

Immunological And Biochemical Changes In Draft Horses Naturally Infested With Gastrointestinal Nematodes With Trail Of Treatment With Fenbendazole In Sharkia Governorate

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ABSTRACT

This study is done to clarify some adverse effects of gastrointestinal nematodes on immunological and biochemical status of draft horses and modulating by fenbendazol. Parasitological examination of 50 faecal samples revealed presence of gastrointestinal nematodes eggs in 38 (76%), horses distributed according to age {<1 year 15 (39.47%), >1-4 years 13 (34.21%), > 4-10 years 10 (26.32%)}, the type of infestations were single 12(31.58 %) or mixed 26 (68.42%). The single infestation were *Strongylus spp* 6 (15.79 %), *Parascaris equorum*, 4 (10.53 %), *Oxyuris equorum* 2(5.26%) while mixed infestation were *Strongylus spp* with *Parascaris equorum* 7 (18.42%), *Parascaris equorum* with *Oxyuris equorum* 5(13.16 %) and *Strongylus spp* with *Parascaris equorum* with *Oxyuris equorum* 14 (36.84%). The treatment with Fenbendazole revealed 100% reduction in egg count/gm on day 9 post treatment.

Gastrointestinal nematodes induce leukocytosis, neutrophilia, eosinophilia, monocytosis insignificant rise in lymphocytes, γ , β , total globulin beside insignificant decrease in α globulin and A/G ratio coupled with significant rise in AST, ALT, urea, creatinine and significant reduction in T.protein, albumin, ALP, trace elements (selenium, copper, iron and zinc) compared with healthy horse.

The present study revealed that fenbendazole had a non significant effect on total and differential leukocytic count and biochemical parameters in healthy draft horses.

In conclusion, the present study showed that gastrointestinal nematodes induce some adverse effects on immune status and biochemical parameters. Those effects were returned to the normal levels at 15 days post treatment with fenbendazole.

INTRODUCTION

In the past and uptill now especially in Egypt, equines did not receive much attention from scientists as compared with other species of animals and many findings about equines are still with unsettled results (1). Equines have the largest collection of parasites of all domestic livestock. These parasites cause damage to the animals both during infection phase and during larval emerged and developed fully to adult parasites (2). Parasitic infestation may be induces alteration in protein absorption ending with hypoproteinaemia (3).

Parasitic infestation may abolish immune system (4). Internal parasites adversely affect health status of horse inducing emaciation and decrease body weight (5) and also several diseases in animals (6). Immature worms migrating through body tissues open the way for bacteria to enter, causing some diseases (7).

Benzimidazole anthelmintic groups are widely used in veterinary medicine against trematode and GI parasites (8). Benzimidazoles have ovicidal effect. It acts by inhibiting the uptake of glucose in the parasite

(9). Fenbendazole is a member of the benzimidazole group of anthelmintics (10).

The objective of the present work was to evaluate the effect of gastrointestinal nematodes on immune state and some biochemical parameters in draft horses.

MATERIAL AND METHODS

Drugs

Fenbendazole (pancure peste) produced by Intervet Company as syringes, each syringe contains 24gm (each 1gm contain 187.5mg fenbendazole) used in a dose of 7.5 mg /kgm body weight orally.

Animals

This study was carried out on 50 draft horses of different ages and sex from different localities in Sharkia Province. Faecal samples from all horses were collected and examined parasitologically for identification of gastrointestinal nematodes (11).

Experimental design

Post parasitological examination, 20 horses were used in this investigation. They were divided into 4 groups (5 each). 1st group as healthy non infested (control), 2nd group healthy horses treated with one dose of fenbendazole (7.5 mg/Kg b.wt.), 3rd group infested with gastrointestinal nematodes did not receive treatment while 4th group infested with gastrointestinal nematodes treated with one dose of fenbendazole (7.5 mg/Kg b.wt.).

Faecal samples

Before and at 1st, 3rd, 6th, 9th and 12th day post treatment faecal samples were collected from rectum in labeled bags and transported to laboratory for parasitological examination by flotation technique (11). Eggs of gastrointestinal nematode were identified morphologically (12) and faecal culture were performed for larval identification (13).

Shedding of eggs/gram of faeces was examined by Modified McMaster egg counting (14). Efficacy % of anthelmintics was calculated on the basis of reduction in egg/gram post treatment (15).

Faecal egg count rate (FECR %) =

$$\frac{\text{Pre-treatment EPG} - \text{Post-treatment EPG} \times 100}{\text{Pretreatment EPG}}$$

Blood samples

Before and at 1st, 7th & 15th day post treatment 2 blood samples were collected from all horses. 1st sample was collected in tube contains EDTA for estimation of leukogram picture (16). 2nd one was taken for estimation of total protein (17), serum protein fractions were estimated (18). Transaminases (AST and ALT) (19), ALP (20), creatinine (21), urea (22), some trace elements (23) were determined..

Statistical analysis

The obtained data were statistically (T test) analyzed (24).

RESULTS AND DISCUSSION

The current study revealed, 38 (76%) out of 50 examined samples were infested with gastrointestinal nematodes, this infection was age depended as they found infection in horses <1 years was 15 (39.47%), >1-4 year were 13 (34.21%) and > 4-10 year were 10 (26.32%) (Table, 1). The same observation was recorded (25) who found that young horses susceptible to nematodes more than adult horses.

Table 1. Parasitological examination of faecal samples for gastrointestinal nematodes of draft horses different ages

No. of examined faecal samples	+ve samples		-ve samples		Prevalence of +ve sample according to horse age					
					< 1 year		> 1 -4 year		> 4 -10 year	
	No	%	No	%	No	%	No	%	No	%
50	38	76.00	12	24	15	39.47	13	34.21	10	26.32

Infestation with gastrointestinal nematodes in our study may be single 12 (31.58%) or mixed 26(68.42 %). Single infestation distributed as {*Strongylus spp* 6 (15.79%) *parascaris equorum*, 4 (10.53%) and *oxyuris equorum* 2(5.26%)} or mixed, (double) 26(68.42 %) {*Strongylus spp* with *parascaris equorum* 7(18.42%), *parascaris equorum* with

oxyuris equorum 5 (13.16%). The mixed, (triple) *strongylus spp* with *parascaris equorum* and *oxyuris equorum* 14 (36.84 %) (table, 2). Nearly same prevalence for gastrointestinal nematodes in horse in Sharkia Province (63%) was recorded (26). Mixed infection of gastrointestinal nematodes is most common in horses (27) .

Table 2. Prevalence of single and mixed infestation with gastrointestinal nematodes in draft horses

No. of +ve samples	Prevalence of +ve samples according type of infestation															
	single infestation								Mixed infestation							
	T.number		Str		Para		Oxy		T.number		Str + Para		Para+ Oxy		Str +Para+Oxy	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
38	12	31.58	6	15.79	4	10.53	2	5.26	26	68.42	7	18.42	5	13.16	14	36.84

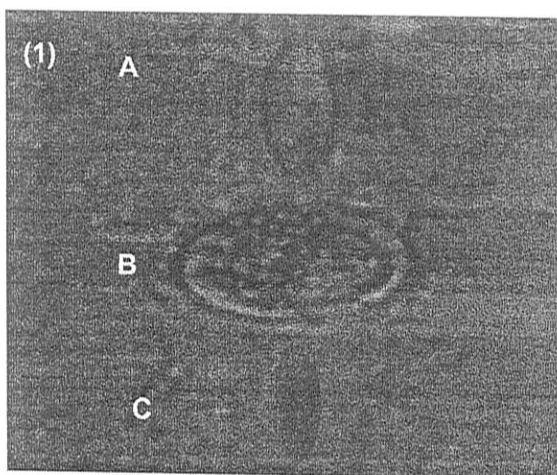
Str = *Strongylus spp*Para= *Parascaris equorum*Oxy = *Oxyuris equi*

Fig.1.

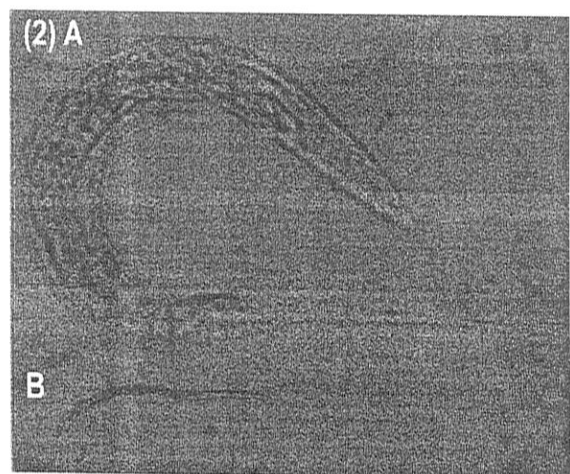
A) Egg of *Oxyuris equi* 40 XB) Egg of *strongylus spp.* X40C) Eggs of *Parascaris equorum* X40

Fig. 2.

A) female of *Oxyuris equi* 40 XB) Larvae of *Strongylus spp.* X40

The healthy horses that received fenbendazole in a dose of 7.5 mg/Kg b.wt. induced a non significant effect in total and differential leukocytic count, protein picture, trace elements, liver enzymes, urea and Creatinine. Similar result was recorded previously (28) they stated that benzimidazol member not induces any toxic effect in dogs at dose below 10 mg/kg bw/day.

In this study, fenbendazole induce 100% faecal egg count reduction at day 9 post

treatment, which agreed (29) who found fenbendazol has 100% efficacy against gastrointestinal nematodes at day 10 post treatment in horses. Fenbendazole was effective against *Strongylus* spp (30). Albendazole induce 100% reduction of gastrointestinal nematodes egg/gm at day post treatment in donkey (31). No gastrointestinal nematodes eggs at 10 day post use albendazole (32).

Table 3. Efficacy of fenbendazol against gastrointestinal nematodes in naturally infested draft horse (n=5)

	Pre treatment	Faecal egg count/ gram									
		Day post treatment									
		1 st day		3 rd day		6 th day		9 th day		12 th day	
No	%	No	%	No	%	No	%	No	%		
G1	00	00	00	00	00	00	00	00	00	00	00
G3	989.93 ±9.16	962.55 ±9.32	00	997.04± 5.33	00	992.39± 9.55	00	914.18±	00	968.55±	00
G4	1052.37 ±7.28	610.41 ±6.80	42	204.4 ±8.28	80.58	35.85 ±7.38	96.59	8.64 00	00	9.54 00	100

Our study revealed leukocytosis, neutrophilia, eosinophilia, monocytosis beside an insignificant rise in lymphocytes in horses infested with gastrointestinal nematodes (table, 4). Same results were recorded (33) in horses infected with strongylus. Leukocytosis in horse infected with gastrointestinal nematodes may be due to inflammation in intestinal wall (34). Same finding was obtained (35) who found that gastrointestinal nematodes induce eosinophilia due to chronic

inflammatory parasitism and harmful effect of toxins produced by parasites on the hemopoietic system. This eosinophilia may be due to chemotactic factors produced by parasites (36). Also, gastrointestinal nematodes in horse induces neutrophilia and monocytosis (37). These changes in leukogram may be due to destructive effective of the parasite on the epithelial cells of the gastrointestinal tract walls (38).

Table 4. Effect of gastrointestinal nematodes and treatment on leukogram of draft horse (n=5)

Parameters	Control group	Healthy fenbendazole treated	Non treated	Diseased		
				Day post treatment		
				1 st	7 th	15 th
TLC(X10 ³)	9.67±0.26	9.86±0.68	10.86±0.33*	10.60±0.24*	10.29±0.12*	9.65±0.18
Neutrophils	3.83±0.09	3.86±0.20	4.13±0.10*	4.10±0.08*	4.03±0.05	3.74±0.12
Lymphocytes	3.23±0.13	3.27±0.23	3.33±0.12	3.26±0.06	3.22±0.08	3.21±0.11
Eosinophils	1.03±0.08	1.05±0.17	1.38±0.10*	1.32±0.06*	1.28±0.07*	1.06±0.09
Basophils	0.47±0.12	0.50±0.08	0.57±0.07	0.54±0.04	0.51±0.06	0.50±0.08
Monocyte	1.11±0.07	1.18±0.12	1.45 ±0.12*	1.38 ±0.10*	1.25 ±0.09	1.14±0.05

*Significant at p < 0.05

Analysis of serum protein of horses infested with gastrointestinal nematodes revealed a significant decrease in protein, albumin beside insignificant decrease in α globulin, A/G ratio coupled with insignificant rise in β , γ and total globulin (Table, 5). Same result was obtained in stallion (39). Gastrointestinal nematodes induce significant decrease in protein, albumin and insignificant decrease in α globulin and A/G ratio (40). Parasitic infection led to reduction feed intake and absorption of required nutrients (41). Nematode induces enteritis and villous atrophy which leads to decrease absorption of

nutrient, total protein and albumin (42) beside albumin leaked from damaged blood vessel due to its small molecular weight (35). and gastrointestinal nematodes induces anorexia and inability of the gut to absorb protein finally led reduction total protein and albumin (5). Strongyles induce insignificant elevation in γ and total globulin (43). Moreover, gastrointestinal nematodes in horse induce insignificant increase in β globulins (44), Rise in γ and total globulin may be due to nematodes infestation and its inflammation (11).

Table 5. Effect of gastrointestinal nematodes and treatment on Protein profile of draft horse (n=5)

Parameters	Healthy Non treated (control)	Healthy fenbendazole treated	Diseased			
			Non treated	Day post treatment		
				1 st	7 th	15 th
T. protein (g/dl)	7.50 ±0.41	7.60 ±0.85	5.95±0.45*	6.15±0.30*	6.93±0.58	7.58±0.42
Albumin (g/dl)	4.20 ±0.53	4.34 ±0.74	2.53±0.45*	2.60±0.41*	3.70±0.33	4.10±0.35
α	1.05±0.09	1.01±0.09	1.00±0.10	1.03±0.11	1.07±0.12	1.16±0.19
Globulin (g/dl)	β 0.99±0.10	0.95±0.10	1.15±0.22	1.13±0.09	1.12±0.13	1.00±0.11
γ	1.26±0.08	1.30±0.08	1.45±0.21	1.39±0.16	1.31±0.18	1.35±0.12
Total	3.30±0.23	3.26± 0.23	3.60±0.42	3.55±0.37	3.50±0.33	3.48±0.43
A/G Ratio	1.27±0.25	1.33±0.21	0.70±0.15	0.73±0.13	1.06±0.32	1.18±0.23

*Significant at $p < 0.05$

The present investigation revealed a significant increase in AST, ALT, creatinine and urea beside a significant decrease in alkaline phosphatase in horse infested with gastrointestinal nematodes (Table, 6). Same results were recorded in stallion (39). Gastrointestinal nematodes induce rise in urea and creatinine in animals (45). Reduction in activity of alkaline phosphatase due to infection with gastrointestinal nematodes may be due to epithelial tissues damage of the intestinal walls by the parasites and their toxins (46).

In our study gastrointestinal nematodes induces a significant decrease in serum selenium, copper, iron and zinc in horses

(Table, 6). Similar results were reported (47) in horses infected with gastrointestinal nematodes. Strongyles and parascaris equorum cause inflammation of mucosa of intestine and diarrhoea leading to malabsorption of nutrients lead to decrease in serum trace elements (48). These changes may be due to gastrointestinal nematodes have deleterious effects on host nutritional status leading to nutritional deficiency (49). Also, gastrointestinal nematodes interfere with copper absorption by increasing PH of gastrointestinal tract (50). Deficiency of a single trace element among animals occurred rarely in field, while combinations of several minerals deficiency are common (51).

Table 6. Effect of gastrointestinal nematodes and treatment on some biochemical parameters of draft horse (n=5)

Parameters	Healthy non treated (control)	Healthy fenbendazole treated	Non treated	Diseased		
				Day post treatment		
				1 st	7 th	15 th
AST(U/L)	189.06±1.65	180.14± 0.97	194.31±1.41*	193.42±1.01*	192.18±1.16	190.04±1.32
ALT(U/L)	17.22± 0.48	18.06±0.86	19.02± 0.37 *	18.83± 0.34*	17.43± 0.20	17.10±0.29
ALP (U/L)	168.20±0.98	169.41± 1.83	164.31±0.92*	164.90±0.68*	166.57±0.86	168.08±0.62
Urea(mg/dL)	14.17±0.90	14.47±0.69	18.07±0.92*	17.25±0.74*	15.34±0.82	14.34±0.69
Creatinine(mg/dL)	1.95±0.32	2.05±0.42	2.97±0.16*	2.85±0.19*	2.42±0.21	2.05±0.49
Selenium(ug/100ml)	16.08±0.89	16.73±0.55	13.61±0.44*	14.15±0.32*	14.30±0.27	15.33±0.68
Copper(ug/dl)	99.21±0.83	99.59±0.61	95.47±0.94*	96.06±0.81*	97.49±0.74	98.98±0.84
Iron (mg/dL)	81.22±1.42	82.42±0.76	75.21±1.43*	77.35±1.10*	79.49±1.16	81.09±1.51
Zinc(ug/dl)	64.16±1.09	65.53±0.95	60.01± 0.87*	61.21± 0.59*	63.57±0.22	63.10±0.31

*Significant at p < 0.05

Treated diseased horse with fenbedazole showed improvement in leukogram and biochemical parameters at 15th day post treatment. Significant improvement in the hemato-biochemical parameters in horses infected with gastrointestinal nematodes and treated with Mebendazole were recorded by (1).

It could conclude that gastrointestinal nematodes in horse induce adverse effect on immune status and biochemical parameters. These adverse effects were returned to normal levels 15th days post treatment by fenbendazole.

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الملخص العربي

التغيرات المناعية والبيوكيميائية في خيول الجر المصابة طبيعياً
بالطفيليات الاسطوانية المعوية مع محاوله العلاج بالفينبندازول بمحافظة الشرقيه

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أقسام (الطفيليات^١ والكيمياء^٢) (٤٣،٢)

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استهدفت هذه الدراسة معرفة اهم الديدان الاسطوانيه التي تصيب خيول الجر ومدى تأثيرها على حاله المناعيه والبيوكيميائية وكذلك دراسه كفاءة الفينبندازول في علاج الإصابة بالديدان الاسطوانيه المعويه. أوضح الفحص الطفيلي لعينات البراز عن وجود بويضات الديدان الاسطوانية المعويه في عدد ٣٨ عينه (٧٦%) موزعه حسب مراحل السن المختلفه (عمر اقل من اسنه إلى عمر سنه فاكثر وجدت البويضات في ١٥ عينه (٣٩,٤٧%)، ١-٤ سنه وجدت البويضات في ١٣ عينه (٣٤,٢١%)، ٤-١٠ اسنه وجدت البويضات في ١٠ عينه (٢٦,٣٢%). وتلك الاصابه موزعه حسب نوع الاصابه الى اصابه بنوع واحد من الديدان ١٢ عينه (٣١,٥٨%) جنس استرونجيلس ٦ عينه (١٥,٧٩%)، الباراسكارس اكوينم ٤ عينه (٦٨,٤٢%)، الاكسيورس اكوينم ٢ عينه (٥,٢٦%) او إصابة مختلطه ٢٦ عينه (٣١,٥٨%) وموزعه (استرونجيلس مع الباراسكارس اكوينم) ٧ عينه (١٨,٤٢%)، (الباراسكارس اكوينم مع الاكسيورس اكوينم) ٥ عينه (١٣,١٦%)، (استرونجيلس مع الباراسكارس اكوينم مع الاكسيورس اكوينم) ١٤ عينه (٣٦,٨٤%). واختفت بويضات الديدان الاسطوانيه المعويه تماما من عينات البراز عند اليوم التاسع من استخدام الفينبندازول.

تشير النتائج أن الطفيليات الاسطوانيه المعويه أدت الى حدوث زيادة معنوية في عدد كرات الدم البيضاء، الخلايا المتعادلة، الخلايا الحامضيه، الملتهمه الكبيرة، الترانس أمينيزسس (AST-ALT)، اليوريا والكرياتينين بجانب زياده غير معنويه في الخلايا الليمفاويه، البيتا جلوبيولين، الجاما جلوبيولين والجلوبيولين الكلى وحدث نقص معنوى في البروتين الكلى، الزلال، الفوسفاتيز القاعدى والعناصر النادره (السلينيوم-النحاس-الحديد-الزنك) و نقص غير معنوى في الألفا جلوبيولين والنسبه بين الزلال والجلوبيولين.

نستخلص من هذه الدراسة أن الديدان الاسطوانيه في خيول الجر أحدثت تأثيرات عكسية على العدد الكلى والتفريقي لكرات الدم البيضاء، القياسات المناعية والبيوكيميائية زتلك القياسات عادت الى معدلها الطبيعى بعد ١٥ يوم من نهايه العلاج باستخدام الفينبندازول بالفم .