Larval cyathostominosis in a working donkey

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Abstract Parasitic infections are one of the most common factors that threaten the health and working performance of donkeys. One of the life threatening parasites is the small strongyles that encyst or burrow into the large intestine and their larvae can initiate severe damage in the lining of the intestine. A 6 years old female donkey with clinical signs of diarrhea and emaciation was necropsied and gross examination of gastro-intestinal tract revealed thin-walled, hyperemic and hemorrhagic cecum. Multifocal petechial hemorrhages were particularly prominent in the submucosa of cecum. Parasitological examination revealed two cyathostomin species included *Cylicocyclus elongatus* and *Cyathostomum pathratum*. At microscopic examination, cross sections of cyathostomins larvae associated with parasitic granuloma were observed in the submucosa of cecum. The lesions were associated with non-suppurative enteritis with infiltration of eosinophils, plasma cells, lymphocytes and macrophages in the intestinal mucosa, submucosa and lamina propria.

Keywords Donkey · Strongyle · Cyathostome · *Cylicocyclus elongatus* · *Cyathostomum pathratum* · Nonsuppurative enteritis

Introduction

In underdeveloped countries donkeys play an essential role in the agricultural economies, but these animals have not yet been given sufficient care and they are subjected to many diseases which affect their viability (Khalifa et al. 1988; Trawford and Getachew 2008). Although donkeys are often described as hardy and resistant animals, they do suffer of health problems (Ayele et al. 2006; Getachew et al. 2010). Studies of helminth parasites in working donkeys have uncovered a diversity of helminth species (Trawford and Getachew 2008). Nematode infection is the main problem reported in donkeys (Ali et al. 2001).

Small strongyles (Nematoda; Strongyloidea; Cyathostominae) also known as cyathostomins, infect donkeys and have the ability to penetrate and localize in the wall of large intestine. Unlike large strongyles, the small strongyles don't migrate through tissues but seriously damage cecum and in some instances other parts of the large intestine of donkeys, horses and zebra (Lyons et al. 1999; Matthee et al. 2000).

In most cases cyathostomins become encysted in the wall of the intestine and result in acute diarrhea, colic and occasionally death due to toxic shock. However, some investigators previously considered cyathostomes as parasites with minimal pathogenic influence on the horses and donkeys (Lyons et al. 1999) but, it has been shown that small strongyles create reactions such as catarrhal inflammation, hemorrhagic typhlitis or fibrinous enteritis in the cecum or other parts of the large intestine that may result in intestinal ulcers and possible perforation of the intestinal wall. The larvae of small strongyles encyst or burrow into deeper parts of the large intestine and this encysted stage remains for long periods of time and produces nodules in the deeper layers (Bodecek et al. 2010). In some instances

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larval cyathostomins is associated with the sudden development of large numbers of cyathostome larvae in the wall of the large intestine and further severe clinical signs and advanced pathological changes.

Presumptive diagnosis of cyathostomins can be obtained based on clinical signs but definitive diagnosis is possible by observing the parasitic granuloma due to the larval stage of this parasite. Several different clinical syndromes such as acute protein losing diarrhea, recurrent diarrhea, rapid weight loss, subcutaneous edema, seasonal or malaise syndrome and non-specific colic have been attributed to cyathostome infection. In addition, small strongyles are probably the causative agents of nonstrangulated intestinal infarcts and caecal tympany (Mair and Pearson 1995; Mair et al. 2000; Love 2002).

The present report describes morphological appearance of two species of small strongyles (*Cylicocyclus elongatus* and *Cyathostomum pathraturum*) and the relevant histopathological findings due to these nematodes in a working donkey.

Materials and methods

A 6-years old female donkey (*Equus asinus*) with clinical signs of diarrhea and emaciation was necropsied and post-mortem examination was conducted. At gross examination the cecum was associated with many parasites. The cecum contained a thin wall which showed prominent edema, severe hyperemia and extensive paint brush and petechial hemorrhages. The intestinal mucosa was excessively folded and small strongyle larvae were burrowed into the lining of the intestine and were encysted in cyst-like structures. No significant gross lesion was observed in other organs. For parasitological examination, the worms were collected from the large intestine, relaxed in hot water, fixed and preserved in a solution composed of 70 % ethanol and 5 % glycerin, cleared in Lacto-phenol and mounted in Glycerin-gelatin for microscopic examination. Tissue samples from the large intestine for histopathological studies were fixed in 10 % neutral buffered formalin, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax and sections of 5 µm in thicknesses were stained by hematoxylin and eosin.

The most important structure for identification of small strongyles are shape and dimension of the wall of buccal capsule, presence or absence of extra-chitinous supports of external leaf crown, and submedian and lateral papillae (Lichtenfels 1975; Tolliver 2000; Eslami and Kiai 2007). Identification and nomenclature of this nematode were based on Eslami and Kiai (2007) practical keys for 18 species of small strongyles of horse. Characteristics of the buccal cavity were measured, using a calibrated eyepiece

micrometer, at a magnification of 800×. The helminths were drawn with camera lucida in this magnification.

Results

Two species of *C. elongatus* and *C. pathraturum* were identified in this donkey. *C. elongatus* which had long and straight esophagus and the walls of buccal capsule looked like chicken leg bone after its meat has been eaten and were slightly bowed (Fig. 1). While in *C. pathraturum*, the walls of the buccal capsule appeared to be segmented. The base of the mouth walls looked like fat little stubs that slightly resemble a kidney bean standing on end. About halfway up into the mouth, the walls pinched almost together like a hinge joint; then, the walls of the mouth curved out and up like a pair of “devil’s horns” (Fig. 2).

Histopathological studies revealed cross-sections of several cyathostome larvae associated with nonsuppurative enteritis and infiltration of eosinophils, lymphocytes and macrophages in the intestinal mucosa and submucosa. Numerous larvae of cyathostome were encysted in a thick fibrous connective tissue capsule within the submucosa of cecum which was usually accompanied by cell debris and edema (Fig. 3). Various combinations of inflammatory cells including eosinophils, lymphocytes, plasma cells and macrophages infiltrated in the cyst’s wall and other neighboring tissues. In some occasions, similar to other nematode infections, focal or multifocal granulomatous reactions surrounded the dead larvae that were previously localized in the mucosa, submucosa or lamina propria. The main cell constituents of these granuloma consisted of eosinophils, lymphocytes, plasma cells and macrophages.

Fig. 1 *Cylicocyclus elongatus*, walls of buccal capsule look like chicken leg bone (arrow), (×800)

Fig. 2 *Cyathostomum pathratum*, the walls of the mouth look like a pair of “devil’s horns” (arrows), ($\times 800$)

Fig. 3 Numerous larvae of cyathostome were encysted in a thick fibrous connective tissue capsule (small arrows) within the submucosa of cecum which was usually accompanied by cell debris (large arrow) and edema (H&E, $\times 200$)

Discussion

Donkeys are found in many areas of Iran where they provide transport or act as draught animals but no information has been available on their parasites. Cyathostomes are non-migrating parasites that live in the large intestine of donkeys, horses and zebra (Matthee et al. 2000; Bodecek et al. 2010). Adult small strongyles live in the large intestine particularly the cecum of donkeys. The larvae have the unique characteristic of being able to burrow into the lining of the large intestine (Peregrine et al. 2005). Shortly after infection, the cyathostomin larvae invade the lining of the cecum and ventral colon where they molt and grow in the deeper parts of epithelium, subepithelial layer or lamina propria of the cecum and in less extent other

parts of the large intestine. During the encysted stage, the larvae are resistant to standard de-worming protocols. The larval stages of small strongyles can cause even more serious problems so that at the start of their invasion they may cause serious damage to the intestinal mucosa (Collobert-Laugier et al. 2002). On the other hand, when the cyst wall is ruptured by an emerging larva, the accumulated excretory and secretory products are released, creating foci of intense mucosal inflammation (Smith 1976).

Diagnosis of cyathostomosis is difficult because the fecal egg counts are often very low and the clinical and laboratory signs are normally variable (Mair 2002; Kaplan and Matthews 2004).

When the eggs released by feces, they develop into larvae in humid areas such as pasture lands and the healthy animals could be infected by contamination of water and food with eggs of these nematodes. The larvae only penetrate the wall of the intestine particularly in the cecum and rarely other parts of the large intestine and normally do not penetrate to other organs. After reaching into the large intestine they attach to the mucous membrane by their head, causing erosion, irritation and inflammation. Large infestations can also be triggered by weight loss, colitis and diarrhea. The most significant damage caused by cyathostomins is associated with synchronous emergence of large numbers of encysted larvae, resulting in diffuse inflammation (hemorrhage and edema) of the cecum and ventral colon (Love et al. 1999). In the large intestine in addition to occurrence of diffuse infiltration of inflammatory cells, parasitic granulomatous enteritis was also observed associated with infiltrations of eosinophils, lymphocytes, plasma cells and macrophages into the mucosa, submucosa and lamina propria of the infected donkey in the present study.

Larval cyathostomosis is a distinct clinical syndrome that occurs with the mass emergence of the encysted larvae from the intestinal wall. Small strongyles are less damaging than the large strongyles since the larval migration is limited to the intestinal lining (Kaplan and Matthews 2004). However, the damage impairs normal gut motility, leading to signs of colic. Prevention and treatment of the small strongyles in donkey can be a serious concern for any donkey owner, because the larval cyathostomosis can threaten the health and working performance of the donkeys.

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