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SELECTED BIOCHEMICAL PARAMETERS IN STRONGYLE INFECTIONS OF HORSES AND ITS RELATIONSHIP WITH THE PARASITE BURDEN

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Abstract

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This study was carried out to determine effect of strongyle infections on some serum biochemical parameters and correlation between biochemical parameters and parasite burden in infected horses. A total of 11 horses of different breed and ages were used as material. Serum concentrations of glucose, urea, creatinine, total cholesterol, triglyceride, total protein, albumin, globulin, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinine kinase (CK), Sodium (Na), potassium (K), Chloride (Cl), phosphorus (P) were measured and compared with reference values. Worm counts in the gastrointestinal tract and identifications were performed in all horses werenecropsied. Correlation between the number of parasite and biochemical parameters were analyzed. An increase LDH and CK activities and a slightly decrease total cholesterol activities compared with reference values were observed. Statistically significant positive correlations were observed between parasite number and K levels ($r = 0,82$, $p<0.01$). The results obtained from this study suggest that some alterations in biochemical profile may form a basis of monitoring infection.

*Key words***:** biochemical parameters, horse, parasite burden, strongyle

Introduction

Nematodes of the family *Strongylidae* which includes large strongyles and small strongyles are the most common parasites of horses. Infections with these parasites are complex and more than 60 species have been documented (Hodgkingson et al., 2001; Lichtenfels et al., 2008). Strongylosis has a huge impact on the wellfare of infected horses and clinical symptomps ranging from mild depression to colic and fatal enteritis that occurs synchronised reactivation of inhibited larvae from the intestinal mucosa (Hodgkinson et al., 2001, Bodecek et al., 2010; McWilliam et al., 2010; Lamb et al., 2012). Diagnosis of strongylosis in practice based on faecal analysis with microscopic identification of eggs and parasite larvae. Determination of eggs and larvae in faeces is the simplest, noninvasive and most commonly used technique for the intravital diagnosis of strongyle infections. However,besides being species identification of eggs is not possible, the identification of larvae is difficult and time-consuming using morphological parameters,suffering from low diagnostic sensitivities. Therefore these techniques can not detect prepatent infection (Hodgkinson et al., 2001; Bodecek et al., 2010; Lamb et al., 2012). Definitive diagnosis of strongylosis can be made by prove the presence of adult or larvae in the gastrointestinal tract during postmortem examinations. But necropsy is time-labour, cost-intensive and can not be applied to living horses (Love et al., 1999; Lyons et al.,2000; Mair, 2002; Kaplan and Matthews, 2004). Forthis reasons alternative sensitive and spesific methods are required for the diagnosis of strongyle infections in living animals(Mair, 2002; Kaplan and Matthews, 2004; Lamb et al., 2012). One of these alternative methods is determination of hemato-biochemical alterations in infected animals. Laboratory data on haematological and biochemical parameters

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are of exteremely importance to confirm presence or absence of a disease, to assess severity of a disease, and to estimate response of therapy. Nevertheless there are a scarce number of investigations regarding the affect of strongyle infections on biochemical profile in horses (Bodecek et al., 2010; Lamb et al., 2012).

Therefore the aim of the present study was to assess alterations in selected biochemical parameters and its relationship with parasite counts of horsesnaturally infected with strongyles, so as to assist the clinicians by providing reference values for an easy diagnosis of infections.

Material and Methods

Animals and post-mortem examinatons

Eleven mixed-breed horses of both sexes, 1 to 14 years of age were used in this study. The animals purchased for another study from farmers different locality in Bursa province (Marmara region). Prior to purchased rectal faecal samples were taken from the horses and examined to determine strongyle infections. Than infected horses brought to the working station at Uludag University and uniquely numbered with ear tags. They were housed and fed with wheat straw and barley. Water was supplied *ad libitum*. Except for one horse all study animals had non specific clinical signs such as weight loss and inappetence. However one horse showed chronic intermittent diarhoea. After 2-3 weeks for another parasitologic study all horses were euthanized humanely and necropsy examinations were performed. Worm counts in the contents of the gastrointestinal tract and identifications were performed using standard procedures(MAFF, 1986;Lichtenfels et al., 2008; Bowman, 2009).The pepsin–hydrochloride digestion method was used to examine the mucosa for larvae and young nematodes.The worms seen during the gross examination or after digestion collected, cleared in phenol alcohol solutions identified (MAFF, 1986; Lichtenfels et al., 2008; Bowman, 2009).

Biochemical analyses

Before the necropsy blood samples were collected from all animals via jugular vein into Vacutainer blood collection tubes and were transported on ice chest to the laboratory for analysis immediately. Sera were collected by centrifugation at 3000 g for 10 min at room temperature. Serum glucose, urea, creatinine, total cholesterol, triglyceride, total protein, albumin, globulin, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinine kinase (CK), Sodium (Na), potassium (K), Chloride (Cl), phosphorus (P) levels were measured using autoanalyzer (Helena Technicon RA-1000 TM System) and accompanying kits.

Statistical analysis

Statistical analysis was performed using the SPSS 17.00 statistical package.Data were expressed as mean and standard error. The relationship between gastrointestinal nematod counts, and biochemical parameters was analysed using Pearson's correlation coefficient and $p<0.05$ was considered as statistically significant.

Results

Necropsy findings showed that 12 different small strongyle and 2 largestrongyle species were determined.*Cyliococyclus insigne* and *C. longibursatus* were the most prevalent species. In addition to this in one horse *Parascaris equorum* was detected. The number of total strongyles in horses has been presented in Table 1. Total parasite count in horses infected with strongyle ranges from 89 to24343 with mean values ± standart error of 3779±200.09 (data are not shown). Mucosal digestion was positive in 9 study animals and a total of 320 parasites obtained.

The mean valuesof different biochemical parameters with the standart errors and the results of the correlation analysis between parasite count and these parameters have been presented in Table 2. The result of blood biochemical analysis revealed that a significant increase in K consantration in sera. A positive relationship can be seen via the correlation coefficient ($r= 0.82$, $P < 0.01$) between the K levels and that of total strongyle counts recovered. Although with the increase of the total parasite counts, an increase was also observed in LDH and CKlevels, this relationship was not statistically significant. On the other hand a slightly decrease total cholesterol activities were observed when compared with reference values. Statistically insignificant negative correlations were determined between the number of parasites and total cholesterol, total protein, creatinine, triglyceride, globulin and Cl.

Table 1
The number of total strongyle in 11 horses

Horse No	The number of total strongyle
1	24343
2	7680
3	6720
4	1322
5	1045
6	491
7	1011
8	57
9	923
10	1649
11	89

Table 2
Mean ± SEM value of some serum biochemical parameters and correlation coefficient of relationships these parameters and the count of parasites in infected horses with *Strongylida*e spp. (n=11)

Analysed Biochemical Parameters	Mean± SEM	Pearson Correlation coefficient
Glucose, mg/dl	80.00±3.73	0.14
Urea, mg/dl	26.33±2.52	0.01
Creatinine, mg/dl	1.05±0.03	-0.29
Total Cholesterol, mg/dl	62.33±4.05	-0.18
Triglyceride, mg/dl	29.00±2.35	-0.30
Total Protein, g/dl	6.90±0.24	-0.18
Albumin, g/dl	2.26±0.07	0.27
Globulin, g/dl	4.63±0.25	-0.25
AST, U/l	210.08±21.66	0.28
LDH, U/l	766.50±10.05	0.10
CK, U/l	371.66±10.01	0.06
Na, mEq/l	140.50±1.96	0.48
K, mEq/l	4.08±0.22	0.82*
Cl, mEq/l	89.25±2.24	-0.08
P, mg/l	2.85±0.22	0.52

***:** statistically significant

Discussion

The present study was conducted to determine, if there are detectable differences in the biochemical parameters in the serum of horses infected with strongyles and itsrelationship with parasite count in the gastrointestinal tract. While faecal analyse estimate parasitic infection during the patent period, analysed biochemical parameters can be usefull diagnostic tolls in evaluations of the pre-patent period of infection of horses with strongyles. Furthermore, there are very few studies regarding biochemical changes in strongyle infections in horses and their relationship with parasite burden. In this study glucose levels were found 80.00±3.73 mg/dl. This result is within reference rangewhich were determined for healty horses (Kahn and Line, 2011). There was no statistically significant correlation between glucose levels and parasite number. Urea and creatinine levels were also in a normal range for a resting horse (Steffey, 1980; Ju et al., 1993; Kahn and Line, 2011). Correlation coefficient between parasite number and urea and creatinine levels were not significant.

Although triglyceride levels was in a normal range for a testing horse, there are negative insignificant (r:-0.30) correlation between triglyceride level and parasite burden.In contrast to our study reduced triglyceride concentrations have been found in donkeys infected with strongyle species (Moore et al., 1994). Lamb et al. (2012) argued that strongyles cause damage to villi of the intestinal wall is likely to interfere with triglyceride absorbtion from the intestinal content and resulted in decreased triglyceride concentration. Moreover these authors (Lamb et al., 2012) reported that if the infection persists, triglycerideconcentrations may increase as fat stores are mobilized.

However mean values of total cholesterol determined in our study was slightly lower than the results of Ju et al. (1993) which were reported that 85.15±14.37 mg/dl. Also, we could not detected significant correlation between parasite countand total cholesterol levels. A previous study revealed that an increase of total cholestorol level in strongyle infected donkeys (Parsani et al., 2011). The increase in cholesterol level in infected animals may be linked to the parasitic stress which might have resulted an increase in the output of epinephrine and corticosteroids.

Total protein concentration in serum is acommon measurement in clinical laboratory diagnosis. Changes in the levels of plasma proteins may result from alteration in synthesis, catabolism or from protein losses. In this study serum total protein, albumin and globulin levels were almost the same with reported by Ju et al. (1993) which were reported that 6.99±0.54g/dl; 2.69±0.32g/dl; 4.30±0.49g/dl, respectively. Esmat et al. (1993) found significant decrease in serum glucose, total serum protein, albumin, globulin in internal nematod infections of horses. In the current study we observed that no correlation between thecount of parasite and total protein, albumin and globulin levels. In contrast to our findings Smets et al. (1999) reported a significant relationship between hypoproteinemia and the presence of cyathostome L4 in the faeces of horses. As postulated Parsani et al., (2011) the reduction in protein levels might be due to fact that poor absorption of the dietary protein. Because strongyles causesdamage of epithelial layer and villous atrophy in the intestine of infected animals leading to reduction of absorption area for degraded protein.

Enzyme profilesare one of main biochemical parameters used in equine medicine to assess muscle and liver functions. While serum titres of CK and AST enzymes are usually measured to evaluate muscular damage (Kanekoet al., 1997), LDH is nonspecific muscle-liver enzyme in the horses. The increasing in the serum concentrations of LDH has been related to the injury on the intestinal mucosa (Reeder et al., 2009). Fransisco et al. (2011) found a positive significant correlation between the numbers of ascarids egg output and the levels of AST and CK as well as between the *Cyathostomine* faecal egg counts and the LDH. In the present study an increase in LDH and CK activities were determined but not in AST activities when compared with reference values

(Kaneko et al., 1997; Kahn and Line, 2011). The rise in the level of these enzymes reflects muscular damage by migratory phase of parasites. Nevertheless in contrast to Fransisco et al. (2011) we couldn't found correlation between the number of parasite and AST, LDH and CK levels. Only potassium level exceeded normal ranges throughout the study, and a positive significant ($p<0.01$) correlation with the number of parasites demonstrated. However, no statistically significant correlation could be found between parasite counts and Na, Cl and P levels in this study. On the contrary of our results-Bodecek et al. (2010) found that decreased levels of potassium in three horses.

In conclusion, in the present study we determined an increase LDH and CK activities and a slightly decrease total cholesterol activities compared with normal ranges. Moreover, we observed a strong correlation between parasite count and K levels. From the results obtained in this study it can be concluded that the potential use of biochemical analysis for diagnosis of *Strongylidae* spp. infections in horses. But in order to exact prove the diagnostic value of biochemical parameters in strongyle infections, it is recommended to carry out further studies using experimentally infected animals.

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