Original Research Article

Prevalence of Gastrointestinal parasites, chemotherapy and haematology of Strongylosis in Donkeys of District Lahore, Pakistan

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A B S T R A C T

Keywords

Prevalence,
Chemotherapy,
Strongylosis,
Donkeys

Prevalence of gastrointestinal parasites, chemotherapy and haematological effects with special reference to strongylosis were studied in donkeys. Three hundred donkeys were examined coprologically for the presence of gastrointestinal (GIT) parasites by using direct smear method and egg per gram (EPG) for strongylus using McMaster egg counting technique. Of these, 167 (55.66%) donkeys were found positive for GIT parasites. Prevalence of *strongylus, trichostrongylus, trichonema, gastrodiscuss* and mixed infection was 28.33, 9.66, 6.33, 6.33 and 5%, respectively. Thirty animals suffering from *strongylus* were randomly divided into three groups of 10 animals each viz. A, B, C and a fourth D comprising 10 healthy animals were kept as healthy non-infected control. Group A was treated with Ivermectin (Ivergen, Symans Pharmaceuticals Pakistan) while group B was given dried *Azadirachta indica* (Neem) leaves. Animals in group C were kept as positive control. The efficacies of Ivermectin and *Azadirachta indica* leaves were 96.42 and 33.33%, respectively. Hemoglobin concentration of *strongylus* positive donkeys was significantly lower than *strongylus* negative animals. Eosinophils were also increased in the animals of group C. It is concluded that *Azadirachta indica* showed significant efficacy for the treatment of strongylosis and the results are comparable with that of standard ivermectin.

Introduction

Strongylosis is one of the most important diseases of equines. Diarrhea, anorexia, weight loss and marked anemia are the clinical signs in infected animals resulting in

huge mortality (Soulsby, 1982). The mixed strongyle infections are common in donkeys with clinical signs including anaemia, diarrhea and unthriftness (Urquhart *et al.,* 1996). Changes also occur in haemotological values such as eosinophilia, monocytopaenia and reduced cell survival due to blood sucking nature of the strongyles (Sipra *et al.,* 1999). For therapeutic trials, it is essential to have a thorough knowledge about the pre-patent and patent phases of parasites which serves as the basis for parasite control program (Irfan, 1984). Broad spectrum activity, wide therapeutic index and shortest residual period are the ideal properties of an anthelmintic (Robert and Edwound 1986). Though the use of ivermectin is not registered in donkeys but many donkey owners use the injection with no side effects and higher efficacy (Seri *et al.,* 2005).

In most of the underdeveloped countries, donkeys serve as a source of energy in agriculture production by way of cultivation and transportation (Seri *et al.,* 2004). Under smallholder farming system like in Pakistan, donkeys are the most precious, suitable and economical animals, because they can be used in areas with coarse topography and underdeveloped roads (Hosseini *et al.,* 2009). Their use in transportation is much more compared to horses (Seri *et al.,* 2005*).* Majority of the people are dependent on donkeys for agriculture and transport (Hosseini *et al.,* 2009). In villages of hilly areas, donkeys are used for carriage purpose and a saddle animal as well (Uslu and Guclu, 2007).

In spite of the fact that donkeys are known as hardy and robust animals, they are exposed to a number of diseases (Hosseini *et al.,* 2009). Surprisingly very negligible work has been done on the parasitic species of donkeys and very few publications show

study on the prevalence of the internal parasites of donkeys (Kheir and Kheir, 1981). Suppositions regarding the epidemiology and pathogenicity of various helminthes species of donkeys are usually guessed from what is known in horses (Wells *et al*., 1998). Helminthes parasites, particularly strongyle nematodes are the common inhabitants of the gastro-intestinal tract of equines and can cause infections with clinical signs from ill-thrift to sudden death (Umur and Acici, 2009). Internal parasites cause significant clinico-pathological changes in donkeys (Lewa *et al.,* 1999). Some parasitic infections even prove fatal if control measures are neglected (Hayat *et al.,* 1987).

In Pakistan, the population density of donkeys is 4.9 million (Anonymous 2012-2013). In view of such a huge population of donkeys, regarding their socio-economic importance and the lack of information about the gastro-intestinal parasites of donkeys, the present study was designed to investigate the prevalence of gastrointestinal parasites in donkeys and to determine the comparative efficacy of Ivermectin and dried leaves of *Azadirachta indica* (Neem) against strongylosis in donkeys. The study also aimed to see the effect of strongylosis on various blood parameters such as haemoglobin (Hb) level and differential leukocyte count (DLC).

Materials and Methods

Source of Animals

Three hundred (300) donkeys were selected randomly from different localities in and around Lahore, irrespective of their age, sex and breed. Animals at the Society for Prevention of Cruelty to Animals (SPCA), Outdoor teaching Hospital of University of Veterinary & Animal Sciences (UVAS), Lahore, Outreach Veterinary Hospital (Sitar

wala) of UVAS, Lahore and its adjoining villages were incorporated in this study.

Sample collection

Faecal samples were collected directly from the rectum of donkeys in separate self-sealing polythene bags and labeled properly for identification. Microscopic examination of the samples was performed in Diagnostic Laboratory of the section of Clinical Medicine of the University for determining the prevalence of strongyloids and other gastro-intestinal parasites in donkeys. Parasitic ova were examined following direct smear method approach (Urquhart *et al.,* 1996).

Direct Smear Method

A small quantity of faecal material was placed on a labeled, clean, grease free glass slide and few drops of water were added and stirred to form a homogenous mixture taking out feacal debris. A cover slip was applied on the faecal smear and examined under the 10-X objective of microscope to detect and identify the parasitic ova. The samples determined positive for strongylus were subjected to McMaster Egg Counting Technique for counting the number of Eggs per gram (EPG) of faeces (Urquhart *et al.,* 1996).

McMaster Egg Counting Technique

Egg per gram (EPG) of faeces counts were carried out on day 0 (pre-medication) and then on days 7 and 14, post-medication. Three gram (3g) of the faecal material was weighed using electronic balance and transferred into a 120 ml wide-mouthed screw capped plastic bottle containing about four dozen small glass balls. Then, 42 ml volume of saturated NaCl solution was poured into it**.** After screwing the cap, the contents were shaken thoroughly for 2-3 minutes to break up the faeces and thus a uniform mixture was obtained. The mixture was then poured through a 100-mesh sieve into small clean beaker. The faecal filtrate was left undisturbed for 8-10 minutes. Then faecal filtrate was agitated and with the help of Pasteur pipette sufficient amount was withdrawn to fill one chamber of McMaster Egg Counting slide. The residue in the pipette was returned to the filtrate, reagitated and again filtrate was withdrawn in Pasteur pipette to fill the second chamber. Both chambers were left undisturbed for 4-5 minutes after filling. Afterwards, corner of the etched lines of first chamber was focused under the microscope and the eggs were counted by moving up and down the columns of lines. Counting in the second chamber was also done in same way as for the first chamber. The total number of eggs counted in two chambers was multiplied by 50 (as per method) to get the number of eggs per gram (EPG) of the faeces.

Therapeutic Trials

Thirty (30) strongylus positive donkeys were selected and randomly divided into 3 groups, i.e. A, B and C comprising 10 animals each. Another Group D comprising 10 healthy animals served as control group. The faecal samples were collected and examined on day 0 (pre-medication) followed by the administration of treatment in each group as per following protocol;
Group A: Inj Ivermectin (Ivergen, Symans Pharmaceuticals Pakistan) was administered S/C (subcutaneously) at the dose rate of 0.2 mg/Kg B. Wt. (1 ml/50 Kg B. Wt. as per recommendation of the company) (Radostitis *et al.,* 2006) only once.

Group B: Dried, ground *Azadirachta indica* leaves were administered orally at 375 mg/Kg B. Wt. (Mahboob *et al.,* 2008) mixed

with wheat bran for four consecutive days.
Group C: The animals in group C were kept as infected control.
Group D: All the animals had already been determined negative for strongyloid infection and thus served as negative control.

Faecal samples from all the experimental animals were examined microscopically to detect any parasitic ova on days 7 and 14 post-medication. Therapeutic efficacies of the drugs were calculated on the basis of reduction in EPG. Percent efficacy (%) was calculated by applying the following formula (Urquhart *et al.,* 1996).
EPG before treatment − EPG after treatment = Reduction in number of ova

$$\% \, Efficacy = \frac{Reduction \; in \; the \; number \; of \; ova}{EPG \; before \; treatment} \times 100$$

Hematological Studies

Blood samples from animals in groups A, B, C and D were collected in EDTA and k3 coated vaccum vacutainers tubes (BD vacutainer plastic K3 EDTA tube) on day 0 pre-medication and then on days 7 and 14 post-medication. Jugular venipuncture was used to collect the blood. The samples collected were labeled and transported to the Medicine laboratory of the University of Veterinary and Animal Sciences, Lahore for haematological examination. Haemoglobin estimation (Hb) and differential leukocyte counts (DLC) were evaluated on day 0 pre-medication and on days 7 and 14 post-medication as described by (Benjamin, 1985).

Haemoglobin Estimation (Hb)

For determining Hb level, decinormal (N/10) Hydrochloric acid (HCl) was added into an empty graduated tube to the level of mark 10. Blood was drawn into the capacity pipette to the 20 cu. mm mark. After wiping the tip of the pipette, the blood was poured into HCl solution in a graduated tube and thoroughly mixed using a stirrer. Then the fluid was diluted with distilled water pouring drop by drop. Mixing continued each dilution till the same colour was obtained as that of the comparison tubes. The Hb level was recorded from the scale by noting the height of the column of the diluted acid hematin.

Differential Leukocyte Count (DLC)

A small drop of blood was placed on a clean grease free glass slide. The end of the spreader slide was placed against the surface of the first slide holding it firmly at an angle of 45º. The spreader slide was drawn gently near the drop of the blood pushing backward and when the blood has spread along two third the width by capillary action the spreader slide was forward to the far end of the horizontal slide with steady and even motion. In this way a thin and even blood smear was prepared which was allowed to air dry.

The dried blood smear was then fixed in methylated alcohol for 2-3 minutes and stained with Giemsa's stain for 25 minutes by putting few drops of the stain on the smear so as to cover it completely. The smear was then washed with water and allowed to dry at room temperature (25 ±3ºC). The stained film was then examined under oil immersion lens (100-X) of the microscope for differential leukocyte counting.

Statistical analysis

The collected data was analyzed statistically by using one way ANOVA followed by LSD (Steel *et al.*, 1997).

Results and Discussion

Prevalence of Gastrointestinal parasites

Of a total of 300 sampled animals, 167 donkeys were found infected with various parasitic species wherein highest infection was that of *Strongylus* (85 donkeys), followed by *Trichostongylus* (29), *Trichonema* (19) and *Gastrodiscuss* intestinal fluke (19) while 15 donkeys were infected with mixed *strongylus* and *gastrodiscuss*. The details are given in Table 1.

Therapeutic evaluation

Therapeutic efficacies of allopathic Ivermectin and *Azadirachta indica* leaves against strongylosis in donkeys were determined on the basis of post-medication faecal egg counts and comparing them with experimental control groups kept under the similar conditions. Faecal egg counts of Group A, on day 0, ranged from 150 to 400 with an EPG of 280±24.94 (Mean ±SEM) while in Group B, EPG was 270±18.55 (Mean ±SEM) with faecal egg counts ranging from 200 to 350. In Group C the faecal egg count ranged from 150 to 350 showing an EPG of 285 ± 19.79. In group D, none of the *strongylus* parasitic ova were observed and thus EPG 0 (Table 2).

Faecal egg counts of the *strongylus* (round worms) recorded on day 7 post-medication in group A, showed a marked reduction in the mean EPG from 280 to 75 wherein four animals of this group recovered with mean EPG at 75±23.86 (Mean±SEM). Thus, efficacy of ivermectin calculated on the basis of reduction in the faecal egg counts (280 to 75) post-medication was 73.2%. At 14th day post-medication in Group A, the egg counts were markedly reduced from 280 to 10 wherein 8 animals recovered with mean EPG of 10±6.66 (Mean±SEM). The efficacy of Ivermectin thus calculated on the basis of reduction (270 eggs reduction) in the faecal egg counts was 96.42% on day 14, post-medication. No serious untoward effects of this drug were observed during the entire trial period except one of the donkey showed swelling at the injection site which resolved by self few days later **(Table 3)**.

Animals of Group B were treated with *Azadirachta indica* which showed a moderate reduction (from 270 to 210) in the mean EPG on day 7 post-medication. None of the animals recovered with mean EPG at day 7 was 210±17.95 (Mean±SEM). Thus, efficacy of *Azadirachta indica* calculated on the basis of reduction in the faecal egg counts (280 to 210) at day 7 post-medication was 22.2%. A moderate reduction (from 270 to 180) in the mean EPG of faeces was recorded on day 14 post-medication. One animal fully recovered with mean EPG of 180±22.60 (Mean±SEM). It showed 33.33 % efficacy of *Azadirachta indica* on 14 post-medication as evidenced by a reduction of 90 faecal egg counts. There were no adverse reactions during the trial period (Table 3).

A slight increase in mean EPG (i.e., from 285-290) was recorded in Group C which was kept as infected control. Thus, the count at day 7 was 290±22.11 (Mean ±SEM). On the other hand, a moderate increase (from 285 to 300) in the mean number of eggs (300±19.72) per gram of the faeces in group C was noted on day 14, as this group was kept as infected control (Table 3).

Haematology

Blood samples collected on day 0 (pre-medication) and then on days 7 and 14 (post-medication) were subjected to determine blood hemoglobin concentration and differential leukocyte counts in the experimental groups. Marked improvement

in Hb level was observed in animals of Group A, whereas moderate improvement was recorded in Group B animals. The details of all groups are given in Table 4.

No significant changes were observed in monocytes, lymphocytes and basophils in all the four groups. However, eosinophils showed significant increase throughout the trial period in group C as compared to groups A and B. The details of all results are given in Table 5.

The prevalence of GIT parasites was observed as 55.66% (167/300) in the current study. Out of 167 infected donkeys, 85 were infected with s*trongylus*, 29 with *trichostrongylus*, 19 with *trichonema*, 19 with *gastrodiscus* and 15 donkeys were found infected with mixed infection. The prevalence of *strongylus, trichostrongylus, trichonema, gastrodiscus* and mixed infection were 28.33%, 9.66%, 6.33%, 6.33% and 5% respectively. The results are in line with Mfitilodze and Hutchinson (1990) who studied infection rate in horses and documented the prevalence of four large strongyles being 28%, 22%, 22% and 30% for *S. vulgaris*, *S. edentates*, *S. equinus* and *T. serratus*. Sipra *et al.,* (1999) studied the mean prevalence of strongylosis being 31.7 % in horses in Faisalabad, Pakistan in a previous report. Seri *et al.,* (2004) also reported the infection rate of *strongylus* being 35.8 % after examining 1200 donkeys in Sudan. In an earlier report by Aftab *et al.,* (2005) who reported the prevalence of endoparasites being 53.33% in horses in Lahore, Pakistan. There is a little variation while comparing our results with the study of Pereira and Vianna (2006), who reported the prevalence of nematodes being 100% in 20 equines in Brazil. Sonone *et al.,* (2013) reported the gastrointestinal helminthes load of horses in Nagpur city of India with prevelence of *Strongylus sp* being (44.12%), *Anoplocephala sp.*(30.88%),

Parascaris equorum (14.71%), mixed infection *(Strongylus + Parascaris equorum)* (5.88%) and lowest one was *Gastrodiscus sp.* (4.41%). The prevalence is different in different parts of the world which may be due to variations in ecological conditions and access to worms control program.

The percent efficacy of Ivermectin (Ivergen, Symans Pharmaceuticals Pakistan) determined in the current study on the basis of reduction in number of eggs per gram of the faeces was 73.21% and 96.42 % respectively on day 7 and 14 post-medication. In a previous report by Sipra *et al.,* (1999), who administered Ivermectin at the dose rate of 0.4 mg/kg and 0.2 mg/kg body weight against strongylosis in equines in Faisalabad, Pakistan and found that ivermectin completely eliminated the egg burden after day 14 post-treatment, which are more close to our report. Seri *et al.,* (2005) employed ivermectin at the dose rate of 0.2 mg/kg against gastrointestinal nematodes in donkeys in Sudan and found that ivermectin was 100% effective in reducing the faecal egg count of nematodes species including *strongylus* spp, small strongyles, *Trichostrongylus axei* etc.

The results of our study are in line with Binev *et al.,* (2005) who administered ivermectin at the same dose rate against strongyloids control in donkeys in Bulgaria and found that ivermectin was 96% effective in terms of faecal egg count reduction. Aftab *et al.,* (2005) evaluated the ivermectin against ecto and endo-parasites in horses and found the efficacy of ivermectin was 95.17% against endoparasites. The results of our study are also in line with Hassan *et al.,* (2005) who administered ivermectin at the same dose rate against ecto and endo-parasites in horses in Lahore and found that the efficacy of ivermectin was 100% against roundworms respectively.

Group B was treated with *Azadirachta indica* dried leaves at the dose rate of 375 mg/kg body weight mixed with wheat bran for four consecutive days. The efficacy of *Azadirachta indica* based on faecal egg count reduction was noted to be 22.22 and 33.33% respectively on day 7 and 14 post-medication. Iqbal *et al.,* (2010) used *Azadirachta indica* seeds in the form of crude methanolic extract (CME), crude powder (CP) and crude aqueous extract (CAE) at 1 and 3 g/kg against gastrointestinal nematodes in sheep. CP and CME were found effective at 3 g/kg with maximum reduction of 29.3 % and 40.2 % in EPG after day 15 post treatment. Begum *et al.,* (2010) used *Azadirachta indica* leaves extract at 20 mg/ml against ascariasis in chiken and reported that *Azadirachta indica* leaves extract have the anthelmintic activity. Akhtar and Riffat (1984) used *Azadirachta indica* fruit powder at the dose rate of 30 mg/kg against gastrointestinal nematodes in goats and reported a 99.4±12% reduction in EPG. Mahboob *et al.,* (2008) administered *Azadirachta indica* dried leaves at 375 mg/kg b. wt once against strongylosis in horses and reported the efficacy of *Azadirachta indica* to be 6.89 and 8.62% on day 7 and 14 post-treatment.

These haematological findings of the current study exhibited valuable improvement in Hb level while treating with ivermectin as well as *Azadirachta indica.* Changes were also recorded in Differential leukocyte count. These results are comparable with some previous studies as reported by Hopfer *et al.,* (1984), Sipra *et al.,* (1999), Lewa *et al.,* (1999), Saleem *et al.,* (2000) and Sonone *et al.,* (2013). Changes in haematological values may be due to the blood sucking nature of the parasite.

Table 1: Prevalence of gastrointestinal parasites

GIT parasites	No. of Positive Cases	Prevalence (%)
Strongylus	85	28.33
Trichostrongylus	29	9.66
Trichonema	19	6.33
Gastrodiscuss	19	6.33
Mixed infection	15	5
Overall	167	55.66

Table 2: Faecal Egg Counts in the experimental groups at different days

	Mean EPG (Mean±SE) on days			
	Day 0 (Pre-medication)		Day 7 (Post-medication)	Day 14 (Post-medication)
Groups	Range	Avg. Mean	Avg. Mean	Avg. Mean
A	150-400	280 ±24.94	75±23.86	10±6.66
B	200-350	270 ±18.55	210 ±17.95	180±22.60
C	150-350	285 ±19.79	290 ±22.11	300±19.72
D	00-00	0.00 ±0.00	0.00 ±0.00	0.00±0.00

Table 3: Faecal Egg Counts and Efficacy of therapeutic agents at Day 7 & 14

Groups	Day 0 Mean ±SE	Day 7 Mean ±SE	Decrease or increase in number of eggs at day 7	Efficacy %	Day 14 Mean ±SE	Decrease or increase in number of eggs at day 14	Efficacy %
A	280 ±24.94	75 ±23.86	205	73.2%	10±6.66	270	96.4%
B	270 ±18.55	210 ±17.95	60	22.2%	180±22.60	90	33. 3%
C	285 ±19.79	290 ±22.11	05	-	300±19.72	15	-
D	0.00 ±0.00	0.00 ±0.00	0.00	-	0.00±0.00	0.00	-

Group-A = Treated with Ivermectin; Group-B = Treated with *Azadarch indica*; Group-C = Positive Control; Group-D = Negative Control

Table 4: Haemoglobin Estimation

Groups	Drugs	Haemoglobin values (g/dl)		
		Day 0	Day7	Day14
A	Ivermectin	8.98±0.20	10.65±0.25	12.12±0.15
B	*Azadirachta indica*	8.92±0.21	9.75±0.32	9.93±0.30
C	Positive control	9.22±0.44	8.99±0.43	8.84±0.41
D	Negative control	12.06±0.19	12.06±0.18	12.06±0.18

Group-A = Treated with Ivermectin; Group-B = Treated with *Azadirachta indica*; Group-C = Positive Control; Group-D = Negative Control

Table 5: Differential Leukocyte Counts in Groups A, B, C & D

Leukocytes	Differential Leukocytic Counts in Groups at different days											
	Group A			Group B			Group C			Group D		
	Day 0	Day7	Day14	Day 0	Day7	Day 14	Day 0	Day7	Day 14	Day 0	Day7	Day 14
Neutrophils	34.50 ±0.85	36.10 ±0.70	38.20 ±0.72	33.20± 0.99	34.20 ±0.92	35.50± 1.00	34.30± 1.22	33.90± 1.27	33.80± 1.14	36.80± 0.85	36.60± 0.79	36.80± 0.67
Lymphocytes	54.40 ±1.44	54.10 ±1.08	54.00 ±1.13	56.30± 1.55	55.40 ±1.53	54.20± 1.51	52.30± 1.78	52.20± 1.80	51.20± 1.48	54.00± 1.06	54.40± 0.83	54.30± 0.68
Monocytes	2.80± 0.51	3.10± 0.40	3.40± 0.37	3.10± 0.43	3.50± 0.45	3.70±0 .42	2.70± 0.26	2.60± 0.22	2.80± 0.24	3.60± 3.30	3.90± 0.40	3.80± 0.38
Eosinophils	7.60± 1.05	5.80± 0.62	4.30± 0.57	6.80± 0.92	6.10± 0.73	5.60± 0.68	12.80± 0.57	10.40± 1.18	11.40± 1.11	4.40± 0.30	4.20± 0.24	4.10± 0.23
Basophils	0.70± 0.15	0.90± 0.23	1.10± 0.27	0.60± 0.22	0.80± 0.20	1.00± 0.25	0.90± 0.17	0.90± 0.17	0.80± 0.20	1.20± 0.24	0.90± 0.23	1.00± 0.21

It is evident from the above discussion that the prevalence of gastro-intestinal parasites in donkeys is 55.66% with *strongylus* being 28.33%. *Azadirachta indica* showed significant efficacy for the treatment of strongylosis and the results are comparable with that of standard Ivermectin. Therefore, further research in exploring the anthelmintic activity of *Azadirachta indica* is recommended.

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