



CASE REPORT

Fatal Encephalitis in Cattle Associated with *Acanthamoeba* Infection in Egypt

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ABSTRACT

A 15-month-old bull suffered from pyrexia, anorexia, convulsion, blindness and ataxia, which died after 6 weeks of onset of symptoms. Hematological and CSF examination indicated a preacute inflammatory condition although bacteriological examination gave a negative result. Histopathological examination of brain and cerebrospinal fluid analysis (CSF) showed evidence of amoebic infection. This is the first report which documented *Acanthamoeba* spp. as a causative agent of fetal encephalitis in cattle in Egypt with special attention for its diagnosis.

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INTRODUCTION

Free-living amoebae of genera *Acanthamoeba*, *Balamuthia*, and *Naegleria* are protist pathogens that are ubiquitously distributed in the environment as they are commonly found in soil, dust, fresh water, sewage and swimming pools (Coşkun *et al.*, 2013). In Egypt, *Acanthamoeba* spp. was isolated from the majority of environmental samples in different Egyptian governorates (Mahgoub, 2010). *Acanthamoeba* spp. is capable of causing a blinding keratitis and fetal granulomatous encephalitis in human and animals (Kinde *et al.*, 2007, Qvarnstrom *et al.*, 2013). Although there are only few papers that reported amoebic encephalitis, it should be included among differential diagnosis of central nervous system (CNS) diseases in cattle during summer in tropical or subtropical areas. So far there is only one case of cattle with primary amoebic encephalitis reported in Costa Rica due to infection with *Naegleria fowleri* (Morales *et al.*, 2006). This paper is considered the first report that documented amoebic encephalitis in cattle from Egypt.

History and clinical examination: A 15-month-old, mixed Frisian bull in the farm of Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt, had a past history of lethargy, anorexia and fever. It fed on ad libitum paddy straw and 1.5 kg concentrates twice daily with multi-vitamins supplementation and drinking from fresh-water pools. The clinical examination revealed fever 39.5 C, and reduced ruminal motility without any marked respiratory or neurological signs. It was treated with

analgesics, antipyretic (Dipyrone 0.5g/25kg BW (Analgin 50%, EL-Nasr pharmaceutical chemicals Co. Egypt), anti-inflammatory (Diclofenac Sodium 2.5% 1mg/kg BW (Pharma-SWEDE, Egypt) and long acting antibiotic (Oxytetracycline Hydrochloride 1ml/kg BW; Oxytertracycline 5%, El Nasr pharmaceutical chemical Co, Egypt). After 5 weeks of treatment, signs of convulsion as muscular tremors, falling down and struggling; in addition to fever and blindness were clinically observed on the bull. The bull showed left-side turn of head and neck, ataxia, head pressing, unilateral blindness, exophthalmia, eye opacity with eye and nasal discharge.

A complete neurological examination revealed a failure of papillary reflex and a failure of controlling the balance while there was not any abnormality in spinal reflexes. The case was tentatively diagnosed based on the clinical signs as space occupying lesion (brain abscess) based on the clinical signs. The animal was treated with antimicrobial "Sulphadimidine Sodium 33.3% injection 1mg/10kg BW (El Nasr pharmaceutical chemicals Co. Egypt)", non-steroidal anti-inflammatory, vitamin E and selenium "Viteselen 15 0.5ml/kg BW (ADWIA Co. Egypt)" and vitamins AD3E (50/25/20) 15ml/large animal (Intermedicavet, Egypt). Fever persisted for one week without any response to treatment. It was noticed that the response to treatment with Dimenazine Acetate (Berenil, Hoechst) (10mg/kg IM) was surprising. The body temperature returned to normal and the animal showed slight appetite but ataxia and blindness persisted. The animal was isolated during the period of treatment. During the isolation, two blood samples were taken from

the bull with 3 weeks interval. CSF was collected from lumbosacral space into heparinized tubes for cytological examination and tubes without anticoagulant for biochemical and bacteriological examination.

Abnormal laboratory findings in blood picture were an isocytosis and acanthocytosis. Leukocytic count was $16.3 \times 10^3/\mu\text{l}$ in first sample and decreased to $5 \times 10^3/\mu\text{l}$ in the second one (normal range $4-12 \times 10^3/\mu\text{l}$). Neutrophile count was $11.4 \times 10^3/\mu\text{l}$ in first sample and reduced to $0.8 \times 10^3/\mu\text{l}$ in second sample (normal range $0.6-4 \times 10^3/\mu\text{l}$). Band cells were $0.8 \times 10^3/\mu\text{l}$ in first sample and $1.6 \times 10^3/\mu\text{l}$ in second one (normal range $0-0.1 \times 10^3/\mu\text{l}$). The WBCs morphology showed cytoplasmic basophilia and vacuolation. Laboratory examination of CSF revealed low cellularity ≤ 20 cells/ μl and mononucleated cells were predominant. Bacteriological examination of CSF and serum using direct microscopic examination and bacteriological culture for aerobic and anaerobic bacteria gave negative result. The serological tests for *Toxoplasma gondii* in two serum samples were negative for infection.

After two weeks of second blood sample, the health condition of bull was deteriorated. The bull was in lateral recumbency and in opisthotonus position, unilateral blindness, and exophthalmia, tympany, increased heart and respiratory rate (Fig. 1).

After the death of animal, postmortem examination was performed and there were no detectable pathological changes in internal organs. The head of animal was taken immediately after death for Computed Tomographic (CT) examination of brain. CT was carried out using multi-detector row CT unit (Asteion super 4, Toshiba, Tokyo, Japan). Obtained CT data were reconstructed by image processing workstation (Virtual Place, AZE, Tokyo, Japan), and observed transverse and dorsal plane images, which showed normal brain findings without remarkable space-occupying lesion on the brain (Fig. 2).

Macroscopic examination of the brain showed congestion, edema of the cerebral hemispheres with focal small grayish white softened areas in the cerebral cortex and cerebellum (Fig. 3). On cross section, cerebral hemispheres show multiple foci of encephalomalacia involving the cerebral cortex, subcortical white matter. Cerebellum and brain stem were more severely affected than the cerebral hemispheres. The cut surface was moist and shining and parenchyma was soft (Fig. 3).

Histopathological examination of brain showed multiple areas of malacia and edema. The blood vessels

within the area of malacia revealed endothelial and adventitial proliferation. In addition to the above mentioned lesion, multiple amebic cysts with double wall and prominent nucleus especially around and within areas of malacia were observed (Fig. 4).

DISCUSSION

In the presented case, the signalment, clinical signs appeared on the animal and neurological examination revealed presence of an infectious disease in the forebrain based on the depression, incoordination, head pressing, head turn, unilateral failure of optic reflex and vestibular disease which appear clinically as unilateral ataxia with a continuous fever.

The hematological examination of the first blood sample showed a neutrophilic leukocytosis with left shift together with monocytosis which indicates an inflammatory condition. In second blood sample which was taken after 3 weeks later, the leukogram showed degenerative left shift with decrease leukocytic count and monocytosis which commonly seen in severe per-acute inflammatory condition in cattle owing to smaller marrow reserve of segmented neutrophils in cattle. Monocytosis usually appears in intracellular bacterial or protozoal infection. In addition to cytoplasmic basophilia and vacuolation in leukogram which indicated parasitic infection (Cotter, 2001).

Laboratory evaluation of CSF is the most direct antemortem method of diagnosis although the cause of CNS disorder often cannot be determined. Total mononucleated cell count (TNCC) was ≤ 20 cell/ μl which was slightly increased in comparison to normal reference value in healthy cattle (TNCC ≤ 10 cell/ μl). Total protein (80 mg/dl) was also increased in comparison to reference value in healthy cattle (≤ 67 mg/dl). According to the above mentioned data the laboratory examination of CSF indicated mild inflammation (Stokol *et al.*, 2009).

Mild mononuclear pleocytosis and mild increased protein concentration were characteristic CSF findings in cattle with listeriosis and abscess although bacteriological examination of CSF and serum in this case give negative result to bacterial infection. It is possible that granular lymphocytes increase in animal with lymphocytic vasculitis or liquefactive necrosis (Garma-Aviña and Tyler, 1999).



Fig. 1: The bull suffering from fatal encephalitis was in lateral recumbency, opisthotonus position (a); with exophthalmia before death (b).

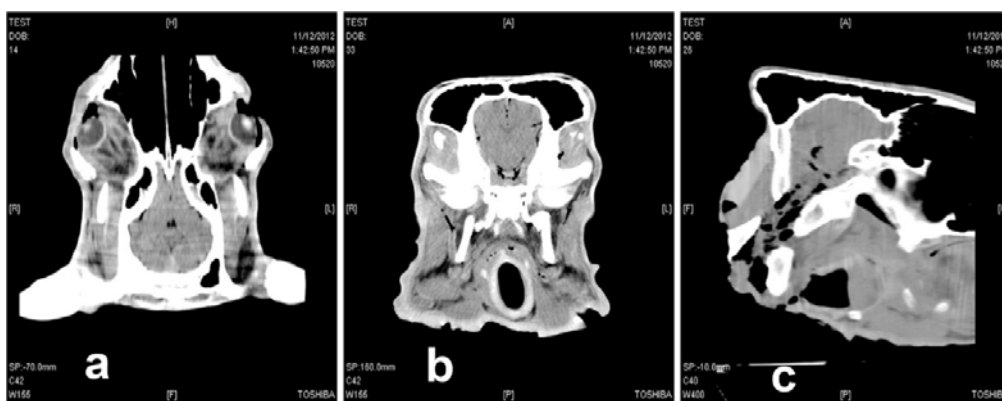


Fig. 2: Dorsal (a), transversal (b) and lateral planes (c) of CT examination of bull (suffering from (Fatal encephalitis associated with *Acanthamoeba*) brain showing normal brain findings



Fig. 3: Brain bull (suffering from (Fatal encephalitis associated with *Acanthamoeba*) showing congestion of cerebral hemispheres (a) and softened areas in cerebellum (a&b). Cross-sections showed multiple hemorrhagic spots in subcortical white matter (c).

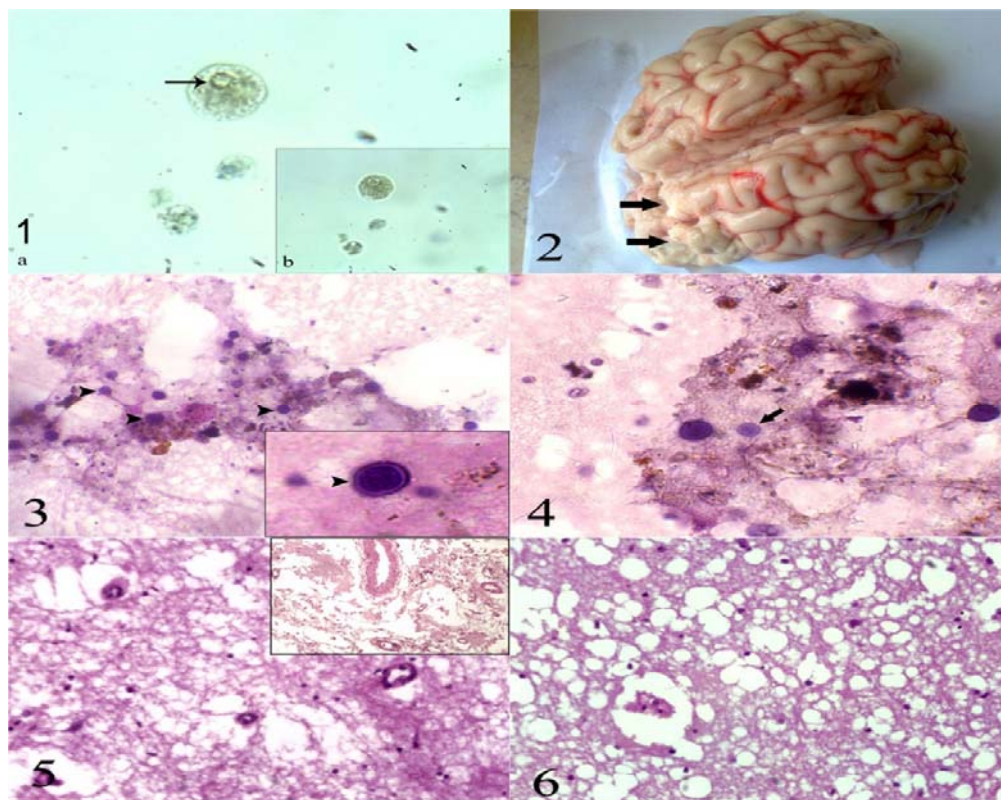


Fig. 4: 1) Showing *Acanthamoeba* spp cysts which are uninucleate (arrow) and have an ectocyst and an endocyst with accompanying polymorphonuclear (PMN) leucocytes in wet mount of CSF smear (a&b); 2) showing congestion, edema of the cerebral hemispheres with focal small grayish white softened areas in the cerebral cortex with multiple parasitic cysts (head arrow). Higher inset showing cyst with double wall (X 400); 3) showing area of malacia with multiple parasitic cysts, one of them (arrow) showing clear nucleus; 4) the brain showing multiple parasitic cysts, one of them (arrow) showing clear nucleus; 5) the brain showing area of malacia; Higher inset, within the area of malacia, the blood vessels reveal endothelial and adventitial proliferation (X 400) and 6) the brain showing pericellular edema. Giemsa stain: 1; H&E stain: 3-6. 1: X1000; 3&5-6: X200. 4: X400.

CT scanning of the brain showing unmarked abnormalities or normal brain findings on plain CT: similar to those non-specific or normal findings of brain was reported in recent studies on amebic encephalitis. Therefore, amebic encephalitis should be considered in any case whenever these non-specific or normal brain findings on CT are accompanied by clinical signs and laboratory findings of encephalitis and history of exposure to fresh-water pools. Moreover, CSF cytology of wet mount becomes mandatory in such case (Naqi and Azeemuddin, 2013).

The histopathological examination of brain tissue and microscopical examination of wet mount of CSF preparation confirmed the presence of mature double wall cysts in highly edematous brain tissue and CSF. Based on the appearance of both trophozoites and cysts in the tissue, the diagnosis should be *Acanthamoeba* infection as cysts are not formed in case of *Naegleria* infection and *Balamuthia* had never been isolated from CSF (Nagi and Azeemuddin, 2013). Unfortunately, there no more samples available for indirect immunofluorescent staining of brain tissue sections or PCR techniques.

A growing body of evidence based on clinical signs, laboratory examinations, favorable response to treatment with Berenil, negative bacteriological examination, CT scanning and confirmed by identifying amebic trophozoite and cyst on histopathological examination of brain tissue and CSF that indicated amebic infection caused by *Acanthamoeba* spp. which is the cause of fatal encephalitis in this bull.

Conclusions: *Acanthamoeba* spp. can be a cause of encephalitis and it must be included in differential diagnosis of nervous diseases causing encephalitis in cattle in Egypt especially in summer season.

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