative pathology of equine phycomycosis. Vet. Pathol. 21:235.
- 28-Meireles MC, Reit-Correa F, Fischman O, Zambrano AF, Zambrano MS & Ribeiro GA (1993):** Cutaneous pythiosis in horses from Brazil, Mycoses, Mar-Apr; 36(3-4):139-42.
- 29-Worster AA, Lillich JD, Cox JH & Rush BR (2000):** Pythiosis with bone lesions in a pregnant mare. J Am Vet Med Assoc, 1;216 (11): 1795-8.

الملخص العربي

الظواهر الإكلينيكية والعناية الجراحية للتقرح الفطري (بزيوزيس) في الفصيلة الخيلية

أحمد السيد بحيرى

قسم الجراحة كلية الطب البيطري جامعة الزقازيق

أجريت هذه الدراسة على اثني عشرة حالة مرضية وردت إلى قسم الجراحة كلية الطب البيطري جامعة الزقازيق في الفترة من ٢٠٠٣-٢٠٠٧ وكانت هذه الحالات تعاني من ورم حبيبي قيحي متقرح بأسفل البطن في إحدى عشرة حصان وخلف مفصل الرسغ في حمار واحد. وكانت شكوى أصحاب تلك الحالات عدم استجابة هذه الأورام للعلاجات التقليدية سواء كانت جراحية أو بالأدوية. تم توصيف الأعراض الإكلينيكية و تسجيل تاريخ حالة المرض والعلاجات السابقة وأعمار الحيوانات المصابة وأوقات ورودها على مدار شهور العام. أجريت فحوصات للدم وأخذت عينات للفحص الميكروبيولوجي والهستوباثولوجي قبل التدخل الجراحي بأسبوعين.

تبين من الفحوصات الميكروسكوبية أن الميكروب المسبب للآفة المرضية هو هيفومييسز دستريونس (*hyphomyces destruens*) أحد الفطريات التي تسبب ورم حبيبي قيحي (pythiosis) في الفصيلة الخيلية

تم علاج هذه الحالات بالتدخل الجراحي واستئصال الجزء المصاب من الأنسجة السليمة المحيطة مع استخدام مضادات الفطريات عاما وموضعا. وإثناء التدخل الجراحي تم استخدام طرق مختلفة من جراحات التقويم للتغلب على قصور الجلد الكبير بعد استئصال هذه الأورام التي يصل قطرها إلى ٢٥سم. تم متابعة علاج الحالات لمدة أربعة أشهر حيث أظهرت طرق العلاج المذكورة شفاء تام لمعظم الحالات فيما عدا حالتين أحدهما لحصان ساءت حالته بعد العملية والأخر لحمار أصيب بالتهاب قيحي في مفصل الرسغ.