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EFFECT OF AN THE LMENTIC THERAPY ON HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN DONKEYS

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ABSTRACT

Parasites are the most common cause of colic and other significant health conditions in equines, among them is the strongyles species. Donkeys in this study were not initially given treatment. A number of fecal and blood samples (whole blood and serum) were taken from donkeys of the current study. The fecal samples were subjected to fecal flotation test. The donkeys were positive for strongyloid egg and negative for other eggs. The donkeys were examined by McMaster technique yielding an average of 790 EPG (egg per gram) and fecal culture demonstrating *Strongylus equinus* larvae. The whole blood samples collected from donkeys were used for hematological examination. The sera collected from donkeys were used for biochemical examination. The donkeys were tested again after using pyrantel tartarate by means of McMaster technique with an average count of 272 EPG and by means of hematological and biochemical parameters. The presented results in this study revealed that treatment with anthelmintic confirms the effect of gastrointestinal nematodes infestation, with special reference to Strongylus species, in Egyptian environmental conditions on biochemical and hematological profile of equine species, which act as metabolic biomarkers.

Key words:

Strongylus, Mac master, Biochemical, Hematological, pyrantel tartarate

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. Strongylosis has a huge impact on the welfare of infected horses and clinical symptoms ranging from mild depression to colic and fatal enteritis. Diagnosis of strongylosis in practice based on fecal analysis with microscopic identification of eggs and parasite larvae **(Guzel *et al.* 2014).**

Strongylosis and cyathostomosis can affect all equids (horses, donkeys, zebras and mules) of any breed or gender. Infection can occur in animals of any age, but the clinical signs are more noticeable and severe in younger and unexposed animals. The reduction in sensitivity to strongyles infection in adults suggests the development of a degree of acquired immunity. However, the immune response can take a long time to develop, even in cases of prolonged parasite exposure, remains incomplete. Equine strongylosis is the most common parasitic infection seen in horses worldwide, having a massive impact economically and on animal welfare. Small strongyles burdens consist of more than 90 per cent larvae and more than 50 per cent of the larval population consists of inhibited early larval stage three (EL3). *Strongylus vulgaris* was once considered to be the most important parasite in horses, although its significance has reduced since the introduction of treatment regimens with current anthelmintic compounds. The prevalence of strongylosis caused by *S. vulgaris* is now much lower than cyathostomosis. For the same reasons, there has also been a reduction in prevalence of other large strongyles (*S. edentatus* and *S. equinus*), with large strongyles eggs now representing a small proportion of total strongyles eggs found in fecal analysis **(Elsheikha and Hallowell, 2014)**.The donkey (*Equus acinus*), popularly known as a 'beast of burden' play an important role in socio-economic development by providing transportation in areas that are inaccessible to motorized vehicles or where modern means of transportation are absent. They are the cheapest and easiest means of transport for small, marginal and poor farmers in tropical countries like India. Although donkeys are often regarded as sturdy and resistant species, they are also vulnerable to an array of infectious and non-infectious diseases including parasitic infections. A heavy internal parasitic burden adversely affects the health of the donkeys, particularly when they are undernourished and put to hard work. Equids are the host of more than 75 species of helminths. The most common helminths of equines are strongyles, tapeworms (*Anoplocephala perfoliata*), ascarid (*Parascaris equorum*), pin worm (*Oxyuris equi*) and the lung worm (*Dictyocaulus arnfieldi*). Among the helminths, strongyles are considered to be major cause of illness in donkeys. Parasitic infections are also known to cause considerable morbidity and mortality in donkeys. Although the importance of donkeys as pack animals is not debatable in developing countries, still they receive very little attention from research point of view. Laboratory data on hematological and biochemical parameters are of utmost importance to confirm presence or absence of a disease, to assess severity of a disease, and to estimate response of therapy. Furthermore, results of hemato-biochemical

analyses when combined with thorough physical examination and history form a basis for the veterinarians to arrive at a final diagnosis of a disease **(Parsani *et al.* 2011)**. Accordingly, the aim of the current study is to assess alterations in major hematological and biochemical parameters of donkeys infected naturally with helminthes, so as to assist the clinicians by providing reference values for an easy diagnosis of helminth infections in donkeys and determining effective therapy.

MATERIAL AND METHODS

Animals and samples:

A total of 5 donkeys were used in this experiment without rotational periodic treatment with anthelmintics.

Fecal samples:

A total of 5 fecal samples from 5 donkeys were collected from rectum (to ensure that they are completely fresh) in clean labeled plastic bags and examined directly for gastrointestinal parasites. The positive samples undergo fecal culture.

Blood samples:

Two blood samples were taken from each animal by vein puncture of jugular vein for hematological and biochemical studies.

a- Whole blood samples: A total of 5 whole blood samples were collected from each animal and 2 ml of blood of each sample was placed in Lavender stopper tubes (vacutainer tubes) containing anticoagulant.

b- Blood serum samples: A total of 5 whole blood samples were collected from each animals and 10 ml of blood of each sample was placed in Red-stopper tube (vacutainer tubes) without anticoagulant. The samples were left for 30 minutes at room temperature until clotting and then centrifuged at 3000 rpm for 20 minutes. The obtained clear sera were transferred to Eppendorf tubes and stored in a deep freezer at -20°C for biochemical analysis.

Faecal Analysis:

The 5 fecal samples were examined by fecal flotation test for parasites egg determination. The positive donkeys were tested by McMaster technique to determine fecal egg count and used before and after the treatment. Moreover, the fecal culture was used for larval identification.

Blood analysis:

The whole blood samples were used for hematological examination; RBCs total count, WBCs total count, differential leucocytic count, hemoglobin concentration & PCV.

Biochemical analysis:

The sera were analyzed for the measuring of serum total protein, albumin, globulin, A/G, ALT, GGT, BUN, creatinine, calcium and phosphorus using commercial test kits supplied by Spectrum Bioscience.

Statistical analysis:

The results were analyzed by Wilcoxon Test and were considered significant when $p \leq 0.05$. The computer software, SPSS version23 for windows was used for analysis.

RESULTS

Fecal analysis results are illustrated in Fig. (1Aand 2A) and (Table 1). For fecal flotation test, the 5 donkeys were positive for strongyloid egg and negative for ascaris egg as shown in Fig. (1).

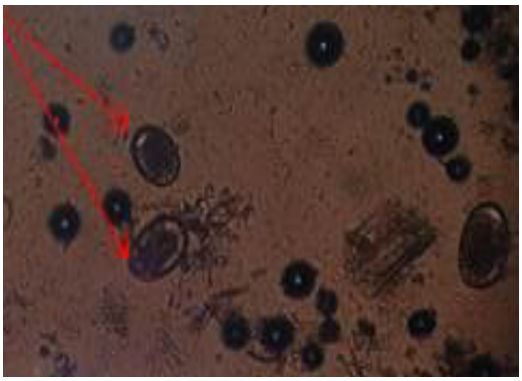

Fig. (1 A): Demonstration of strongyloid egg as shown by arrow

For Fecal egg count (McMaster technique), the 5 donkeys that were positive for fecal flotation test were tested for fecal egg count (FEC) where the total number of eggs in both sides of the chamber was multiplied by 50. The total number will be expressed as eggs per gram of feces (EPG) as shown in (Table 1) and also EPC was calculated after treatment with antithelmentic. Further investigation using fecal culture for the positive samples, demonstrating *Strongylus equinus* larvae as shown in Fig. (2).

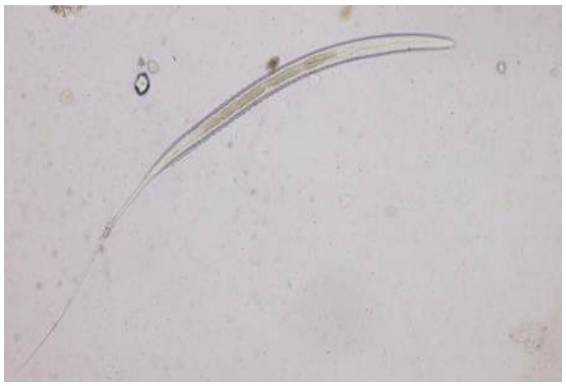

Fig. (2 A): demonstration of *Strongylus equinus* larvae whole worm.

Table (1): Showing fecal egg count expressed as eggs per gram of feces.

	N	Mean	Std. Deviation	Minimum	Maximum	P-value
Total Count (EPG) before treatment	**5**	**790**	**438**	**400**	**1450**	**0.042**
Total Count (EPG) after treatment	**5**	**272**	**172**	**130**	**550**	

The bold numbers indicate the high significance p≤ 0.05.

Hematological analysis results. Are illustrated in (Table 2 and 3) Hematological examination before the treatment yielded average readings as follows; (RBCs 4.7× 10^6/µl, PCV 29.2%, hemoglobin 9.9 g/dL, WBCs 10.6× 10^3/µl, neutrophils 45.4%, lymphocytes 37.2%, eosinophils 12.6%, basophils 0.4%, monocytes 4.4%, MCV 62.7 fL, MCH 21.2 pg and MCHC 33.9 g/dL), while after treatment using pyrantel tartarate gave average readings as follows; (RBCs 5.5× 10^6/µl, PCV 31.8%, hemoglobin 10.7 g/dL, WBCs 10.7× 10^3/µl, neutrophils 46.2%, lymphocytes 39.2%, eosinophils 10.6%, basophils 0.4%, monocytes 3.6%, MCV 57.7 fL, MCH 19.3 pg and MCHC 33.5 g/dL). The significance between the hematological parameters readings of the animals before and after the treatment as displayed in (Table 2).

Biochemical analysis results:

Regarding to the biochemical results at table 3, the sera collected from donkeys used for biochemical examination before treatment yielded an average readings as follows. (total protein 4.1 g/dL, albumin 1.8 g/dL, globulin 2.4 g/dL, A/G 0.8, ALT 46.4 U/L, GGT 83.6

U/L, BUN 22.8 mg/dL, creatinine 0.9 mg/dL, calcium 9.0 mg/dL and phosphorus 3.4 mg/dL). while after treatment using pyrantel tartarate the biochemical examination gave average readings as follows; (total protein 4.3 g/dL, albumin 2.0 g/dL, globulin 2.3 g/dL, A/G 0.9, ALT 58.9 U/L, GGT 115.6 U/L, BUN 25.2 mg/dL, creatinine 1.3 mg/dL, calcium 7.2 mg/dL and phosphorus 4.8 mg/dL). The significance between the biochemical parameters readings of the animals before and after the treatment as displayed in (Table 3).

Table (2): significance between haematological parameters of infested donkeys before treatment and after treatment with using pyrantel tartarate.

	N	Mean	Std. Deviation	Minimum	Maximum	P-value
RBCs (106/µL) BT	**5**	**4.7**	**0.507**	**4.1**	**5.2**	**0.042**
RBCs (106/µL) AT	**5**	**5.5**	**0.647**	**4.9**	**6.4**	
PCV (%) BT	**5**	**29.2**	**1.483**	**27.0**	**31.0**	**0.042**
PCV (%) AT	**5**	**31.8**	**1.789**	**30.0**	**34.0**	
Hemoglobin (g/dL) BT	**5**	**9.9**	**1.003**	**8.3**	**11.0**	**0.043**
Hemoglobin (g/dL) AT	**5**	**10.7**	**1.013**	**9.3**	**11.8**	
WBCs (103/µL) BT	**5**	**10.6**	**2.359**	**7.6**	**13.0**	**0.683**
WBCs (103/µL) AT	**5**	**10.7**	**2.268**	**7.9**	**13.4**	
Neutrophils (%) BT	**5**	**45.4**	**5.505**	**37.0**	**50.0**	**0.414**
Neutrophils (%) AT	**5**	**46.2**	**4.147**	**41.0**	**51.0**	
Lymphocytes (%) BT	**5**	**37.2**	**2.588**	**34.0**	**41.0**	**0.059**
Lymphocytes (%) AT	**5**	**39.2**	**3.271**	**35.0**	**44.0**	
Eosinophils (%) BT	**5**	**12.6**	**4.506**	**8.0**	**19.0**	**0.039**
Eosinophils (%) AT	**5**	**10.6**	**3.209**	**7.0**	**15.0**	
Basophils (%) BT	**5**	**0.4**	**0.548**	**0.0**	**1.0**	**1.000**
Basophils (%) AT	**5**	**0.4**	**0.548**	**0.0**	**1.0**	
Monocytes (%) BT	**5**	**4.4**	**1.140**	**3.0**	**6.0**	**0.102**
Monocytes (%) AT	**5**	**3.6**	**1.673**	**1.0**	**5.0**	
MCV (fL) BT	**5**	**62.7**	**4.841**	**56.8**	**69.0**	**0.043**
MCV (fL) AT	**5**	**57.7**	**3.473**	**53.1**	**61.2**	
MCH (pg) BT	**5**	**21.2**	**1.313**	**20.0**	**23.3**	**0.043**
MCH (pg) AT	**5**	**19.3**	**0.823**	**18.4**	**20.3**	
MCHC (g/dL) BT	**5**	**33.9**	**1.898**	**30.7**	**35.5**	**0.144**
MCHC (g/dL) AT	**5**	**33.5**	**1.488**	**31.0**	**34.7**	

The bold numbers indicate the high significance p≤ 0.05.

Table (3): Significance between biochemical parameters of infested donkeys before treatment and after treatment using pyrantel tartarate.

	N	Mean	Std. Deviation	Minimum	Maximum	P-value
Total protein(g/dL) BT	**5**	**4.12**	**0.45**	**3.6**	**4.6**	**0.063**
Total protein(g/dL) AT	**5**	**4.28**	**0.33**	**4**	**5**	
Albumin(g/dL) BT	**5**	**1.76**	**0.15**	**1.5**	**1.9**	**0.042**
Albumin (g/dL) AT	**5**	**2.02**	**0.19**	**1.8**	**2.3**	
Globulin(g/dL) BT	**5**	**2.36**	**0.38**	**1.8**	**2.7**	**0.285**
Globulin(g/dL) AT	**5**	**2.26**	**0.23**	**2**	**3**	
A/G BT	**5**	**0.78**	**0.13**	**0.7**	**1.0**	**0.102**
A/G AT	**5**	**0.88**	**0.11**	**0.8**	**1.0**	
ALT(u/L) BT	**5**	**46.38**	**13.38**	**33.5**	**67.0**	**0.042**
ALT(u/L) AT	**5**	**58.98**	**12.17**	**49**	**79**	
GGT(u/L) BT	**5**	**83.58**	**73.30**	**5.8**	**172.5**	**0.043**
GGT (u/L) AT	**5**	**115.64**	**79.31**	**28.1**	**202.0**	
BUN(mg/dL) BT	**5**	**22.80**	**4.01**	**18.2**	**27.4**	**0.041**
BUN(mg/dL) AT	**5**	**25.20**	**4.17**	**19**	**30**	
Creatinine(mg/dL) BT	**5**	**0.90**	**0.16**	**0.7**	**1.1**	**0.104**
Creatinine(mg/dL) AT	**5**	**1.28**	**0.40**	**0.9**	**1.9**	
Calcium(mg/dL) BT	**5**	**9.00**	**2.10**	**5.7**	**11.0**	**0.059**
Calcium(mg/dL) AT	**5**	**7.20**	**1.25**	**6**	**9**	
Phosphorus(mg/dL) BT	**5**	**3.36**	**0.61**	**2.7**	**4.1**	**0.034**
Phosphorus(mg/dL) AT	**5**	**4.76**	**0.72**	**3.9**	**5.7**	

The bold numbers indicate the high significance p≤ 0.05.

DISCUSSION

Equine worldwide are exposed to an array of gastrointestinal nematodes, which are considered very dangerous and life-threatening causing colic, weight loss, disease and performance problems when present in large numbers in animals grazing on contaminated pasture and are not treated with effective anthelmintic **(Matthews, 2014)**. The fecal flotation test of equines in our study revealed the presence of strongyloid egg and absence of ascaris egg in the examined donkeys. The donkeys that were positive for fecal flotation test were tested for fecal egg count (McMaster technique) and the mean egg count was 790 per gram of feces, which is ranked as high contaminators **(Kaplan and Nielsen, 2010)**. The donkeys were then treated with pyrantel tartrate at a dose of 12.5 mg/kg at an interval of 14 days and then FEC was repeated resulting in an average count of 272 per gram of feces. The fecal egg count reduction test (FECRT) is considered the gold standard for clinical diagnosis of anthelmintic resistance in horses **(Kaplan, 2002**), the fecal egg count reduction test in this study was equal to 65% and so anthelmintic resistance is generally considered to be present as FECRT is less than 95% (**Mckenna 1990 and Mckenna 1994**). Using fecal culture, the positive strongyloid egg in donkeys in our investigation demonstrated *Strongylus equinus* larvae which have migratory pathway and adverse effect on equine health **(McCraw and Slocombe, 1985**). The current experiment was conducted to evaluate the effect of gastrointestinal nematodes infestation on biochemical and hematological profile of equine species, which further confirms that the larvae of nematode in equines induces biochemical and hematological changes in affected equine. The growth of all parasitic nematodes, including *Strongylus spp.* requires energy and macromolecular precursors derived from the metabolic network of the host animal and this means during infection and development of the *Strongylus*, the horses' body undergoes certain metabolic changes. This indicates that the larval strongylosis can alter the horses' metabolic profile and this can be associated with specific metabolic biomarkers. In the current study, the relation between animals readings of the hematological and biochemical parameters before and after treatment were analyzed by Wilcoxon Test. Regarding to the hematological parameters, the RBCs count, PCV% and Hg concentration were less than normal levels, These findings may be due to blood loss from ingestion of erythrocytes as the large strongyles are voracious blood suckers and probably due to reduced haemopoiesis as a result of poor metabolism, damaging intestinal lining and poor quality nutrition status **Parsani *et al.*** (**2013**). These findings were found to be in disagreement with

those of **Wosu** (**2014**) who have reported the hematological parameters fell within the normal range of values in the examined equine despite high parasite burden. The horse is unique in comparison to most other mammalian species in that its spleen is a very capacious organ, storing up to one third of the RBCs, and this reserve can be mobilized by stress (including those caused by infection) and can rapidly be transferred into circulation in response to stress (infection) and this compensatory response phenomenon could explain why most of the blood parameters remained within the normal range of values despite high parasite burden. The mean value of RBCs, PCV and hemoglobin was evaluated on day 90 post medication with 6 doses of pyrantel tartrate at a dose of 12.5 mg/ kg 2 weeks apart without change in the quality of nutrition. *Statistically* by using Wilcoxon Test revealed significant improvement in these parameters and suggest that, the cause of anemia may be due to strongyles infection after discard the nutritional factor. The mean of WBCs count and the mean percent of neutrophils, lymphocytes, basophils and monocytes was analyzed by Wilcoxon Test and revealed no significant change. The mean percent of eosinophils which is considered higher than normal percent reported by **Yakubu *et al.* (2008), Burden *et al.* (2016), Parsani *et al.* (2011) and Laus *et al.*** (**2015**). The high eosinophil counts in infested animals due to migratory larvae and due to the extremely elevated levels of IgE in parasitized animals which mediate mast cell degranulation thereby stimulate release of eosinophil chemotactic factor of anaphylaxis. This material, in turn mobilizes the body's eosinophil pool resulting in the release of large number of eosinophil into circulation **(Sipra *et al.*, 1999)**. By using Wilcoxon Test revealed significant decrease in these parameter and suggest that the cause of eosinophilia may be due to strongyles infection after discard the nutritional factor. For the biochemical parameters, the mean value of total protein, albumin, globulin and A\G ratio were found to be less than normal. This result was in agreement with **Esmat *et al*. (1997), Parsani *et al*. (2011) and Guzel *et al.*** (**2014**) who have reported that there is significant decrease in total protein, albumin, globulin and A\G ratio in infected donkey with strongyles. These parameters were evaluated on day 90 post medication with 6 doses of pyrantel tartrate at a dose of 12.5 mg/ kg 2 weeks apart without change in the quality of nutrition, were analyzed by Wilcoxon Test and revealed significant change in albumin and insignificant change in other parameters.These results are suggestive of poor absorption of the dietary constituent from intestinal tract, which might have been due to gastrointestinal disturbances caused by parasite infection, as strongyles are known to cause necrosis and desquamation of

the epithelial layer of the intestinal wall leading to reduction of absorption area for degraded protein, beside low absorption of protein coupled with less dietary intake as a result of anorexia during infection period **(Parsani *et al.*, 2011)**. The mean value of GGT was in disagreement with normal value recorded by **Laus *et al.* (2015) and Burden *et al.*** (**2016**). This parameter was evaluated on day 90 post medication with 6 doses of pyrantel tartrate at a dose of 12.5 mg/ kg 2 weeks apart without change in the quality of nutrition and there was further significant increase by using Wilcoxon Test The mean value of ALT was in disagreement with normal value recorded by **Seri *et al.*, (2006), Simenew *et al.* (2011), Sow *et al.*, (2012) and Laus *et al.* (2015).** This result was in agreement with **Parsani *et al.*** (**2011**) who has reported that there is significant increase in ALT in infected donkeys with strongyles due to migratory larvae to the liver This parameter was evaluated on day 90 post medication with 6 doses of pyrantel tartrate at a dose of 12.5 mg/ kg 2 weeks apart without change in the quality of nutrition and there was further significant increase by using Wilcoxon Test. At the same context, the mean values of BUN, creatinine, phosphorus and calcium were found to be in the normal range **Seri *et al.*, (2006).** Finally, further studies are recommended to establish an accurate reference standard on a large scales across Egypt for hematological and biochemical profiles of equines in relation to parasitic infestation in Egyptian climatic and environmental conditions. Also, a regular and routine system for screening parasitic infestation required to ensure periodically that, the program implemented is the optimized program for control and prevention of parasitic infestation.

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