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Author(s)	Ali, Abdel-Moneim; Fathy, Ashraf; Ibrahem, Rania et al.
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Pathological findings of cutaneous periocular habronemiasis in a horse in Egypt

Abdel-Moneim Ali[1,*)], Ashraf Fathy[2)], Rania Ibrahem[2)], Mohamed Gomaa[3)] and AL-Gabri Naif [1)]

[1)]Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, Egypt
[2)]Department of Pathology, Animal Health Research Institute, Egypt.
[3)]Department of Surgery, Faculty of Veterinary Medicine, Zagazig University, Egypt
*Corresponding author: E-mail: abdelmoneim.ahmedali@yahoo.com.

Abstract

A 14 years old horse was admitted in June 2014 with an unhealed ulcer near to the medial canthus of the left eye. The ulcerated skin lesion was 3 cm in diameter reddish in color with hazy irregular borders and containing grayish and yellowish necrotic tissues with offensive odor. Histopathological examination of tissue sections showed parasitic elements embedded in multiple granulomas with caseated centers with degenerated *habronema* larvae containing masses from granulated eosinophil in the center followed by eosinophilic and basophilic necrotic debris beside numerous macrophage and giant cells followed by fibrous capsule. The covering skin revealed large deep cutaneous ulcer containing purulent or serous exudate with living and degenerated neutrophils. The inflammatory process was extended into deeper area forming pyogranuloma containing multinucleated giant cells and fibrous tissues. Alsothe adjacent tissues revealed aggregations of microfilaria in dermis (Onchocerciasis), and in other section from behind the granuloma we saw nonseptated mycelium (Cutaneous Phaeohyphomycosis) within the purulent exudate as an opportunistic infection.It could be concluded that complicated cutaneous Habronemiasis consider one of the parasitic cutaneous lesions which is rare among horses in Egypt. Immunological reactions of the parasitic element may be one of the etiological agents of skin ulcers and fungal invasions.

Key Words: Horses, Cutaneous *Habronema, microfilaria* in dermis, *Onchocerciasis, Phaeohyphomycosis*.

Introduction

Habronemiasis (also called summer sores, granular dermatitis, jack sores, bursati, and other terms) is a complex parasitic disease of donkeys, horses, mules, zebras, and dogs and is most commonly encountered in temperate, subtropical, and tropical regions[9,18)]. It has a seasonal distribution, with occurrences predominately in the spring and summer and coinciding with fly activity[22)]. Habronemiasis is caused by theinvasion of Draschiamegastoma, *Habronemamajus, and H. muscae* nematodes which transmitted by house flies, face flies, and stable flies respectively. The adult nematodes live in thehorse's stomach, laying eggs, which pass out in the horse's feces. The most common aberrant forms (Ocular and cutaneous habronemiasis) are associated with the nematode larvae being deposited inthese areas, not completing its life cycle, with resultant signs probably associated withlocal hypersensitivity[14)]. Diagnosis is based on gross lesions and the seasonal

nature of the disease and may be confirmed by histopathology or identification of larvae from the lesion. A rapid onset and. exuberant, pruritic granuloma at the medical canthus of the eye with small yellow, calcified concretions embedded in it are considered pathognomonic for ocular habronemiasis. A definitive diagnosis is established by histopathology as a granulation tissue infiltrated by eosinophils, mast cells, neutrophils, macrophages and an occasional *habronema* larva[12]. Lesions are usually reddish-brown single or multiple subcutaneous nodules, mostly bloody and purulent discharge and contained numerous caseous granules, with occasional ulceration. They often are "greasy," serosanguineous, and contain yellow calcified "rice grain-like" material and some cases reveal extensive epidermal erosion and focal ulceration with dermal necrosis and other description as a single or multiple ulcerated timorous masses with eosinophil, epithelioid cells infiltration and scattered necrotic debris.Moreover, diffuse and heavy dermal infiltration of numerous eosinophils and fewer neutrophils were noted[14,2,22]

In cutaneous habronemiasis, skin lesions are ulcerated; granulomatous masses that bleed easily appear as gritty plaques as a pathognomonic sign and characteristic histological lesions consist of eosinophilic dermatitis and coagulative necrosis with or without the degeneration of nematode larvae in the center [20,15]. Typical signs include no healing skin lesions, ulceration of moist areas, intense itching, and formation of exuberant granulomatous tissues[9,18]. Ulcerated fungating growths very liable to hemorrhage they consist of exuberant fibrous tissue enclosing few or many larvae of *Habronema* spp. accompanied by dense infiltration ofeosinophil's, which form small esinophils-cells abscesses due to immobilized and dead larvae[8].

Cutaneous habronemiasis in 15 horses and 5 donkeys was described by[11]; the lesions were distributed in many parts of the body involving the medial canthus, shoulder and pectoral regions, knee and fetlock joints, abdominal wall and prepuce. Some animals had more than one lesion. The lesions were ulcerative and filled with soft light red granulation tissue. When curetted, the deeper layers revealed a dense fibrous tissue with calcified foci.

In dogs, ulcerative, non-healing skin lesions and the formation of exuberant granulation tissue were observed as in horses. Microscopically, the epidermis was ulcerated and overlaid with a thin layer of necrotic cellular debris admixed with neutrophils. The dermis and subcutis were markedly edematous and contained extensive infiltrates of inflammatory cells, predominantly eosinophils, with fewer plasma cells, macrophages, and lymphocytes. Variably-sized tracts of necrotic cellular debris admixed with fragmented collagen and large numbers of degenerate eosinophils were also present in the dermis[18].

A 6-year-old castrated dromedary camel (*Camelus dromedarius*) was presented with a non-healing, severely pruritic, ulcerative fibrotic plaque located at the medial canthus. Histological examination of surgical biopsies identified degenerating nematode larvae within eosinophilic granulomas. A biopsy of this lesionrevealed multiple granulomas, areas of patchy ulceration, and proliferative granulation tissue with moderate eosinophilic inflammation extending from the dermis to the subcutaneous muscle[13].

In one case with the signs of bilateral conjunctivitis on the Rhinoceros, the histological evaluation of the excised conjunctiva showed reactive fibrosis and diffuse eosinophilic infiltration throughout the substantia propria. Larger eosinophil-rich granulomas surrounding transverse and vermiform longitudinal sections of parasites were also observed[7].

Phaeohyphomycosis is a general term for disorders caused by melanized fungi with hyphal histopathology. It covers a wide variety of clinical forms, including cutaneous and subcutaneous, central nervous system and disseminated infections. The mycoses should be distinguished from mycetoma and chromoblastomycosis, which

are also caused by dematiaceous fungi[17], it is considerable opportunistic infection that occurs through contamination of wounds 1). In other others the microscopically lesions described as an epithelioid macrophages with fewer multinucleated giant cells, neutrophils, lymphocytes, and plasma cells infiltrated the dermis and sub cutis in vague coalescing granulomas. Numerous intra- and extracellular brown-pigmented fungi were present which characterized by coalescing pyogranulomas involving the dermis and subcutis. Within these pyogranulomas, pigmented, septate, fungal hyphae with multiple globose dilatations were identified. The characteristic feature of this group of fungi is their pigmented appearance in section with dark colored hyphae visible on H&E stained sections[6].

Cutaneous Onchocercosis can affect horses[4]. Adult forms and larvae of *Onchocerca* are responsible for the troubles as in study in the one hundred and sixty skin biopsies on horses under the study from Poland, France and Spain in order to detect *Onchocerca* larvae. Six foreign horses were found positive (5/95 Polish and 1/18 Spanish). Equine Onchocerciasis is a parasitic infection of horses, with world-wide distribution, caused by a filarial worm of the genus *Onchocerca*. The adults of *Onchocerca cervicalis* are found in the ligamentous tissue adjacent to the nuchal attachment of the thoracic vertebral spinous processes and in and around the supra spinous bursa[10].

There are no literatures on cutaneous habronemiasis in equine in Egypt, so this study aimed to describe the pathological features of a complicated cutaneous granuloma in a horse.

Materials and Methods

A 14 years old mare admitted in June 2014 to the Surgery Department, Faculty of Veterinary Medicine, Zagazig University, Egypt, with an unhealed ulcer skin near to the medial canthus of the left eye. The ulcerated skin lesion was 3 cm in diameter reddish in color with hazy irregular borders and containing grayish and yellowish necrotic tissues with offensive odor with (Fig. 1A). The animal was subjected to nasolacrimal duct lavage with normal saline 0.9% to exclude nasolacrimal duct obstruction. Biopsy of lesion was taken under tranquilization of the animal by 1.1 mg/kg I/V (Xylazine Hcl 2% (Xylaject® Adwia) and fixed in 10% neutral buffered formalin solution, dehydrated in ascending concentrations of ethanol (70-100%), cleared in xylene, and embedded in paraffin. Five-micron thick paraffin sections were prepared and then routinely stained with hematoxylin and eosin [19], and then examined microscopically. Other specimens were sending for microbiological identification. An extensive debridement of the lesion around the medial canthus of the left eye was performed under deep narcosis using Chloral hydrate 10% 5 gm/ 50 kg B.wt I.V.

Results

Grossly, large single red brown protruded masse with diameter 0.5 to 1 cm was seen from unhealed ulcerated skin near to the medial canthus of the left eye. The ulcerated skin lesion was 3 cm in diameter reddish in color with hazy irregular borders and containing grayish and yellowish necrotic tissues with offensive odor (see Fig.1A).

Histopathological examination of the biopsy specimen showed multiple parasitic granulomas in the dermal tissue represented by caseous centers with or without degenerated *habronema* larvae containing masses from granulated eosinophil in the center followed by eosinophils and basophils necrotic debris beside numerous macrophage and foreign body giant cells, lymphocytes and plasma cells followed by fibrous capsule on the periphery (Figs 1 B, C, D). Also the surrounding tissue of skin, subcutis and muscles had massive eosinophil infiltrations. Also dermal collagen fiber suffered from collagenolysis represented by fragmented and distorted (necrosis) or hyalinzed fibers

infiltrated by numerous inflammatory cells mainly living and degranulated eosinophils. The skeletal myocytes muscle cells showed vacuolation and edema with more esinophilic sarcoplasm beside pyknosis of their nuclei. Large deep cutaneous ulcer containing purulent or serous exudate with living and degenerated neutrophils, sometimes admixed with identified fungi elements (mycelia) (phaeohyphomycosis) over the surface of parasitic granuloma described as a septate, fungal hyphae with multiple globose dilatations were identified (Fig.1E). Moreover, the inflammatory process was extended deeper forming pyogranloma containing multinucleated giant cells and fibrous tissues. Other biopsy specimen from the fore mentioned lesion of the skin mainly in vicinity of sebaceous glands and muscle revealed aggregations of microfilaria (Onchocerciasis) in dermis with or without inflammatory reactions mainly eosinophils and mononuclear cells representing Onchocerciasis (Fig.1F).

Discussion

In this report the case was diagnosed by the history, clinical signs and histological finding. This is a complicated case of Cutaneous Habronemiasis in horse with Phaeohyphomycosis and Onchocerciasis. The exact pathogenesis of Habronemiasis is unknown but it is highly probable that the disease involves as a hypersensitive reaction to dead or dying larvae[15,21)]
In our work the Cutaneous *Habronema* located in preiocular region near the medial canthus of left eye.This site was a common location. This finding was in agreement with[15,5)] who mentioned that the common sites for infestation are limbs, ventral aspect of the abdomen, prepuce, external genitalia males (penis, urethral process), ocular and peri-ocular areas (conjunctiva, medial canthus, nasolacrimal duct) and commissure of the lips. Habronemiasis in this report occurred in summer season and related to fly activity and this result was coincided with that mentioned by[22)].
In our histopathological findings we observed multiple parasitic granuloms in the dermal tissue represented by caseous centers with degenerated *Habronema* larvae. This lesion was observed in previous studies[15,20,12)]. Similar microscopic features of the larvae and the inflammatory response were also previously reported in Cutaneous Habronemiasis of dog[18)], in camel[13)], in Rhinoceros[7)] and in donkey[11,22)].
Clinical diagnosis is unreliable as there is a range of equine skin diseases that should be considered as differential diagnoses. These include proliferative granulation tissue, sarcoids, squamous cell carcinoma, fungal granulomas, pyogranulomas and foreign body granulomas. Differential diagnoses for ophthalmic Habronemiasis include ocular neoplasia, foreign body granuloma, ocular Onchocerciasis and Phycomycosis[15,16)]. The exact pathogenesis of Cutaneous Habronemiasis is unknown, however, a hypersensitivity reaction to the dead or dying larvae and the marked tissue eosinophilia are presumed to be involved[22)]. The larvae were degenerated at the time the biopsy was performed, possibly as a result of the marked tissue eosinophilia induced by the parasite and the ivermectin dose given previously by the referring veterinarian[13)]. In this study we suggest that intense eosinophil's infiltration accompanying larvae due to the role of eosinophil's and their granules in the caseated center and the immunological response for focal granuloma. Also macrophages and Giant cell usually infiltrating of the granuloma formation due to the role of the detectable T-helper lymphocytes. Exuberant fibrosis and collaginolysis usually seen in the vicinity of the dermal immunological reactions of larvae.
Grossly the skin overlying of parasitic granuloma in our case revealed large single unhealed ulcerated skin with hazy irregular borders and containing grayish and yellowish necrotic tissues with offensive odor. These lesions were in accordance with[18,9,20,14,22)] also described lesion as a

reddish-brown subcutaneous nodules which were "greasy," serosanguineous, and contain yellow calcified "rice grain-like" material also our results agree with[18] in dog,[13] in camel,[7]in Rhinoceros and[11,22] in donkey.

Microscopically skin above parasitic granuloma in our case revealedlarge deep cutaneous ulcer containing purulent or serous exudate with living and degenerated neutrophils, sometimes admixed with fungal elements (mycelia) (Phaeohyphomycosis). This finding is in agreement with[3,6] which described the Cutaneous Phaeohyphomycosis as an ulcerated skin with dark colored hyphae covered by neutrophils and dermis diffusely infiltrated by numerous epitheloid macrophages admixed with multinucleated giant cells, neutrophils, lymphocytes, plasma cells and rare eosinophils. In this study, aggregations of microfilaria in dermis with or without inflammatory reactions mainly eosinophils and mononuclear cells were found and appeared in vicinity of sebaceous glands of skin and muscle. Similar results were reported in horse's survey studies in France by[4]. It could be concluded that complicated Cutaneous Habronemiasis consider one of the parasitic cutaneous lesions which is rare among horses in Egypt. Immunological reactions of the parasitic element are one of the etiological agents of skin ulcers and fungal invasions.

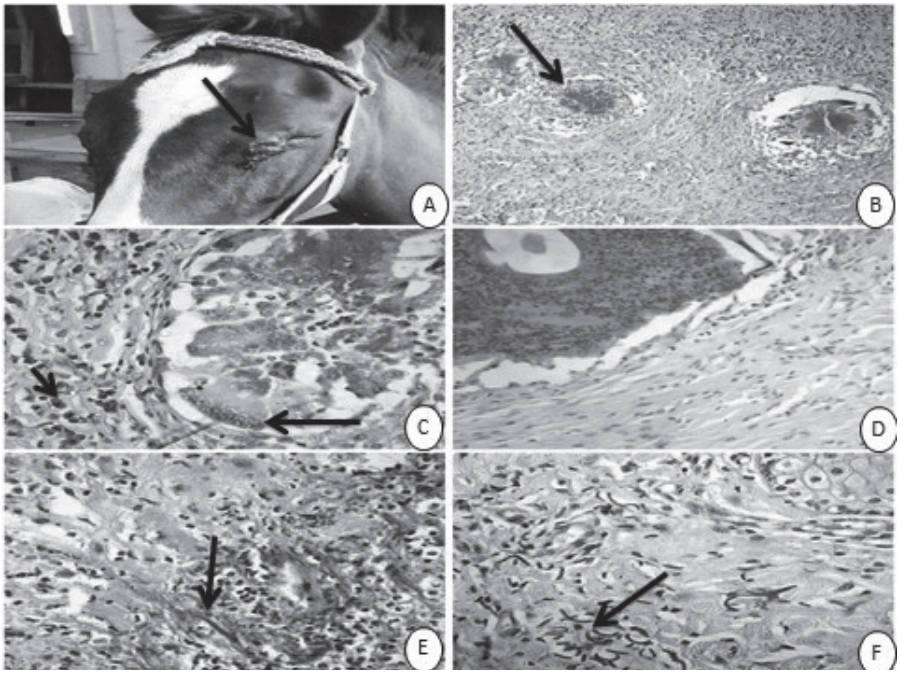

Fig 1: A) Ulcerated lesion on the skin at medial canthus of the left eye of a horse. **B)** Skin of horse showing three multiple degenerated *habronema* larvae surrounded by granulomatous reactions. H&E (X 300). **C)** High magnification of the previous picture to show numerous macrophages and giant cell (arrow) encircled by fibrous capsule infiltrated by eosinophils (short arrow). H&E(X 1200). **D)** Skin of horse showing degenerated *habronema* larvae embedded in caseated tissue. H&E (X 800). **E)** Skin of horse showing fungal filament (arrow) with intense neutrophils infiltrations within the inflammatory exudate. H&E

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