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Original Scientific Article

ANTHELMINTIC RESISTANCE AND ASSOCIATED MANAGEMENT PRACTICES IN LOCAL HORSES IN SOKOTO METROPOLIS, NIGERIA

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ABSTRACT

This study was carried out to assess the management practices used in the control of gastrointestinal (GI) nematodes of horses and to determine the efficacy of three anthelmintics commonly used in Sokoto metropolis. A questionnaire was administered on management practices, while an anthelmintic efficacy test was carried out using 15 horses. The 15 horses were divided into three groups (A, B and C) comprising of 5 each and treated with albendazole, ivermectin and fenbendazole, respectively. The faecal egg count reduction test (FECRT) was used to determine the efficacy and faecal culture was used to determine the parasite species. Majority of the respondents (80%) claimed to have worm control strategies, but only 32.5% used anthelmintics for the control of GI parasites. 62.5% of respondents designed their deworming plan, while only 25% relied on veterinarians. Most of the treatments were done by the horse owners and/or handlers and they largely depended on visual judgement in dosage determination. Their selection of anthelmintics was based on familiarity and 52.5% of the respondents dewormed their horses six times a year using a particular class of anthelmintic or herbal remedies. Resistance against albendazole as well as suspected resistance against fenbendazole by the GI nematodes identified was observed, while ivermectin demonstrated high efficacy against all nematodes isolated. In conclusion, a single dose of subcutaneous injection of ivermectin was highly effective against gastrointestinal parasites in horses, while the worm control strategies employed by respondents enhanced the selection of nematode resistance to albendazole and fenbendazole.

Key words: anthelmintic efficacy, horses, gastrointestinal parasites, Sokoto

INTRODUCTION

Horses have played a very important role in the northern part of Nigeria. Majority of the horses are local breeds, kept under traditional husbandry practices and depend mostly on grasses, hay and grains as feed sources with minimum health care interventions (1). Of all the infectious diseases, parasitic diseases are the major obstacle in the growth and development of animal health all over the world (2). It is also one of the most important problems for equids in developing countries. Several nematode species infest horses and other equids, but the prevalence of other species is usually lower than that of strongyles, particularly cyathostomins (3, 4).

The control strategy for parasitic nematodes has mostly relied on the use of anthelmintics or the combination of anthelmintics and pasture management, although studies have demonstrated the presence of nematodes resistant to one or more anthelmintics in different regions of the world (5-9). The development of resistance among nematodes has largely been attributed to their considerable population sizes, high levels of genetic diversity, relatively rapid generation rates and common practice of interval-based treatment programmes (10, 11).

High prevalence of gastrointestinal (GI) nematodes, particularly strongyle infestations in equids through coprological analysis have consistently been reported in Nigeria (12-15), and reports on

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resistance to anthelmintics in ruminants in Nigeria have been documented (16). However, the status of anthelmintic resistance in horses is not known. In addition, there are no published studies describing the factors that affect the prevalence of the various parasitic nematode species, and the development and spread of anthelmintic resistance in horses to the best of our knowledge. In order to address this lack of information, this study was conducted to assess the management practices used in the control of helminth infestation and to determine the efficacy of anthelmintics commonly used in Sokoto metropolis to control GI nematodes of horses.

MATERIAL AND METHODS

Study area

The study was conducted in Sokoto State, Nigeria. Geographically, Sokoto is located in the north-western part of Nigeria, between 4^0 8' to 6^0 54'E and 12^0 to 13^0 58'N. It has an estimated population of 3.7 million people whose main occupations are farming, animal husbandry and fishing (17). It is the second largest State in Nigeria, the livestock population is estimated to be about 1.18 million cattle, 2.90 million goats, 1.98 million sheep, 2.0 million chickens, 45 000 camels, 34 532 horses and 51 388 donkeys (18).

Questionnaire survey

Questionnaires aimed at determining the management practices used in the control of helminth infestation in horses were administered to horse owners/handlers within Sokoto metropolis. Components of the questionnaire include: demographic information, worm control strategies, utilization of anthelmintics and stable management practices. The questionnaire comprised of 21 questions: 5 open- and 16 close-ended multiple-choice questions with the opportunity to include additional comments at the end of each question.

Study animals and management

The study animals comprised of Nigerian local horses kept in 40 stables or farms. Each stable or farm has an average herd size of horses ranging from two to twenty, comprising mainly of males (colt or stallion). The management system of most of the stables was based on traditional practices with the horses kept tethered on an open field, using sand as bedding with or without provision for housing. They were kept purposely either for racing, traditional purpose (durbar), companion and/or polo and each horse has its own watering and feeding trough. They were fed with millet, wheat bran, hay and crop residues.

Study design and faecal egg counts

The study was conducted under strict adherence to the principles of care and use of farm animals in research, teaching and testing (19). A preliminary faecal screening test was performed on 100 faecal samples collected per rectum using clean gloves. The samples were positive for Strongyle eggs, with eggs per gramme (EPG) ranging from 50 to 3 475. Horses with greater than 200 EPG of faeces that had not received any anthelmintic agent in the last three months were considered for the anthelmintic efficacy test. A total of 15 horses were selected from three stables (A, B, C) with similar management practices, five horses per group (stable). The criteria for group formation were the owner's acceptance of the study, as well as the actual possibility of faecal sampling, both for pre-treatment and post-treatment. The management practices (as identified in the questionnaire) considered were types of feed, mode of feeding (in container on the ground), weekly feeder cleaning and daily stable/manure cleaning. Information about the individual horse such as name, age, identification marks was recorded. Each group was then randomly assigned to an anthelmintic agent with albendazole administered to horses in group A, ivermectin administered to horses in group B and fenbendazole administered to horses in group C. The approximated weight (W) of each horse was estimated from body measurement data (20). The treatments were given based on registered label dose rate recommended by the manufacturers (Table 1).

Table 1. Description of the anthelmintic drugs used in the FECRT for efficacy evaluation

Trade name	Composition	Dosage mg/kg	Route	Batch No	Manufacture Date	Expiry Date
A-zole®	Albendazole 2.5%	5.0	Orally	EAZ1520	06/2015	05/2018
Herbeiivermec®	Ivermectin 1%	0.2	Subcut	160512	05/2016	05/2019
Fenbzole®	Fenbendazole 2.5%	7.5	Orally	LP16002	01/2016	12/2018

Sample collection and faecal analysis

Faecal samples were collected rectally, before the anthelmintic treatment on day 0 and day 14 post-treatment, to determine the faecal egg counts (FEC). The samples were either immediately processed or stored at 4^0C for 24 hours before processing. Simple flotation technique was used to demonstrate the presence of helminth eggs and the EPG of faeces were determined using a modified McMaster method with a sensitivity of 25 EPG by dissolving 4 grammes of faeces in 26 ml flotation fluid for each sample (21). Pooled faecal cultures for each treatment group were done based on 5 g of faeces collected from each animal in a group before treatment (day 0) and on day 14 after treatment. The faecal samples were incubated at room temperature (approximately 28 to 30°C) for 12 days, regularly aerated and checked for desiccation, and moistened when necessary. The larvae were recovered by baermannization, quantified and identified as described by MAFF (21). The identification was done using the number, shape and arrangement of intestinal cells (22).

Anthelmintic efficacy test

The resistance to anthelmintic drugs was determined using the Faecal Egg Count Reduction Test (FECRT) (23). The arithmetic mean and prevalence of pre-treatment (Pre) and post-treatment (Post) helminth parasitism was calculated based on EPG. The efficacy of the anthelmintic treatment was calculated using the pre-treatment and post-treatment results obtained, according to the following equation:

$$FECR = 100 \times [(1 - FEC\ Post) / (FEC\ Pre)]$$

The 95% confidence interval (CI 95%), as well as the lower confidence level (LCL) was determined. The results obtained using FECRT was interpreted based on the faecal egg reduction percentage, as well as on the lower limit of the 95% confidence interval (LCL 95%). In accordance with the methods recommended by the World Association for the Advancement of Veterinary Parasitology for the detection of anthelmintic resistance in horses and ruminants (23).

i. resistance is present (R) if FECR < 90% and the LCL 95% <90%
ii. resistance is suspected (S) if FECR ≥ 90% and/or LCL 95%< 90%
iii. no resistance (N) if FECR ≥ 90% and LCL 95% > 90%

Statistical analysis

The data generated from the questionnaire was entered into Microsoft Excel and analyzed using descriptive statistics (percentages and tabulations). The software used was SPSS for Windows, version 14.0. The efficacy of anthelmintics was evaluated based on the reduction in faecal egg counts. Calculations of the arithmetic means, percentage of reduction between pre- and post-treatment faecal nematode egg outputs and 95% upper and lower confidence limits for the reduction were conducted according to the method described by Coles et al. (23).

Table 2. Worm control strategies employed for the control of gastrointestinal nematodes in horses within Sokoto metropolis, Nigeria (total of analysed questionnaires n = 40)

Description	No. respondents	Response rate (%)
Parasitic control method		
Anthelmintics	13	32.5
Herbal	5	12.5
Both method	22	55.0
Presence of deworming plan		
Yes	32	80.0
No	8	20.0
Designing of deworming plan		
Horse owner/handler	25	62.5
Veterinarian	10	25.0
Don't have	5	12.5
Basis for deworming		
FEC	0	0.0
Clinical sign	22	55.0
Anytime	18	45.0

RESULTS

Questionnaire survey on management practices

A total of 40 responses were analyzed. All the respondents were male and their age ranged between 13 and 55 years, with 12 (30%) being young adult (<20 years), 19 (47.5%) adult (21-40 years) and 9 (22.5%) were (> 40 years) old. Based on the primary occupation, respondents were horse handlers (47.5%), civil servants (20%) and 32.5% traders. Sixty percent of the respondents had secondary school certificates, 15% were degree holders, 10% had primary school certificates and 15% had no formal education.

Eighty percent of the respondents claimed to have a deworming plan, however, only 32.5% used anthelmintics, while 55% combined the use of anthelmintics and herbal medicines in their worm control strategies (Table 2). According to 62.5% of respondents, designing of the deworming plan was done by the horse owners/handlers, while only 25% relied on veterinarians. Most of the respondents identified helminth infestation by signs of a rough coat, weight loss and presence of worms in faeces.

Table 3. showed the utilization of anthelmintics for the control of gastrointestinal parasites in horses in the study area, with albendazole (80%) being the most commonly used anthelmintic. The common herb used is neem (*Azadirachta indica*) leaves and stem. The neem leaves and/or stem are usually either soaked in water over night or boiled and about 1 to 2 litres/horse of the extract is administered orally. Ninety percent of respondents

Table 3. Utilization of anthelmintics for the control of gastrointestinal parasites in horses within Sokoto metropolis, Nigeria (total of analysed questionnaires n = 40)

Description	No. of answers	Proportion of answers (%)
Dewormer administration		
Horse owner	30	75.0
Horse handler	5	12.5
Paravet	1	2.5
Veterinarian	4	10.0
Dosage determination		
Visual judgement	36	90.0
Weight of individual horse	4	10.0
Weight estimation		
Visual estimation	38	95.0
Weight bands	1	2.5
Body measurements	1	2.5
Selection of anthelmintics		
Familiarity	34	85.0
Low price	1	2.5
Horse handler recommendation	1	2.5
Vet recommendation	4	10.0
Formulation of dewormer		
Drench	35	87.5
Paste	2	5.0
Drench & paste	2	5.0
Drench & Injectable	1	2.5
Frequency of deworming		
Once a year	1	2.5
Twice a year	4	10.0
Thrice a year	4	10.0
Four times a year	7	17.5
Six times a year	21	52.5
None	3	7.5
Alternate use of anthelmintic classes		
I do change	10	25.0
Change occasionally	9	22.5
I don't change	21	52.5

Table 4. Stable management practices employed for the control of gastrointestinal parasites in horses within Sokoto metropolis, Nigeria (total of analysed questionnaires n = 40)

Description	No. of stables	% of stables
Type of feed		
Grains & hay	7	17.5
Grains & grasses	3	7.5
Grains, hay& grasses	30	75.0
Mode of feeding		
Feed on ground	10	25.0
In container on the ground	25	62.5
In a raised container	5	12.5
Feeders cleaning		
Don't clean	9	22.5
Daily&before every feed	16	40.0
Weekly	10	25.0
Anytime	5	12.5
Cleaning drinkers		
Daily	28	70.0
Weekly	2	5.0
Anytime	9	22.5
Don't clean	1	2.5
Stable/manure cleaning		
Daily	19	47.5
Weekly	1	2.5
Anytime	20	50.0
Use of bedding		
Yes	15	35.0
No	25	62.5

use visual judgement in dosage determination in case of anthelmintic. Seventy five percent of the respondents, who are horse owners and 12.5% who are horse handlers administer the anthelmintics by themselves. According to 85% of the respondents, anthelmintics selection is based on familiarity and is usually in drench form. Fifty two point five percent of the respondents deworm their horses six times a year using either anthelmintic or herb, but they do not change the class of the anthelmintics used in their stables. Furthermore, they had no schedule on the choice of the type of dewormer (chemical anthelmintic or herb) to be used at any deworming period.

Based on the stable management practices employed (Table 4), daily cleaning of stables or

Table 5. Faecal egg count reduction test in horses naturally infected with gastrointestinal nematodes from 3 stables within Sokoto metropolis, Nigeria

Treatment groups	A (n=5)	B (n=5)	C (n=5)
Pre-treatment mean EPG	1850	1750	1300
Post-treatment mean EPG	700	0	100
FECR (%)	62.2	100.0	92.3
Upper 95% Cl (%)	78.0	-	98.0
Lower 95% Cl (%)	36.0	-	72.0
Interpretation	**Resistant**	**No resistance**	**Resistance suspected**

Group A = Albendazole; Group B = Ivermectin; Group C = Fenbendazole; Mean EPG = arithmetic mean of faecal nematode egg counts; FECR = faecal egg count reduction

Table 6. Infective larva (L3) from faecal cultures from the horses before and after treatment

Infective larva (L3)	A		B		C	
	Pre-treat	Post-treat	Pre-treat	Post-treat	Pre-treat	Post-treat
Strongylus vulgaris	Yes	Yes	Yes	No	Yes	Yes
Strongylus edentatus	No	No	Yes	No	No	No
Strongylus equinus	Yes	Yes	No	No	Yes	Yes
Cyathostomum spp type A	Yes	No	Yes	No	No	No
Cyathostomum spp type C	Yes	Yes	Yes	No	Yes	Yes
Cyathostomum spp type D	No	No	Yes	No	No	No
Cyathostomum spp type G	Yes	Yes	No	No	Yes	Yes
Gyalocephalus capitatus	Yes	No	No	No	No	No
Trichostrongylus axei	Yes	No	No	No	Yes	No

Group A = Albendazole; Group B = Ivermectin; Group C = Fenbendazole

manure was not regular. Daily cleaning of the feeding and watering container was practiced by 40% and 70% of the stables respectively. The use of sand bedding is a common practice among 62.5% of the stables.

Anthelmintic efficacy test and larval identification

The result of faecal egg count reduction tests (FECRT) is shown in Table 5. Nematode infective larvae (L3) recovered in pre- and post-treatment pooled faecal cultures of all treatment groups during the FECRT are presented in Table 6. The majority (90.26%) of the larvae in the culture were *Cyathostomum* spp. The mean percentages of other infective larvae identified were *Strongylus vulgaris* (2.96%), *S. edentatus* (2.02%), *S. equinus* (3.19%), *Trichostrongylus axei* (4.37%) and *Gyalocephalus capitatus* (3.20%). Albendazole and fenbendazole were unable to kill most of the large and small strongyles identified, while they were all susceptible to ivermectin.

DISCUSSION

The need to minimize parasitic diseases, as well as to address the threat of wide spread anthelmintic resistance in equine nematodes necessitates a change in the approach to control parasitic infestation in order to preserve anthelmintic efficacy. This study therefore provides information on the management practices employed and about the efficacy of albendazole, fenbendazole and ivermectin on GI nematodes of horses within Sokoto metropolis.

The use of anthelmintics and herbal remedies were the main parasitic control method employed in the study area. Although the efficacy of some of these herbal remedies against ruminant nematodes has been tested *in vivo* and *in vitro* (24, 25), their efficacy in horses has not been published from the study area to the best of our knowledge. The effectiveness of neem against parasites was attributed to the compound called azadirachtin. Azadirachtin interferes with the central nervous system of parasites via inhibition of excitatory cholinergic transmission and partly blocks the calcium channel resulting in expulsion of parasites from the body of hosts (26, 27). This is the same mechanism employed by some of the anthelmintics used. A higher percentage of the respondents had a deworming plan, however, only 25% relied on veterinarians in designing the plan. This could be the reason why most deworming is done at any convenient time of the year or following manifestation of clinical signs as indicated by a larger percentage of respondents. Despite the presence of anthelmintic resistant nematodes for several decades, and the importance of faecal egg count in the targeted dosing method of helminth control, there has been concern regarding poor dissemination of this information among equine veterinarians and horse owners (28). Low level of veterinary involvement in parasite control plan was also reported in a majority of racing yards in the UK (29). This finding possibly contributed to the non-implementation of FECs, which was evident in this present study, with no single respondent using FEC test in designing their worm control strategies. More so, neither systematic drenching nor FEC-based drenching systems was practiced by any of the respondents. One can therefore imply the already described reluctance of ruminant breeders to implement selective drenching (30, 31) to apply for horse owners too. It is therefore important for

veterinarians to provide more integrated parasite control advice to the horse owners and handlers.

The respondents based selection of anthelmintics on familiarity with the drug and majority of them gave a particular type or class of anthelmintic. The administration of the dewormer, which was usually based on visual weight estimation, was largely performed by the horse owners and handlers. This is in accordance with previous studies which showed that objective weight estimation was under-utilized, with 87 to 94% of the owners relying on visual estimation of weight alone (8, 32). The practices of self-treatment of horses by the horse owners and/or handlers may be the reason for their selection and buying of anthelmintics formulation for oral route administration which is easy to administer. More so, the need to pay an added value to veterinarians may become pointless as the importance given to costs of veterinary care is one of the most important obstacles among pet owners to veterinary visits (33). This practice could obviously lead to treatment failure as a result of inappropriate dosing due to underestimation of the live weight. Also, considering the frequency of deworming per year with a particular anthelmintic type and wrong dosing, development of resistance to those particular classes of anthelmintics is not very unlikely.

The management practices, particularly the type of feed, mode of feeding and inconsistence in the cleaning of feeder and stable/ manure, employed by most of the respondents may also encourage high load of parasites in the environment as previously reported (34). In addition, studies from different parts of the world and Nigeria in particular have documented helminth diseases as the most prevalent disease in horse population, of which strongyles had the highest prevalence (2, 3, 4, 13, 14, 15). Previous coprological analysis of horses from Sokoto-Nigeria revealed a prevalence of 84.4% for helminths, with 75.5% being strongyles (12). Sixty eight point eight percent prevalence rate for strongyles was reported in Kaduna, Nigeria (35), while Biu et al. (36) reported two major helminths: *Parascaris equorum* (48.6%) and *Strongyles* (42.9%) in Maiduguri, Nigeria. The percentage prevalence of larvae in the culture are similar with what was reported by previous researchers, particularly with cyathostomins being the most common species in horse population (4, 37, 38).

The use of herbal remedies (neem) may also contribute to the high burden of the nematodes in the horses examined and possibly encourage resistance to common anthelmintics. This is because most of the respondents had no schedule on which dewormer (anthelmintic or herb) is to be used at any deworming period, effectiveness of the neem extract used is not known, and probably horses may not be receiving required doses. More so, studies have showed varying degree of efficacy of neem leaves on equine helminths. Ali (39) documented 53.6% reduction against *Gastrodiscus* spp, while Mahboob et al. (40) reported 6.89% and 8.62% reduction on day 7 and 14 post treatment and Yadav (41) reported 21% reduction for strongyles.

The anthelmintic efficacy screening showed that albendazole and fenbendazole were unable to kill most of the large and small strongyles identified, while their susceptibility to ivermectin was 100%. Although the most pathogenic species of large strongyle (*S. vulgaris)* was identified, however, no clinical signs due to its infestation were observed in any of the studied animals.

The resistance of most nematodes identified in this study to albendazole and to a lesser extent fenbendazole is a true reflection of what was observed in the questionnaire-based survey, where the choice of anthelmintic selection among horse owners or handlers was largely dependent on familiarity with a particular anthelmintic and practice of using a particular type or class of anthelmintic (benzimidazoles). The low efficacy observed with fenbendazole was a bit higher than what has been reported by in other studies from other parts of the world (8, 42, 43, 44). The resistance of nematodes in horses to albendazole in this study, with an efficacy value of 36%, was very low when compared with previous studies (7, 45). Contrary to our findings on the efficacy of ivermectin, some studies reported anthelmintic resistance against macrocyclic lactones, particularly of small strongyle (46, 47, 48). However, reduction in naturally infected horses observed in this present study confirms that ivermectin has excellent anthelmintic efficacy against many species of GI nematodes of horses as previously reported (48, 49). Also considering the management practices (feeding on grains, hay and grasses in containers on the ground, weekly cleaning of feeders and daily clearing of manure) used as inclusion criteria for the selected stable and the EPG recorded pre- and post-treatment, we strongly believe the differences in efficacy observed in this study are due to the anthelmintics used and the stable management has no influence on the outcome of the study.

CONCLUSION

To the best of our knowledge, this is the first study of management practices and anthelmintic efficacy in horses conducted in Sokoto, north-western region of Nigeria. The worm control strategies employed by horse owners and/or handlers possibly enhance the selection of nematodes resistant to albendazole and fenbendazole, while a single dose of subcutaneous injection of ivermectin was highly effective against gastrointestinal parasites in horses. The resistances observed were associated with risks of under dosing, continued use of a class of anthelmintic irrespective of its efficacy status, as well as non-use of FECs and veterinary involvement in the control of parasites.

CONFLICT OF INTEREST

The authors declared that they have no potential conflict of interest with respect to the authorship and/or publication of this article.

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