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Case Report

Disseminated acanthamoebiasis in a horse

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Abstract

Amoebic infections have been described in humans and animals, causing lesions in specific organs or as systemic infections. In both animals and humans, immunocompromised patients are predisposed to the infection. The present report described the macroscopic and histologic findings in a Quarter horse filly who presented with a systemic infection caused by *Acanthamoeba* spp. that was characterized by multinodular lesions in lung, kidney, heart, submandibular lymph node, and lip. Histopathology revealed dense inflammatory infiltrates composed of neutrophils, histiocytes, occasional multinucleated giant cells, and numerous intralesional trophozoites. Immunohistochemistry was positive for *Acanthamoeba spp* trophozoites in lungs, kidneys, heart, submandibular lymph node, and lip. No predisposing conditions were identified.

Key words: amoebae, disseminated amoebic disease, lung, *Acanthamoeba*, *Naegleria*, *Balamuthia*.

Introduction

Microorganisms belonging to the genera *Acanthamoeba*, *Naegleria* and *Balamuthia* are free-living amoebae that cause important infections in human and animals (5, 25, 29). *Acanthamoeba* species have worldwide distribution and can be frequently found in soil, dust, air, fresh water and salt water, as well as cellular culture contaminants (5). Additionally, they can be found in other contaminated sources such as swimming pools, drinking water, ventilation systems, dialysis apparatus, hydrotherapy areas in hospitals, dental irrigation systems, bottled mineral water, distilled water bottles, insects, vegetables, and surgical instruments (6).

Acanthamoeba spp. infections occur more frequently in immunocompromised patients, although immunocompetent patients are not totally excluded from infection. This amoeba can induce three clinical syndromes in humans: 1) granulomatous amoebic encephalitis (GAE), an opportunist disease with mortality around 97-98% and with clinical symptoms including lethargy, stiff neck, hallucinations, changes in body temperature, seizures, nausea, and vomiting; 2) amoebic disease disseminated to different tissues and organs, which is seen frequently in AIDS patients and has mortality around 70% with clinical symptoms such as simple or multiple nodules in the skin, weight loss, decreased respiratory efficiency, pulmonary edema; and 3) amoebic keratitis (AK), which is a painful syndrome characterized by clinical symptoms including severe eye pain, blurred vision, edema of the conjunctiva and eyelids and inflammation of the lacrimal gland and extraocular muscle (6, 16, 19, 24).

Infections with amoebae generally occur through inhalations of cysts or exposure of skin lesions to contaminated elements of the environment. *Acanthamoeba* may grow and multiply in the nasal mucosa, followed by hematogenous spread to the rest of the animal, especially to the central nervous system (3, 17, 24, 30). *Acanthamoeba* infections are characterized by relatively high mortality despite low incidence (19). Additionally, multiple factors contribute to these infections such as parasite biology, genetic diversity, environment spread and host susceptibility (6).

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The aim of this case report is to describe the gross, histopathological and immunohistochemical findings of a case of disseminated acanthamoebiasis in a Quarter horse filly.

Case report

A 1-year-old, 321 kg, Quarter horse filly was presented for necropsy. This animal was originally from Riverside county in Southern California, where it was raised for racing purposes together with approximately other 100 horses. No other animal species were present on the property. This animal was fully vaccinated and shared a pen with other 10 yearling horses. Ten days before the necropsy, the filly had a "swollen face" with a presumptive diagnosis of rattlesnake bite, which was treated by the owner with Excede® and Dexamethasone. After a few hours, the face was even more swollen, so a veterinarian began treatment with other antibiotics and corticoids. After five days of treatment, the swelling started to reduce and at day 8, the owner thought the animal was going to recover. On day 10 the horse had a seizure-like event and died, and was submitted for necropsy. This was the only animal affected on the property. All animals received a similar diet and the water was provided by the city (tap water).

At necropsy, the carcass had good general body condition. Necropsy findings included diffuse edema of the inferior lip as well as an enlarged, firm and dark red submandibular lymph node. All lung lobes were dark red and firm with multifocal white firm nodules of approximately 5 to 8 mm diameter, surrounded by a dark red halo (Fig. 1). Similar nodules were also identified in the kidneys and in the mesentery. The pericardium showed several petechial hemorrhages in proximity to the coronary vessels and paragonal groove. No significant findings were detected in other organs.



Figure 1. Lung: Multiple, 0.5 – 1.0 cm diameter, well demarcated, firm white areas surrounded with a red halo are present along the visceral pleura of all lung lobes.

Representative samples of multiple organs were collected and fixed in 10% neutral buffered formalin. After paraffin embedding, 4 µm thick hematoxylin and eosin (H&E) sections were prepared by routine techniques. In addition, Gram stain and immunohistochemistry for *Acanthamoeba* spp., *Balamuthia* spp. and *Naegleria* spp. was performed in lung, kidney and heart, submandibular lymph node and skin of the lip.

Histopathologic examination of the lung revealed multifocal inflammatory infiltrates composed of large numbers of viable and degenerated neutrophils, histiocytes, occasional multinucleated giant cells, admixed with necrotic cellular debris, hemorrhage and fibrin. Within these areas, numerous round eosinophilic protozoal structures were identified, approximately 15 to 20 µm in diameter, with an eccentric single round nucleus (trophozoites) (Fig. 2). Kidney, mesentery and heart had lymphoplasmacytic to pyogranulomatous infiltrates, plus a few intralesional trophozoites. In the lymph node numerous plasma cells, histiocytes, Mott cells, fewer neutrophils and occasional multinucleate giant cells were present, with occasional trophozoites.

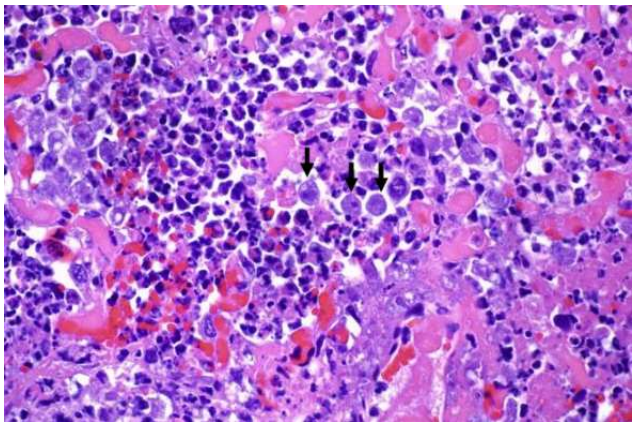


Figure 2. Lung: Numerous *Acanthomoeba* trophozoites present in the alveolar space (arrows), together with numerous viable and degenerate neutrophils, all immersed in edema and fibrin. Hematoxylin and eosin, 600X.

In order to correctly identify the agent, immunohistochemistry was performed for *Acanthamoeba* spp., *Balamuthia* spp. and *Naegleria* spp. on lung sections following the standard procedures of the California Animal Health and Food Safety laboratory, San Bernardino branch, UC-Davis, as previously reported.[18] Sections were counterstained with Mayer hematoxylin and coverslipped.

The immunohistochemistry was positive for *Acanthamoeba* spp. (Fig. 3) and was negative for *Balamuthia* spp. and *Naegleria* spp. in lungs, kidneys, heart, submandibular lymph node and lip. No bacteria were highlighted with Gram stain in the tissues mentioned above. Based on these findings, a diagnosis of disseminated amoebic infection, caused by *Acanthamoeba* spp, was reached.

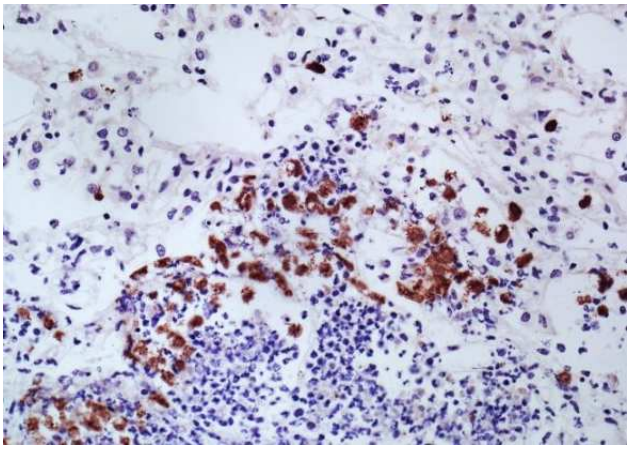


Figure 3. Lung: *Acanthamoeba* spp. immunohistochemistry, with numerous trophozoites with positive immunoreactivity to Acanthamoeba spp. antibody. Mayer's hematoxylin counterstain, 200X.

Discussion

Amoebic infections have been reported causing disease in humans and animals. Both *Acanthamoeba* spp. and *Naegleria* spp. genera are amphizoic organisms because they can be free –living organisms or pathogens and opportunistic parasites. *Acanthamoeba* has two stages in its life cycle: a) an active trophozoite stage with vegetative growth, observed under favorable conditions, and b) a dormant cyst stage, under harsh conditions, with minimal metabolic activity. Both stages can be found in infected tissues of the host and in the environment (7, 19, 24, 25).

In animals, natural infection by *Acanthamoeba* spp. has been described in different species, with inflammatory processes in various organs in association with trophozoites and cysts. Cases of acanthamoebiasis have been reported in animals such as macaques, a bull, kangaroos, Indian buffaloes, reptiles, amphibians, fishes and invertebrates (24, 25, 30). In other species, such as dogs, it has been associated with granulomatous encephalitis (2, 8, 23) or multisystemic amoebic infection with structures present in the skin, nasal mucosa, lung, heart, liver, pancreas, spleen, intestines, adrenals, kidneys, lymph nodes, prostate as well as cornea and peritoneal cavity (2, 6, 8, 9, 15, 21, 26, 27). In cats it has been associated with keratitis, similar to that described in humans (12, 22). In horses, it is associated with granulomatous encephalitis and systemic infection affecting organs such as brain, lung, stomach, kidney, uterus, placenta, lymph nodes, and spinal cord (3, 17). As observed in the present case, reports of *Acanthamoeba* spp. infections in horses suggest the systemic dissemination of the infectious agent affecting multiple organs and causing embolic lesions in association with intralesional trophozoites. However, the clinical history of the previous reports mainly describes the neurologic signs prior to the death of the horses, which in the present case was not reported other than the terminal seizure. The few reports in horses are mostly concentrated in southern California, however the reason for this cluster is not entirely clear (17).

Immunocompromised animals are more predisposed to natural infection and dissemination of *Acanthamoeba* spp. with presentation of GAE or disseminated amoebic disease syndromes, while AK syndrome have been described mainly in experimental infections (9, 17, 19, 26, 30). In the present case, neither the medical history nor the necropsy revealed immunological conditions predisposing to infection by amoebae. At the time of the submission of this report, no other animals in the premises were reported to be ill with a similar condition and after interviewing the owners, no other similar cases have been observed in 20 years of horse breeding.

Amoebic infections can predispose to bacterial diseases, through the so called ´Trojan horse theory´ since amoebae can act as a vector of many pathogenic microorganisms (such as fungi, protozoa and viruses) (4, 11, 19, 25). In humans, amoebic infections principally occur in immunocompromised patients, causing GAE or disseminated amoebic disease developing into encephalitis, meningitis, pneumonia, and dermatitis. In the described keratitis cases, the use of contact lenses has been described as the main predisposing factor and can occur in both immunocompromised and immunocompetent patients (5, 6, 13, 16, 24, 28).

Currently, 22 *Acanthamoeba* genotypes (T1-T22) have been described, with T4 the most commonly strain isolated from humans and animals with GAE and AK (1, 13, 19, 22, 25). The pathogenesis of *Acanthamoeba* infection is still controversial. It has been shown that adhesion to host cells is one of the crucial steps for the pathogenicity to allow other pathogenic mechanisms such as secretion of proteases and phagocytosis, resulting in host cell death by apoptosis (5, 25). The pathogenicity is also directly associated with the secretion of specific enzymes with cytotoxic effects and a high activity of collagenase, elastase, peroxidase but a low activity of superoxidase dismutase, thus the role of oxidative stress in the pathogenicity of acanthamoebiasis is a key and determining factor in the host-amoeba interaction (16, 19, 20).

Trophozoites range from 15 to 35 µm, contain a single and large nucleus and a central nucleolus (karyosome), numerous mitochondria, abundant Golgi apparatus and endoplasmic reticulum, a large contractile vacuole, and occasionally acytoplasmic pseudopodia called acanthopodia, which are responsible for amoeboid movement, adhesion to surfaces or prey capturing. After a period of intensive growth or as a result or harsh conditions, the trophozoites have minimal metabolic activity and transform into a cyst that is highly resistant to many physical and chemical factors. The cystic form can survive for long periods of time at low temperatures (0°-

2°C) and for more than 20 years in *in vitro* conditions. The double-walled cyst ranges from 15 to 20 µm with an undulating or wrinkled outer wall (ectocyst) and a smooth inner wall (endocyst), with variably electron-dense cytoplasm with numerous electron-dense vacuoles (14, 16, 19, 30). Other diagnostic techniques such as immunohistochemistry and PCR allow confirmation of infection (7, 9, 17, 30). Usually the number of trophozoites and cysts can be abundant but these can be difficult to identify.

The diagnosis of amoebiasis is based on the morphologic features of trophozoites and cysts under the light and electron microscopy. Frequently inflammation contains numerous cellular elements that can interfere with their identification, as observed in this case. The differentiation of trophozoites from macrophages requires experience in recognizing the characteristic nuclear morphology, particularly in areas of intense inflammation and necrosis. Because of this, immunohistochemistry is a helpful tool to identify the agent, quantify the degree of infection and precisely identify its location in the affected tissues, particularly in necrotic areas (10, 14). The diagnosis of *Acanthamoeba* in lung, brain and other organs suggest the importance of including differential diagnosis of amoebiasis when the animals show respiratory and progressive neurological signs or systemic disorders (9, 17). There are a few cases of systemic acanthamoebiasis in horses reported in the literature (17). In cases of infection and embolic dissemination of bacteria and fungi in the lung and brain, the clinical signs and lesions may be similar, therefore the microscopic diagnosis with routine techniques in association with immunohistochemistry plays a key role in the correct identification and diagnosis of amoeba infections. The present case allows a better understanding of the range of lesions that can be present, adding a very important differential diagnosis for systemic diseases in horses.

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