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Original Article

A study on selected anthelmintics on strongylosis along with haematology in horses in Quetta region

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Abstract

The present study was conducted to determine the prevalence rate, worm load, effects of strongyles infestation on blood parameters and comparative efficacy of Oxfendazole, Ivermectin and Pyrantel anthelmintics against strongyles in the horses at Quetta and suburbs. For this purpose, 100 horses were randomly selected and kept untreated for anthelmintics for 3 months before commencement of study. Overall, prevalence was found to be at 48%. Among them, 40 positive horses were randomly divided into 4 groups namely A, B, C and D comprising 10 horses in each group. Group A was treated with Oxfendazole, group B with Ivermectin, group C with Pyrantel while group D was kept an untreated (No Anthelmintic). The efficacy of anthelmintics was evaluated on reduction of eggs per gram (EPG) of the faecal sample on day 7 and 14 post-medication. The efficacy of Oxfendazole in Group A was 91.43% and 97.14%, efficacy of Ivermectin was 95.24% and 97.62% whereas efficacy of Pyrantel was 87.18% and 94.87% on day 7th and 14th day post-medication respectively. All the anthelmintics administered were effective against strongylosis with ivermectin having comparatively better efficacy followed by oxfendazole and pyrantel. Worm load had direct relation on blood parameters and its reduction showed significant improvement in total erythrocyte count (TEC), Haemoglobin (Hgb) and Packed Cell Volume (PCV) on day 7 and 14. Efficacy of routinely used anthelmintics should be regularly determined through faecal examination for EPG count to cater for development of resistance.

Keywords: Horse, Anthelmintic, Strongylosis, Haemoglobin, Total erythrocyte count, Packed cell volume

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Introduction

Pakistan has a population of 0.4 Million horses (Ministry of Finance, GOP, 2018) and this vast population is prone to certain diseases due to their peculiar anatomical features and managemental constraints. Horses are prone to be infected by multiple species of parasites and strongyles are one of the most significant internal parasites affecting them. The strongyles belonging to the family strongylidae have been reported to affect more than 80% equines in the world (Shite et al., 2015). The strongylosis is a common disease of horses throughout the world and causes deaths when control measures are not taken (Radostits et al., 2006). During course of clinical illness, ill-thrift, poor hair

coat, impaired performance, weight loss and anemia are signs associated with a 'wormy' horse.

Haemoglobin estimation and packed cell volumes of the blood are some of the factors for the assessment of infection with strongyles (Radostits et al., 2006). Intermittent colic, low grade fever and diarrhea are also noted (Soulsby, 1982). Due to blood sucking nature of the strongyles changes also occur in haematological values such as eosinophilia, monocytopaenia and reduced cell survival (Sipra et al., 1999). These changed haematological parameters help in establishing the extent of infestation and subsequent damage.

Anthelmintics are effective against both larvae and adult strongyles which amongst others include avermectins (ivermectin), benzimidazoles (oxfendazole) and tetrahydropyrimidines (pyrantel) (Radostits et al., 2006). Deworming at desired intervals (4-8 weeks) with an effective anthelmintic provides sufficient guard against these parasites (Urquhart et al., 1996).

There is little information available on epidemiology and prevalence of gastrointestinal parasites of horses in Balochistan in general and Quetta in particular. Quetta is the provincial capital of Balochistan province, and 9th largest city of Pakistan. Horses and donkeys are kept by local people for carting purposes. Moreover, horses are also used for equestrian activities like polo, tent pegging and racing. Besides it, a sizeable population of horses is being maintained by government agencies in Quetta and suburbs where they are mainly used for various equestrian activities. The animals are mostly kept under intensive system of management where internal parasites can maintain their life cycle without any major environmental and managemental hindrance. Drastic effects of strongylosis on health and performance of horses necessitated that prevalence of disease in the region be studied and effective chemotherapeutic measures be devised to curtail such losses. The current study was conducted on horses being maintained by government agencies in Quetta and suburbs to provide groundwork for future research in the region.

Material and Methods

The present study was conducted from August to October 2018 on 100 horses kept by government agencies at Quetta and suburbs, to determine the prevalence of strongyle parasites, their effects on blood parameters and efficacy of different anthelmintics on decreasing the worm load and subsequently improving the blood parameters. The animals were not dewormed 3 months before start of study. All the animals were on same feeding scales and stabled in similar housing conditions. The animals were tested / screened for presence of strongyles through direct smear and salt floatation methods of fecal examination after collecting 10 gm of faecal samples directly from the rectum of animals in separate polythene (plastic) bags. Those found positive through either of the methods were tested for eggs per gram using McMaster Egg Counting Technique (Soulsby, 1982). 40 horses from positive cases were randomly divided into 4 groups namely Group A, B, C and D of 10 horses each irrespective of sex, age and breed. Each animal of group A was treated with Oxfendazole at10 mg/kg PO, group B with Ivermectin at 0.2 mg/kg PO, group C with Pyrantel at 0.6 mg/kg PO while group D was kept as control (No Anthelmintic). Weight of each animal was estimated before chemotherapy to administer the anthelmintic at correct dose by following formula (Higgins and Snyder, 2006):-

$$W = [(hg)^2 \times l] / 11877$$

Where, W = Weight (kg), hg = Heart girth (cm), l = Length of body (cm) from point of shoulder to point of buttock. The blood parameters (Total Erythrocyte Count-TEC, Haemoglobin Estimation-Hb and Packed Cell Volume-PCV) were tested as per the procedures described by Benjamin (1985) after collecting the blood in sterilized commercially available Ethylene Diamine Tetra Acetic Acid (EDTA) coated vacutainers. Blood parameters and fecal egg counts per gram were tested premedication (day 0) and then on day 7 and 14 to determine the efficacy of drugs on reduction of worm load and improvement of blood parameters. 4th group (positive untreated) was kept as control. Percent efficacy of each drug was calculated by measuring fecal egg count reduction by day 14 and applying the following formula (American Association of Equine Practitioners: AAEP parasite control guidelines, 2017): -

$$\% \text{ Efficacy (FECRT)} = \frac{\text{EPG (pretreatment)} - \text{EPG (14 day post treatment)}}{\text{EPG (pretreatment)}} \times 100$$

Statistical analysis

The data regarding efficacy of drugs on EPG reduction and resultant effects on blood parameters were

analyzed statistically through one-way ANOVA technique. Difference among various treatments was determined by applying the Least Significant Difference (LSD) test for the comparison of the groups (Steel et al., 1997).

Results and Discussion

Prevalence and worm load

Results regarding prevalence of strongyles in horses of government agencies in areas of Quetta and suburbs revealed that 48 (n = 48) samples out of 100 tested were found positive. Prevalence was therefore observed to be 48%. The results of current study revealed moderate prevalence (48%) of strongyles in horses. The results found are in conformation with Gras et al. (2011) who studied the prevalence of strongylosis in horses in Italy and found that 44% of the horses were affected. The results of current study are also in close consonance with those found by Sonone et al. (2013) who found prevalence of strongyles in horses at 44.12%. The results are also in line with Melkamu (2012) who conducted a study for prevalence of strongylosis in equines in Ethiopia and found 47.7% horses positive for strongylosis. The results found are also in closer conformation with the results of Saleem et al. (2000), Fatima et al. (2007) and Mahboob et al. (2008) who studied the prevalence of strongylosis and found the prevalence rate at 56.66%, 60% and 57.47% respectively. The results, however, are not in agreement with Morariu et al. (2016) who studied the prevalence, abundance and distribution of strongyles in horses in western Romania and found 100% horses to be affected. A slightly lower prevalence rate may be ascribed to better managemental practices and parasite control programmes adopted by government agencies.

The horses affected in the current study were found to be moderately contaminated with mean EPG count of 381.25 ± 33.98 (200-500 EPG level for moderate contamination as per guidelines of American Association of Equine Practitioners). The results are not in agreement with the contamination level found by Fatima et al. (2007) who found the horses to have severe contamination of strongylosis with EPG ranging from 800-2000. Mean EPG found is towards higher side than that found out by Pilania et al. (2013) who studied the intensity of gastrointestinal parasitic infections in horses in Rajasthan and found that fecal samples showed an overall mean EPG count of 201.20 ± 12.28 for strongyles. The results are also towards a higher side than those studied by Saeed et al. (2008) who found mean EPG in strongylosis affected horses to be at 237. The difference can be explained by the managemental/ environmental variations and difference in parasite control programmes in study areas.

Comparative efficacy of anthelmintic drugs

Efficacy of Oxfendazole (Group A) was noted at 91.43% and 97.14% at 7 day and 14 day respectively; while the efficacy of Ivermectin (Group B) was 95.24% and 97.62% at day 7 and day 14 respectively. Efficacy of Pyrantel (Group C) was 87.18% and 94.87% at 7 day and 14day respectively.

The results suggested that Ivermectin proved to be the most effective in controlling strongylosis in horses with highest average efficacy of 97.62%, followed by Oxfendazole 97.14% and Pyrantel 94.87%. Parasitic load from day 0 to 7th and 14th showed gradual reduction in all three groups. Control (Group D) exhibited relative increase in EPG (Table 1).

Pre-medication EPG count in the faeces of animals in group A was in the range from 100 to 800 (350 ± 68.77); while the EPG range in group B was 100 to 900 (420 ± 77.23) and in C group the pre-medication EPG was in range of 100 to 1100 (390 ± 98.33), whereas the pre-medication EPG count in group D was in the range of 100 to 800 (380 ± 71.23). However, analysis of variance revealed that statistically the differences in EPG load between various groups were non-significant ($p>0.05$) with $F\ 0.05\ (3,36) = 2.866 > 0.13$ (Table 1).

Table-1: EPG and Comparative Efficacy of Various Drugs on Day 0, Day 7 and Day 14

Group	No of Horses	Anthelmintics	Day 0		Day 7		Day 14	
			EPG (Mean ± SE)	Efficacy %	EPG (Mean ± SE)	Efficacy %	EPG (Mean ± SE)	Efficacy %
A	10	Oxfendazole	350.00 ± 68.77	-	30±21.36	91.43	10±10.0	97.14
B	10	Ivermectin	420.00 ± 77.23	-	20±13.34	95.24	10±10.0	97.62
C	10	Pyrantel	390.00 ± 98.33	-	50±22.38	87.18	20 ±13.34	94.87
D	10	Control	380.00 ± 71.23	-	420±72.78	-10.53	480+75.77	-26.32

The parasitic load examined by EPG (egg per gram) count at 7th day post medication in animals of group A, B, C and D showed significant effect of anthelmintic drugs on parasitic load. On the basis of efficacy percentage of anthelmintics tested, Ivermectin (Group B) showed the best anthelmintic effect on strongyles, where the EPG count in faeces at day 7 post-medication reduced to 20 ± 13.34 against pre-medication EPG count of 420.00 ± 77.23 showing a reduction of 400 EPG, suggesting that Ivermectin efficacy was 95.24%. Oxfendazole also showed remarkable anthelmintic effect on strongyles (Group A) where EPG count in faeces was reduced to 30 ± 21.36 at day 7 post medication against pre-medication EPG count of 350.00 ± 68.77 showing reduction of 320 EPG, suggesting that efficacy of Oxfendazole was 91.43%. Pyrantel (Group C) was ranked 3rd in efficacy against strongyles where the EPG count in faeces at day 7 post-medication reduced to 50 ± 22.38 against pre-medication EPG count of 390.00 ± 98.33 showing a reduction of 340 EPG, with efficacy of 87.18%. Whereas in Control (Group D) EPG count at day 7 increased to 420±72.78 against pre-medication count of 380.00 ± 71.23 (Table 1).

The parasitic load examined by EPG count at 14th day post medication in animals of group A, B, C and D also showed significant effect of anthelmintic drugs on parasitic load. On the basis of efficacy percentage of anthelmintics tested, Ivermectin (Group B) showed the best anthelmintic effect on strongyles, where the EPG count in faeces at 14th day post-medication reduced to 10 ± 10.0 against pre-medication EPG count of 420.00 ± 77.23 showing a reduction of 410 EPG, suggesting that Ivermectin efficacy was 97.62%. Oxfendazole also showed remarkable anthelmintic effect on strongyles (Group A) where EPG count in faeces was reduced to 10 ± 10.0 at day 14 post medication against pre-medication EPG count of 350.00 ± 68.77 showing reduction of 340 EPG, suggesting that efficacy of Oxfendazole was 97.14%. Pyrantel (Group C) was ranked 3rd in efficacy against strongyles where the EPG count in faeces at day 7 post-medication reduced to 20 ± 13.34 against pre-medication EPG count of 390.00 ± 98.33 showing a reduction of 340 EPG, with efficacy of 94.87%. EPG count in Control (Group D) increased to 480+75.77 at day 14 against pre-medication count of 380.00 ± 71.23. Analysis of variance showed that statistically the differences in EPG load between various groups were significant ($p>0.05$) with $F\ 0.05\ (3,36) = 2.866 > 0.13$. LSD test was utilized to determine any significant difference between the 3 treatment groups. There was no significant difference between treatments at Group A, B and C because means of all three groups had difference of less than the computed LSD (LSD at 0.05 computed by Fischer's method was 112.61). However, all the 3 groups had difference of means greater than LSD when compared with the control.

The results of current study with efficacy of Oxfendazole, Ivermectin and Pyrantel at day 7 and 14 at 91.43% and 97.14%, 95.24% and 97.62%, and 87.18% and 94.87% respectively are in closer consonance with the data collected by Mahboob et al. (2008) who studied chemotherapy of strongylosis in horses of district Layyah and found that efficacy of oxfendazole at days 7 and 14 post administration was 96.82 and 98.14% respectively. Similar results were also found out by Saleem et al. (2000) who found efficacy of Oxfendazole at day 14 post administration at 98.7%. The results are also in conformation with those collected by Fatima et al. (2007) who found efficacy of ivermectin against Strongyloids at 96.5% and 99.3% on day 7 and 14 post medication. The results are also in harmony for Ivermectin with those collected by Saeed et al. (2008) who studied the efficacy of Ivermectin against strongylosis in horses and found it to be 98% at day 14 post medication. However, the results are not in agreement with the same authors for Oxfendazole which in the current study are higher (97.14%) than those determined by the authors who found it to be at 94% for Oxfendazole at day 14 post medication. The results of current study are towards better side for Ivermectin than those studied by Waqas et al. (2014) who evaluated the anthelmintic efficacy of Ivermectin and found it to be 73.2% and 96.42% on day 7 and 14 respectively. The results of present study are also on a higher side than those observed by Binev et al. (2005) who found the efficacy of parenteral administration of ivermectin against strongyles to be at 96% at 14 days post administration. The efficacy of ivermectin and Pyrantel in current study is little lower than that found by Sanna et al. (2016) who found the efficacies at 100% and 98.9% respectively for both drugs at day 14 post medication. The differences in efficacies, though negligible, may be attributable to differences in frequency of use of the said anthelmintics in parasite control programme. However, none of the anthelmintics used in the current study showed any signs of parasitic resistance.

Effects of decrease of worm load on blood parameters

A gradual improvement in blood parameters was observed with decrease in worm load which was measured through decrease in number of EPG. Table 2 summarizes the results of effects of all 3 anthelmintics on EPG reduction and subsequent effects on blood parameters through reduction of worm load. Means of all the three blood parameters tested (TEC, Hb and PCV) were non-significant between the groups at day 0 but were significant at day 7 and 14 post medication due to decrease in number of EPG. Blood parameters improved towards normalcy in treated groups whereas the increase in worm load in untreated control group further deteriorated the blood parameters thereby implying that reduction of worm load had direct effect towards normalization of blood parameters.

The present study established that anthelmintics treatment resulting in decreased egg count (worm load) also resulted in parallel improvement in TEC, Hb and PCV. It was also established that in the absence of anthelmintics treatment in control group the blood parameters further dropped showing a direct link between Strongyloids load and resultant blood parameters. TEC in treatment groups increased from 4.52 ± 0.21, 4.26 ± 0.13, 4.05 ± 0.09 (10^6/µl) at day 0 to 5.74± 0.19, 5.89± 0.13, 5.32 ± 0.11 (10^6/µl) and 7.00 ± 0.10, 6.95 ± 0.11, 6.74 ± 0.11 (10^6/µl) at day 7 and 14 for Oxfendazole, Ivermectin and Pyrantel respectively. The same figures for control (Infected, untreated) dropped further from 4.37 ± 0.17 at day 0 to 4.29 ± 0.16 and 4.07 ± 0.12 (10^6/µl) at day 7 and 14 respectively thus establishing the link between worm load and resultant blood picture. Similarly, Hb concentration in treatment groups increased from 9.68 ± 0.15, 9.58 ± 0.18, 9.22 ± 0.19 (gm/ dl) at day 0 to 10.74± 0.14, 10.52± 0.13, 10.38 ± 0.21(gm/ dl) and 12.10 ± 0.19, 12.02 ± 0.15 and 11.4 ± 0.24 (gm/ dl) at day 7 and 14 for Oxfendazole, Ivermectin and Pyrantel respectively. The same figures for control dropped further from 9.9 ± 0.25 at day 0 to 9.68 ± 0.24 and 9.44 ± 0.25 (gm/ dl) at day 7 and 14 respectively. Likewise, PCV % in treatment groups increased from 32 ± 0.58, 31.6 ± 0.50, 32.1 ± 0.53 (%) at day 0 to 35.4 ± 0.45, 35.2 ± 0.39, 35.3 ± 0.40 and 39.3 ± 0.30, 39.0 ± 0.21, 38.6 ± 0.27 (%) at day 7 and 14 for Oxfendazole, Ivermectin and Pyrantel respectively. The same figures for control dropped further from 32.8 ± 0.42 at day 0 to 32.3 ± 0.45 and 31.5 ± 0.40 (%) at day 7 and 14 respectively.

The results of current study match with those found by Mahboob et al. (2008) who studied the haematology of strongylosis in horses of district Layyah and found that horses affected with strongylosis showed reduction in haematological parameters. They established that TEC, Hb and PCV values were lower in affected horses and post treatment the blood parameters showed marked increase towards normalcy. Similar findings were also noted by Sipra et al. (1999) who evaluated haematological parameters of strongylosis in horses and found that values of TEC, Hb and PCV were low in infected untreated animals than those of infected treated animals. The results of present study are also in line with Sonone et al. (2013) who found out changes in Haemoglobin, PCV, TEC and DLC in infected horses before and after treatment. The findings of current study are also in consonance with Saleem et al. (2000) who studied the effects of strongylosis on haematological parameters and established that affected animals showed significant reduction in TEC, Hb level and PCV. Likewise, the findings are also in line with Waqas et al. (2014) who evaluated post treatment haematological parameters for animals infected with strongylosis and observed marked improvement in Hb concentration in animals treated with ivermectin.

Table-2: Mean EPG, TEC, Hb and PCV of all Groups at Day 0, Day 7 and Day 14 Post-Medication

Groups	Anthelmintics	Day 0				Day 7				Day 14			
		EPG	TEC x 10^6/µl	Hb gm/ dl	PCV %	EPG	TEC x 10^6/µl	Hb gm/ dl	PCV %	EPG	TEC x 10^6/µl	Hb gm/ dl	PCV %
A	Oxfendazole	350.00 ± 68.77	4.52 ± 0.21	9.68 ± 0.15	32 ± 0.58	30 ± 21.36	5.74 ± 0.19	10.74± 0.14	35.4 ± 0.45	10 ± 10.0	7.00 ± 0.10	12.10 ± 0.19	39.3 ± 0.30
B	Ivermectin	420.00 ± 77.23	4.26 ± 0.13	9.58 ± 0.18	31.6 ± 0.50	20 ± 13.34	5.89 ± 0.13	10.52± 0.13	35.2 ± 0.39	10 ± 10.0	6.95 ± 0.11	12.02 ± 0.15	39.0 ± 0.21
C	Pyrantel	390.00 ± 98.33	4.05 ± 0.09	9.22 ± 0.19	32.1 ± 0.53	50 ± 22.38	5.32 ± 0.11	10.38 ± 0.21	35.3 ± 0.40	20 ± 13.34	6.74 ± 0.11	11.4 ± 0.24	38.6 ± 0.27
D	Control	380.00 ± 71.23	4.37 ± 0.17	9.9 ± 0.25	32.8 ± 0.42	420 ± 72.78	4.29 ± 0.16	9.68 ± 0.24	32.3 ± 0.45	480 ± 75.77	4.07 ± 0.12	9.44 ± 0.25	31.5 ± 0.40

Conclusion

All the anthelmintic drugs used in the study reduced parasitic load effectively when compared with pre-medication EPG counts. Ivermectin proved to be most effective against strongyles with efficacy of 97.62% followed by Oxfendazole at 97.12% and Pyrantel at 94.87%. Moreover, reduction in worm load led to improvement in blood parameters. It is recommended that periodic deworming of horses is necessary to decrease the worm load, maintain blood parameters and improve their athletic performance. Efficacy of routinely used anthelmintics should be regularly determined through faecal examination for EPG count to cater for development of resistance.

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Contribution of Authors

Baloch MIH: Experimental design, Data collection, Data analysis, Write up.
Tunio MT: Experimental design, Data collection, Data analysis, Write up (As Supervisor)