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Original article

Treatment of cyathostominosis with ivermectin and its influence on selected blood biochemical parameters

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Abstract

Infections caused by nematodes of the subfamily *Cyathostominae* affect nearly 100% of pastured horses. Despite of an absence of pronounced symptoms, cyathostominosis can have very serious health consequences. The aim of this study was to monitor changes in total protein levels and concentrations of selected microelements and macroelements in the blood of horses before and after ivermectin treatment. In healthy horses infected by the studied parasites, total blood protein levels were below the physiological norm, but iron (Fe), magnesium (Mg), calcium (Ca) and phosphorus (P) concentrations were within normal limits. Ivermectin treatment reduced the number of excreted parasite eggs (FEC) by 100%, and dead parasites were observed in feces. Decreased iron (Fe) concentrations and an insignificant increase in total blood protein levels were reported. A progressive decline in iron levels was observed when parasite eggs reappeared in feces 60 days after treatment. Iron loss takes place as a result of bleeding from the large intestine when adult nematodes affected by the drug are removed from intestine and fourth-stage larvae leave parasitic nodules in the intestinal wall. A drop in iron levels could be an indirect indicator of the severity of cyathostominosis.

Key words: horses, ivermectin, *Cyathostominae*, micro- and macroelements

Introduction

Infestations caused by strongylid roundworms of the subfamily *Cyathostominae* are prevalent in horses and they may affect up to 100% of pastured animals (Tolliver et al. 1987, Lyons et al. 1999, Osterman et al. 2003). In adult horses, the disease is generally asymptomatic or it is observed in sub-clinical form. In animals that are not treated, or are treated irregularly, parasite counts can reach hundreds of thousands

(Chapman et al. 2003, Gasser et al. 2004, Gawor 2009). Larvae consumed with grass shed their exuviae in the gastrointestinal tract and penetrate the large intestinal mucosa. Ecdysis takes place in parasitic nodules and young nematodes enter the intestinal lumen where they mature. The cylindrical buccal capsule of nematodes is armed with inner lamellae and rows of small denticles which enable adults to become attached to the intestinal mucosa. Parasites damage epithelial cells and sometimes blood capillaries of the

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host (Slocombe 1985, Murphy and Love 1997). Secretions from their pharyngeal glands have anticoagulant or even hemolytic properties (Stefański 1963), which could lead to anemia in heavy infections. Clinical symptoms may involve colic and persistent diarrhea with possibly lethal consequences. Larvae penetrate the wall of the large intestine to form parasitic nodules in the mucosa or submucosa (Gasser et al. 2004, Steinbach et al. 2006). The above could lead to larval cyathostomiasis which results in progressive weight loss and health deterioration due to diarrhea and impaired nutrient absorption (Giles et al. 1985, Mathews et al. 2004, Gawor 2009). A decrease in total protein levels and neutrophilia are observed in the blood biochemical profile of most animals infested by *Cyathostominae* (Giles 1985, Kelly and Fogarty 1993, Murphy and Love 1997, Love et al. 1999, Steinbach et al., 2006). Other changes in the blood profile, such as anemia, eosinophilia and fluctuations in enzyme activity, are less frequently encountered (Murphy and Love 1997, Love et al. 1999). There is a general scarcity of data relating to the effect of *Cyathostominae* infection on the blood levels of microelements and macroelements in warm-blooded horses. The aim of this study was to monitor changes in selected biochemical indicators, in particular iron levels, in horses infested with *Cyathostominae* before and after ivermectin treatment.

Materials and Methods

The presence of *Cyathostominae* eggs was determined in feces samples collected from 85 warm-blooded horses, aged three to 15 years, in the spring of 2010 and 2012. Feces samples of 100-200 g were obtained directly from the rectum and stored in plastic containers. Every feces sample was thoroughly mixed before three specimens of 1 g were acquired from each one. They were analyzed on the day of collection by the floatation method with the use of Darling's solution, as modified by McMaster (Whitlock 1948). The number of parasite eggs was counted for each horse and the results were presented as the mean of three measurements. Eggs were identified according to the method proposed by Ziomko and Cencek (1999) based on morphological analyses (shape, thickness and structure of the eggshell, number of blastomeres) performed under a light microscope at 400x magnification. Egg size was measured under the Olympus CX31 microscope with a digital camera, using the Quick PHOTO MICRO 2.3 application for image acquisition and visualization. To confirm the diagnosis of *Cyathostominae* infection, fecal samples from day 0 were cultured on the Petri dishes

(incubation period: 7 days at 37°C). The Baermann technique was used to obtain L3 larvae from 5 g of cultured feces. Five ml of larvae-containing fluid was poured into the Petri dish and larvae were immobilized with 10% iodine solution. From each sample three drops of larvae-containing fluid were applied on the glass slide and observed under a light microscope at 100x magnification in five fields of view. In every field of view the intestinal cells of all visible larvae were counted. All recovered larvae possessed eight intestinal cells.

Upon the determination of *Cyathostominae* eggs in feces samples, 55 horses excreting the highest number of eggs (250-700 eggs/g feces), aged three to twelve years, with body weight of 480 to 550 kg, were selected for successive parts of the study. *Cyathostominae* infestations were treated with commercially available paste for oral application containing 1% ivermectin (Grovermina®, Biovet- Drwalew, PL) in doses recommended by the manufacturer (0.2 mg/kg BW) and determined individually for every animal. 30 horses excreting <200 eggs per 1g feces remained untreated and comprised the control group. The horses had *ad libitum* access to water and pasture throughout the experiment.

Feces samples were collected 24 hours after the administration of ivermectin, on treatment days 2, 3, 4, and every 10 days until the reappearance of *Cyathostominae* eggs in feces.

Blood was collected from the jugular vein into heparinized test-tubes using a closed loop sampling system on the day of ivermectin administration (day 0) and on treatment days 4 and 60. The samples were centrifuged at 2500 rpm for 10 minutes at a temperature of +4°C. Plasma was poured into test-tubes and stored at -80°C until biochemical analyses which involved the determination of total protein levels (TP) by the Lowry micro method (Sigma Diagnostic Kits), and the concentrations of iron (Fe), magnesium (Mg), calcium (Ca) and phosphorus (P) ions by the kinetic method using the Hydrex-Pointe biochemical kit and the Labsystem Uniskan Diabla plate reader.

The results were processed using the Student's t-test. Difference was regarded as significant at a level of $p \geq 0,01$.

Results

Prior to treatment, an average of 350 strongylid eggs were observed in 1 g of feces. 24 hours after ivermectin administration, the egg count per g of feces decreased to 100 eggs. On the fourth day of treatment, all of the analyzed feces samples were free of parasite eggs. The presence of individual eggs was

determined again on treatment day 60 (Fig. 1). Dead nematodes were excreted from the first to the third day after ivermectin administration.

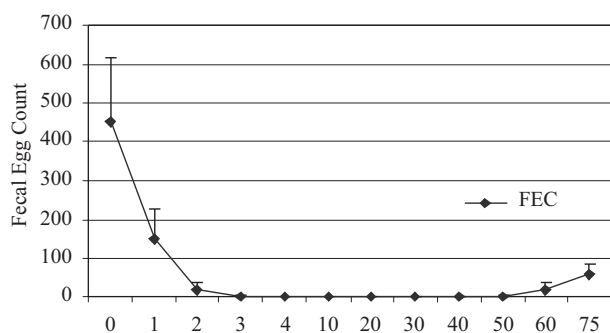


Fig. 1. Fecal Egg Count (FEC) in horses before and after ivermectin treatment.

on day 60. On day 0 the average concentration of iron (Fe) in control horses was determined at 136.05 µg/dl. On day 4 the average level of iron was 131.02 µg/dl, and 115.6 µg/dl on day 60. The average magnesium level on day 0 was 1.90 mg/dl and did not change on day 4. On day 60 it was determined at 2.0 mg/dl. Phosphorus (P) and Calcium (Ca) levels were determined at 3.49 mg/dl and 10.61 mg/dl on day 0 and 4, and at 3.51 mg/dl and 10.68 mg/dl on day 60, respectively (Table 1).

Discussion

The absence of pronounced disease symptoms in infested horses points to the strong compensatory ability of the animals, and the presence of a dynamic balance between the host and the parasites (Love

Table 1. Blood concentrations of selected microelements, macroelements and total protein (TP) in horses before and after ivermectin treatment.

Parameter	Day of examination					
	Day 0		Day 4		Day 60	
	treated (n=55)	control (n=30)	treated (n=55)	control (n=30)	treated (n=55)	control (n=30)
Fe (µg/dl)	135.01	136.05	105.29*	131.02	95.14*	115.6
SD	(35.12)	(31.33)	(21.65)	(15.23)	(22.15)	(37.42)
Mg (mg/dl)	1.89	1.9	1.68	1.9	2.06	2.0
SD	(0.18)	(0.16)	(0.24)	(0.21)	(0.16)	(0.09)
P (mg/dl)	3.51	3.49	3.43	3.49	3.51	3.51
SD	(0.94)	(0.92)	(0.41)	(0.81)	(0.55)	(0.52)
Ca (mg/dl)	10.89	10.61	10.21	10.61	10.62	10.68
SD	(0.16)	(0.21)	(0.14)	(0.16)	(0.12)	(0.09)
TP (g/l)	53.2	53.4	54.16	53.1	52.22	52.91
SD	(2.62)	(2.33)	(2.45)	(2.61)	(2.32)	(2.68)

P ≥ 0.01

Total protein levels (TP) in the analyzed horses reached 53.3 g/l on day 0, 54.18 g/l on day 4 and 52.24 g/l on day 60. The average iron (Fe) concentrations were determined at 135.01 µg/dl on day 0. Iron levels decreased significantly in treated animals to reach 105.29 µg/dl on treatment day 4 and 95.14 µg/dl on day 60. Average magnesium (Mg) concentrations in blood plasma were determined at 1.89 mg/dl prior to treatment and at 1.68 and 2.06 mg/dl on treatment days 4 and 60, respectively. Before treatment, phosphorus (P) and calcium (Ca) levels were 3.51 mg/dl and 10.89 mg/dl, respectively. They were determined at 3.43 mg/dl and 10.21 mg/dl on day 4 pt, and at 3.51 mg/dl and 10.62 mg/dl on day 60 post treatment, respectively.

In 30 untreated horses from the same herd TP levels fluctuated from 53.4 g/l on day 0, to 52.92

et al. 1999). The analyzed 45 horses excreted large numbers of *Cyathostominae* eggs with feces, but they were free of clinical symptoms of disease and their total protein level, blood iron, magnesium, calcium and phosphorus concentrations were within the upper physiological limit. The administration of ivermectin resulted in 100% elimination of parasitic eggs from feces within three days of treatment. Dead nematodes were excreted during that period, and similar observations were made by other authors (Demeulenaere et al. 1997, Osterman Lind et al. 2003, Steinbach et al. 2006, Abbot et al. 2007). In most animals, a drop in blood iron, magnesium, calcium and phosphorus concentrations was noted on treatment day 4, what could not be associated with the influence of nutrition. Iron loss in the first days of treatment could be attributed to bleeding from small but numerous lesions in the

large intestinal mucosa that occurred after the treatment. Peregrine et al. (2006) observed severe microcytosis, what corresponds with iron deficiency, in all examined horses suffering from larval cyathostomiasis. An insignificant increase in total protein (TP) level was also noted on day four after treatment. Similar correlations between total protein levels, calcium and phosphorus concentrations were described by Steinbach et al. (2006) and Pawlas-Opiela et al. (2010). The noted changes were within the physiological norm.

Feces samples were collected at 10-day intervals to capture the moment of parasite recurrence. The developmental cycle of *Cyathostominae* nematodes lasts from eight to 12 weeks (Stefański 1963, Gasser et al. 2004), therefore, it can be assumed that new infestations occurred maximally two weeks after treatment, and that new eggs were produced by young females emerging from third- and fourth-stage larvae undergoing hypobiosis.

The progressive decline in plasma iron levels could be caused by the influence of nutrition at the pasture, but it does not explain the difference between two groups of horses. It is possible that increased blood loss takes place as the great number of awakened larvae exit parasitic nodules at the same time.

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