









Parasite Control Practices Used by Horse Owners in Punjab, Pakistan

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ABSTRACT

Horses are companion animals that are reared for multipurpose such as riding, racing, plowing, pulling carts, etc. Therefore, the population of horses is increasing in Punjab. Horses are very sensitive to internal parasites and may frequently be prone to infection caused by small strongyles. Nematodes are responsible for poor health, reduced growth, weight loss, anorexia, diarrhea, gestational lesions, liver damage, and a high mortality rate. The management strategies practiced by the farm owners have an impact on the prevalence of parasitic infections in animals. Farmers adopt different management practices for the well-being of horses. Anthelmintic drugs are the primary control method for their prevention. The drugs used for parasite control give varying efficacy. Therefore, a study was planned to conduct a questionnaire-based survey in Punjab to estimate the stud farmer's practices for parasite control and to check the efficacy of 6 brands of drugs commercially available in Punjab. The data of the questionnaire was recorded on Google Forms and fecal samples were also collected from various stud farms for egg hatch assay. Based on the survey, Systemax[®] was the most widely accepted brand of anthelmintic by farmers for the treatment of helminth infections in horses (82.98%). In the egg hatch assay, it is estimated that Parazole[®] had the lowest dose (0.2 mg/mL) at which 50% inhibition of cyathostomins egg hatching occurred. While Oxafax[®] showed the highest dose (2.4 mg/mL) at which 50% inhibition of egg hatching occurred. *In vitro*, the efficacy of Oxafax[®] was less as compared to other brands. However, the dose-response of all six brands had a non-significant difference. It is concluded that the different strategies adopted by horse owners had a role in the prevalence of parasitic infections in horses and all six brands had varying efficacy against small nematodes.

Keywords: Efficacy, Cyathostomins, Questionnaire, Egg hatch assay, Equine

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INTRODUCTION

Horses (*Equus caballus*) belong to the family Equidae which plays an important role in carriage, transportation, plowing, amusement, pulling carts, pleasure riding, racing, and for heavy purposes in the fields of agriculture and industries, especially in developing countries (Nansen et al. 1995). In Pakistan, the numbers of horses, donkeys, and mules are 0.4, 5.7, and 0.2 million, respectively in 2021-2022 (Pakistan Economic Survey 2022). Among infectious diseases, internal parasites pose significant risks to the health of horses (Osman et al. 2021; Borovikov et al. 2023). They are more prone to parasitic infections and susceptible to many internal parasites. They harbor various species of parasites at any time (Stoltenow et al. 2003; Sindhu et al. 2022). Parasites are responsible for high morbidity and mortality in horses. Cyathostomins are the small strongyles and most prevalent species of nematodes in adult equids (Sallé and Cabaret 2015; Mughal et al. 2021). About 50 species of genus *Cyathostomins* are recognized out of which 10 species are more prevalent in horses. The prevalence of small strongyles in horses reported in

different districts of Punjab ranges from 32.2 to 58.5% (Saeed et al. 2010; Goraya et al. 2013; Khan et al. 2020). The life cycle of nematodes consists of three stages namely eggs, larvae (L₁-L₅), and adults. The third larval stage (L₃) is the infective stage, and horses become infected when they ingest them with pasture (Corning 2009; Abbas et al. 2020; Nawaz et al. 2022). Clinically the infection is characterized by weight loss, lethargy, debilitation, and diarrhea. There is a condition known as larval cyathostomiasis caused by the migration of the fourth larval stage in the lumen of the intestine. This condition is characterized by severe damage to the gut wall which results in diarrhea, and colic, and ultimately may lead to death (Imran et al. 2020; Junco et al. 2023).

Several studies indicated that different management practices have an influence on the prevalence of parasitic infections in animals which include rotational grazing, open grazing, stall feeding, deworming, and manure removal from the pasture (Scare et al. 2018). The farmers adopt different management practices for the control of parasites in horses. Besides grooming and administration of herbal products to horses, farmers also use synthetic anthelmintics

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for internal parasite control. The use of anthelmintic drugs is the primary method for the treatment of gastrointestinal nematodes (Naseer et al. 2017; Zaman et al. 2017; Sazmand et al. 2020). Previously in Pakistan, no such studies were conducted to assess the management practices, particularly for the control of parasitic infections in horses. Therefore, to better understand the management practices for the control of parasites and the maintenance of the health of horses is important. Moreover, the emergence of drug resistance in small strongyles is a major issue. They show a widespread resistance to benzimidazoles. For designing the optimal strategies for the control of nematode infections in horses, detailed information regarding management strategies being practiced in Punjab must be recognized. Therefore, the purpose of this study is to complete a survey on management practices, parasite control strategies in equines, commercially available different brands of anthelmintic drugs in Punjab, and the evaluation of the efficacy of these brands of anthelmintic drugs against cyathostomins.

MATERIALS & METHODS

Questionnaire Survey

A questionnaire-based study was designed according to Nielsen et al. (2018) with slight modifications and data were collected from 188 stud farms all over Punjab, Pakistan. The data was recorded on Google Forms. During the collection of data, the information collected from the participants includes the type of farms either private, government, or other, breed, age, feeding and deworming practices and fecal examination. These management practices affect the prevalence of parasitic infections in horses.

Evaluation of Comparative Efficacy of Different Brands of Anthelmintics

The fecal samples were collected from animals of private stud farms in zip-lock polythene bags, labeled with collection date, location, farm, and horse name, and preserved at 4°C during transportation to the Department of Parasitology, University of Agriculture, Faisalabad, Pakistan. Anthelmintic activity of six commercially available brands of anthelmintic i.e., Albamax®, Parazole®, Alvenax®, Systemax®, Oxafax®, and Fenzole® was evaluated using egg hatch assay (Johansen 1989). Briefly, five 2-fold serial dilutions of different brands of albendazole and oxfendazole (purchased from the local veterinary pharmacy of district Faisalabad) were prepared in the flat bottom 24 well microtitration plate. The number of eggs was adjusted to 500 eggs per ml by using the McMaster technique. The suspension of eggs of 0.2ml containing approximately 100 eggs was distributed in a flat-bottomed 24-well microtiter plate, and mixed with 1ml volume of different concentrations of drugs (1.875, 3.75, 7.5, 15, and 30 µg/ml). There were only diluent and eggs in negative control wells. The microtiter plates were incubated at 27°C for 48 hours. After 48 hours, 2 drops of Lugol's Iodine solution were added to the solution to stop the hatching of eggs. The eggs were counted in each plate under a stereomicroscope with a maximum magnification of 40X (Coles et al. 1992). Three replicates of this test were performed.

Statistical Analysis

For the egg hatch test, probit transformations were performed to transform a typical sigmoid dose-response curve into a linear function. The drug concentration required to prevent 50% [lethal concentration 50 (LC₅₀)] of the hatching of

eggs was calculated. The data were statistically analyzed using the software Poloplus (LeOr Software 2002).

Table 1: Common management practices followed at stud farms of Punjab for the control of helminths infections in equine

Parameters	Respondents out of 188 stud farms	Percentage
Private farms	74	39.36
Mix farms	114	60.64
Thoroughbred horses	14	7.4
Desi horses	174	92.55
Younger than 5 years	110	58.51
Older than 5 years	78	41.49
Open grazing	2	1.06
Stall feeding	186	98.93
Routine deworming	126	67.02
Deworming by a horse owner	148	78.72
Deworming by a horse handler	24	12.76
Deworming by veterinarian	16	8.51
Systemax®	156	82.98
Mix brands of anthelmintic drugs available in Pakistan	32	17.02
Rotational use of brands	6	3.19
Deworming of newly inducted horses	120	63.83
Simultaneous deworming of all the horses	35	18.62
Deworming twice a year	90	47.87
Removal of manure daily	188	100
Fecal examination before deworming	2	1.06
Daily grooming	180	100
Body condition scoring > 4	13	6.91
Body condition scoring < 4	175	93.09

RESULTS

Pieces of general information was obtained from the owner or manager of the stud farm. Based on the results of the survey, it is estimated that many farmers prefer desi-bred horses (92.55%) over thoroughbred ones (7.4%) because of their local availability and habituation with the environment. Based on the susceptibility, desi-bred horses are less prone to internal parasitic infections as compared to thoroughbred horses. Moreover, the stall grazing of animals also decreases the chances of occurrence of parasitic infections. For the control of parasites, routine deworming is a common practice (67.02%) all over Punjab. However, farmers give preference to specific brands of anthelmintic drugs available in Pakistan based on their knowledge and parasite reduction results. The most preferred brand is Systemax® which contains oxfendazole and about 82.98% of farmers use this brand of anthelmintic for the deworming of their horses. Based on the survey, the common management practices followed by farm owners, or their managers are listed in Table 1.

The egg hatch assay was performed to evaluate the dose-dependent response of different brands of albendazole and oxfendazole commercially available in Punjab. The anthelmintic efficacy of different brands was estimated and compared to check the most effective brand. The lethal concentrations (LC) estimates have been presented in Table 2 and the percentage of egg-hatching inhibition is in Fig. 1. Results indicated that Parazole® had the lowest dose (0.2 mg/mL) at which 50% inhibition of cyathostomins egg-hatching occurred. While Oxafax® showed the highest dose (2.4 mg/mL) at which 50% inhibition of eggs occurred. *In vitro*, the efficacy of Oxafax® was less as compared with other brands. However, the dose-response of all the brands was the same statistically.

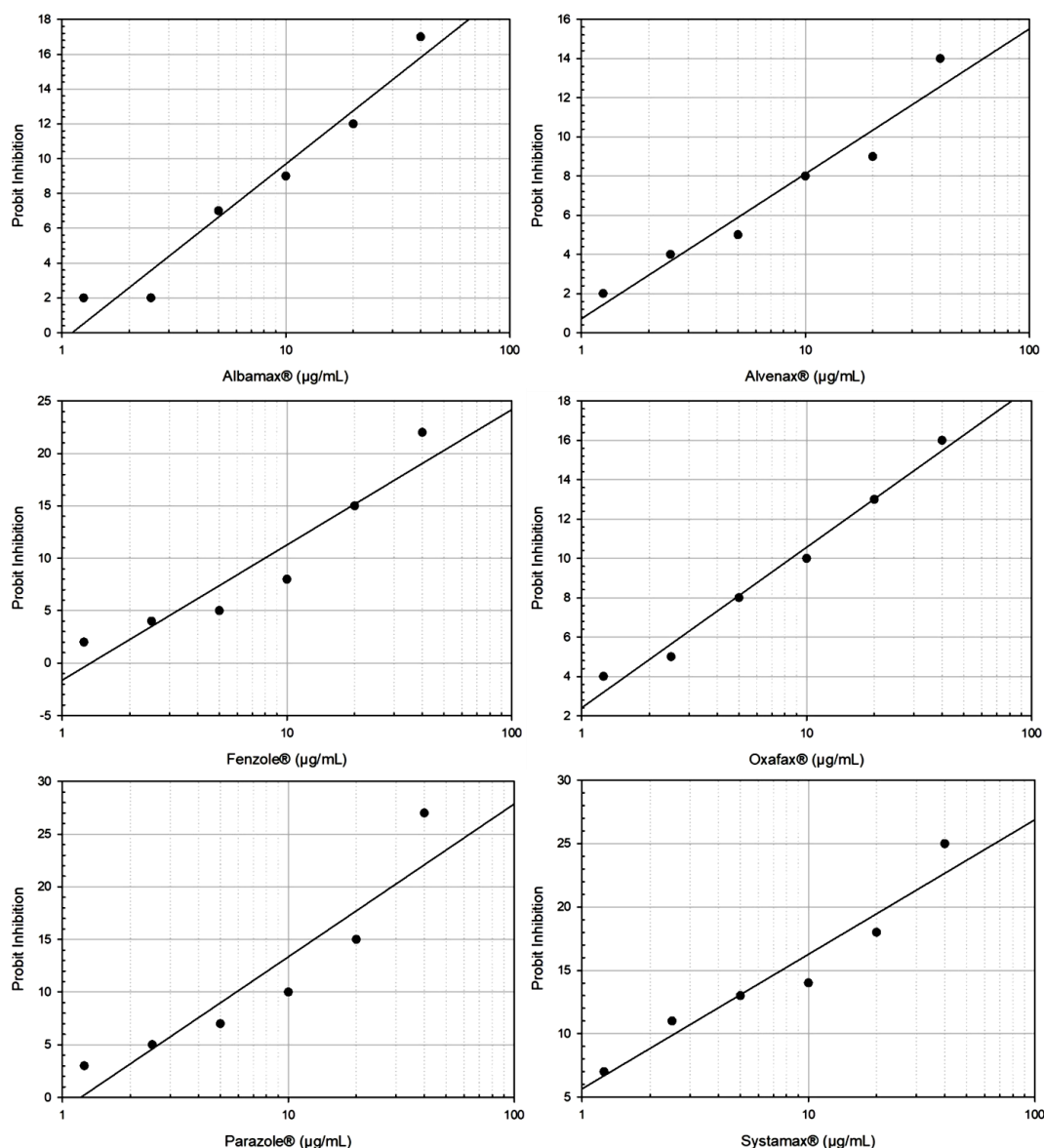


Fig. 1: Probit inhibition × log concentration plot from eggs of cyathostomins submitted to egg hatch inhibition assay.

Table 2: Estimates of LC₅₀, LC₉₀, and LC₉₉ (expressed in mg/mL) of different brands of albendazole and oxfendazole for their anthelmintic activity against eggs of cyathostomins

	Albendazole			Oxfendazole		
	Albamax®	Parazole®	Alvenax®	Systamax®	Oxafax®	Fenzole®
Slope (SE)	0.74±0.16	0.96±0.23	0.67±0.22	0.5±0.18	0.6±0.18	1.002±0.3
X ²	1.15	0.8	0.4	0.7	0.1	0.36
LC ₅₀ % (95% CI)	0.7 (0.19-16.7)	0.2 (0.09-1.8)	1.8 (0.3-7491.6)	1.2 (0.2-336.7)	2.4 (0.3-8112.8)	0.2 (1.0-3.1)
LC ₉₀ % (95% CI)	367.6 (0.31-16697.1)	4.8 (0.8-586.2)	151.7 (4.2-0.0)	425.4 (10.0-0.0)	461.4 (8.6-0.0)	4.8 (0.8-987.8)
LC ₉₉ % (95% CI)	920.1 (30-4721649)	60.3 (4.6-65050.1)	5402.1 (38.5-0.0)	5047.6 (225.6-0.0)	3289.5 (124-0.0)	53.1 (3.9-12984.9)

Parazole® and Fenzole® showed the highest efficacy (4.8mg/mL (0.8-586.2) and 4.8 mg/mL (0.8 – 987.8) at which the 90% inhibition of egg hatching had occurred. Additionally, both concentrations were statistically the same. The least activity was shown by Oxafax® and this brand requires more concentration of active ingredients to stop the 90% hatching of eggs. Again, statistically, all the brands have the same efficacy.

DISCUSSION

Equines are seriously prone to infections caused by gastrointestinal (GI) nematode *Strongyloides*, particularly in tropical, sub-tropical, and even moderate climate regions of the world (Sangster 1999; Getachew et al. 2007). The

advancement in the development of anthelmintic resistance against small strongyles of the equine led to management practices for the control of parasites. The concept of selective treatment and reducing the rate of deworming of animals is recommended in order to delay the occurrence of resistance. The selective treatment is based on the animal's fecal egg count (FEC) and then the animals that cross the threshold are treated (Kaplan and Nielsen 2010). The American Association for Equine Practitioners (AAEP) 2013 published guidelines regarding the management of the occurrence of anthelmintic resistance (Nielsen et al. 2013). However, this survey illustrates the lack of compliance with the guidelines of AAEP and only a small number of farmers follow the recommended guidelines. In the US a survey was conducted which showed that 20% of equine owners adopt the practice of FEC before giving any

anthelmintic drug (Robert et al. 2015). This was the first survey conducted in Pakistan to demonstrate that <1.5% of farm owners perform FEC before giving medication to their horses. If the same practice continues, there will be a chance of rapid occurrence of drug resistance in Punjab as compared to other developed countries. However, farmers adopt different management practices to ensure the health and well-being of horses. Additionally, management practices directly impact the prevalence of parasitic infections in animals. They use broad-spectrum anthelmintics for the control of parasitic infections in animals. The brands of drugs available in Pakistan are prioritized based on the traditional knowledge of farm owners. However, the different brands have varying efficacy against gastrointestinal nematodes. In this survey, we found that farmers preferred different management practices according to their traditional knowledge. Based on the survey, they were daily involved in the grooming, cleaning of manure, and stall-feeding practices which were also reported by Papini et al. (2015). Likewise, a survey was conducted by Murray et al. (2015) presenting the same questions as presented in this survey. Their results coincide with the results presented in this study. The worm burden and types of parasites in foals and yearlings are different from the adult animals and require different parasitic approaches for the control of parasites (Reinemeyer and Nielsen 2017). The scenario of applying different approaches in varying age groups is lacking in Pakistan and farm owners deworm all the animals at the same time (Ahmad et al. 2022). This practice is problematic and requires an immediate shift in treating the different age groups of animals with different approaches so that the health and well-being of the horses will be ensured.

Similarly, many studies have also been conducted to evaluate the efficacy of different anthelmintics. Egg hatch assay (EHT) is the preferred test to validate the efficacy of different anthelmintics against parasitic eggs. Moreover, it could also be used to estimate the resistance developed by parasites against a particular class of anthelmintic. Matthews et al. (2012) conducted *in vitro* study investigating the efficacy and development of resistance by small nematodes of horses against benzimidazole. Similarly, another group performed an egg-hatching test to evaluate the efficacy of benzimidazole. During the standardization of EHT for resistant estimation, they observed resistant strains of small strongyles against anthelmintics (Von Samson-Himmelstjerna et al. 2009). Their results support our trial in such a way that different brands of drugs containing the same active ingredient showed different dose-response in preventing the hatching of cyathostomin eggs. The variation observed in different brands of anthelmintics may be due to different vehicles incorporated with active compounds by different pharmaceutical companies to make their brands effective.

Conclusion

Based on the survey and egg hatch assay, it is estimated that farmers adopt different management practices according to their traditional knowledge and prefer certain brands of anthelmintics because of their results in managing the parasitic ailments in horses. Systemax® is the most preferred brand among owners of stud farms. The efficacy of different brands of anthelmintic drugs varies against cyathostomins however, all six brands were statistically the same.

Author's Contribution

ZuD Sindhu and B Aslam conceived the idea and designed the study. M Niaz, S Ejaz and MK Khan conducted the experiments. RZ Abbas and M Imran performed

statistical analysis. F Munir, ZuD Sindhu, and S Ejaz were involved in the write-up and proofreading of the manuscript.

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