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Effectiveness of ivermectin and moxidectin against cyathostomins in four horse breeding farms in Mexico

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Abstract

This study aimed to evaluate the effectiveness of oral ivermectin and moxidectin against natural cyathostomin infection in four horse farms located in the central regions of Mexico. Of the 445 horses: Warmblood (145), Thoroughbreds (100), and Quarter Horses (200) breeds, aged between 6 months and 27 years, were used. Data on horses and parasite control methods were collected through interviews with farm owners and veterinarians. Using the McMaster technique, fecal samples were processed from all 445 horses, 180 of which were positive for cyathostomins. On each farm, a selection was made of 45 animals meeting the criteria of a Faecal Egg Count Reduction Test yielding results exceeding 150 eggs per gram of strongylid-type nematodes. Subsequently, three separate experimental groups were formed for each farm, each consisting of 15 horses The first group was treated with oral ivermectin 1.87 %; the second group with oral moxidectin 2 %; and the third was the non-treatment control group. Coprocultures were also performed to identify the presence of nematode species. The data obtained were analyzed with RESO.exe©. Three of the four farms achieved a 100 % reduction in eggs per gram with both macrocyclic lactones. One farm achieved 93 % reduction with ivermectin and 87 % with moxidectin. This study demonstrates that macrocyclic lactones effectively reduce cyathostomins in three of the four farms studied. The results suggest potential cyathostomin resistance to macrocyclic lactones, particularly moxidectin, on one farm. Given these findings, sustainable parasite management is required on horse breeding farms in Mexico.

Keywords: Cyathostomins; Ivermectin; Moxidectin; Anthelmintic resistance; Horses.

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Study contribution

Cyathostomins are the most abundant nematodes of horses worldwide and may represent an emerging anthelmintic resistance problem. In Mexico, ivermectin (IVM) and moxidectin (MOX) are effective against cyathostomins populations. This study is the first report in Mexico of suspected MOX resistance to cyathostomins infestation in horses. More studies on the chemical resistance of parasites in horses are required.

Introduction

Cyathostomins are the most frequently reported nematodes in horses, and anthelmintics (AH) have been the main method used to control them.[1, 2] In Mexico, the total estimated equine population is over 6.3 million[3] It has been assessed that only 300 000 horses receive nutritional and medical care (B. Monroy-Hérnandez, personal communication, October 21th, 2021). Approximately, 150 000 horses receive basic treatments including AH, and 45 000 comprise the high-performance group, which is subjected to more continuous deworming (either monthly or every other month) with macrocyclic lactones (ML), as they offer a pharmacologically approved endectocide action.[4]

Regular and non-technical use of AH, derived from customary clinical practice, has favored the selection of cyathostomin populations capable of surviving, thus promoting the anthelmintic resistance (AHR) phenomenon.[5, 6] For more than 50 years, horses have been conventionally dewormed using high-intensity short-term schemes. This practice originally served the purpose of eliminating the somatic larvae of *Strongylus vulgaris*, which causes arteritis and aneurysms in horses.[7, 8] Although it is not common to find serious cases of *S. vulgaris*, these deworming practices have prevailed, subjecting the cyathostomin populations to high selection pressure, enhancing resistant or multi-resistant parasitic populations because of decreased AH effectiveness.[9, 10] While worldwide resistance to ML has not been sufficiently reported, the consequences of AHR are potentially serious because of the scarce treatment options for horses with severe parasite infestation.[11-14]

Materials and methods

Ethical statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Feces were obtained while farm veterinarians were performing breeding evaluations or from the ground in the case of younger animals. As well as the usual application of deworming pastes. No handling involved stress or injury to the animals.

The type of study and farm location

A longitudinal cohort study with convenience sampling was conducted in four horse breeding farms located in the central and central-western regions of Mexico, specifically in the states of San Luis Potosi (farm 1), Guanajuato (farm 2), both with

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a dry climate BSw Köppen[15] scale and the State of Mexico (farms 3 and 4) with a temperate-sub-humid climate: Cw Köppen scale.[15]

Animals

The study was conducted using a cohort of 445 horses distributed as follows: farms 1 and 3 held a total population of 70 and 75 racing Thoroughbreds, respectively; farm 2 held 100 racing Quarter Horses and farm 4 held 200 show jumping Warmbloods with ages ranging from birth to 27 years (Table 1).

Questionnaire

Interviews were conducted with horse-owners and veterinarians of four farms to retrieve information about the breed, age, intended use, frequency of AH administration, type of AH used, and criteria used for parasite control in the past seven years. Geographic location (municipality and state) and climate were also recorded (Table 1).

Table 1. Farm and horse information and deworming programs at four breeding farms in central and west-central Mexico

Farm	Farm municipality/ State	Farm climate	Total horse population	Intended use	Breed	Frequency of deworming (times year)	Anthelmintics used*	Main criteria
1	San Luis Potosí, SLP	Dry (BSw)	70	Racehorses	Thoroughbred	3 o 4	IVM, FBZ, IVM+PRZ MOX	AH rotation, MOX only foals (70 days old)
2	Sierra de Lobos, Guanajuato	Dry (BSw)	75	Racehorses	Quarter Horses	4	IVM, FBZ, MOX, IVM + PRZ MOX + PRZ	Every season
3	Teoloyucan, State of Mexico	Temperate-humid (Cw)	100	Racehorses	Thoroughbred	12: 8-10**	IVM, MOX, IVM + PRZ MOX + PRZ FBZ, PRZ, PYR,	Keep animals free of parasites
4	Valle de Bravo, State of Mexico	Temperate-humid (Cw)	200	Showjumping	Warmblood	6	IVM, MOX, IVM + PRZ MOX + PRZ FBZ	Keep animals free of parasites

BSw and Cw = Köppen scale[15]
*Anthelmintics used in the last seven years.
**Times of the year in which macrocycle lactones (ML) were used.
IVM: ivermectin; MOX: moxidectin; FBZ: fenbendazole; PRZ: praziquantel; PYR: pyrantel pamoate

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Faecal Egg Count Reduction Test

No AH had been administered to the horses for at least 60 days before this study. The effectiveness of IVM and MOX against cyathostomins was determined with a Faecal Egg Count Reduction Test (FECRT) (methodology approved by international organizations).[16] It consists of three stages and its rationale is to measure the reduction of the egg count per gram (EPG) after AH treatment (Figure 1).

Stage 1: Pre-treatment

Fecal samples of the total population of horses (n = 445) were processed using a modified McMaster quantitative technique with a sensitivity index of 50 EPG.[17, 18] Samples of 2g of feces were homogenized in 28 mL of saturated NaCl solution of 1.25 density. The solution was then filtered and transferred to a McMaster chamber to perform an EPG count using an optical microscope (10×) to determine the parasite load. Forty-five horses with a fecal egg count reduction test ≥ 150 eggs per gram of strongylid-type nematodes and who were 6 months or older were randomLy selected per farm.

Stage 2: Treatment

A total of 180 horses (45 horses per farm) were included in the treatment phase. On each farm, three groups of 15 horses were randomLy selected (Figures 1 and 2). The first group was treated with a single dose of IVM 1.87 % oral paste (200 µg/kg BW); the second group was treated with a single dose of MOX 2 % oral gel (400 µg/kg BW); and the third group was the non-treatment control group (Figure 1). The weight was estimated using a morphometric tape according to Wright's recommendations.[19]

Stage 3: Post-treatment

McMaster's modified quantitative technique[20] was performed in all animals (n = 180) fourteen days after treatment (Figure 1). Two assumptions were considered as part of the methodology: 1) if the result was SUSCEPTIBLE, the test would not be repeated and 2) if the result was RESISTANT, the test would be repeated (Figure 2).

Larval culture

Larval culture was performed in fecal samples preserved at 4 °C from stage 2 (treatment) and stage 3 (post-treatment) of all 180 horses (Figure 1). Following the technique of Corticelli and Lai,[21] larvae were collected using a Baermann device to identify the nematode genera.[2, 22] Larval viability was analyzed following two criteria for species counting and identification: 1) larvae were shed in their infective stage (L3), and 2) larvae had vigorous motility. A total of 600 larvae were analyzed per farm, following the recommendations and taxonomic keys of Santos et al.[2] and Bevilaqua et al.[22] to classify the species found.

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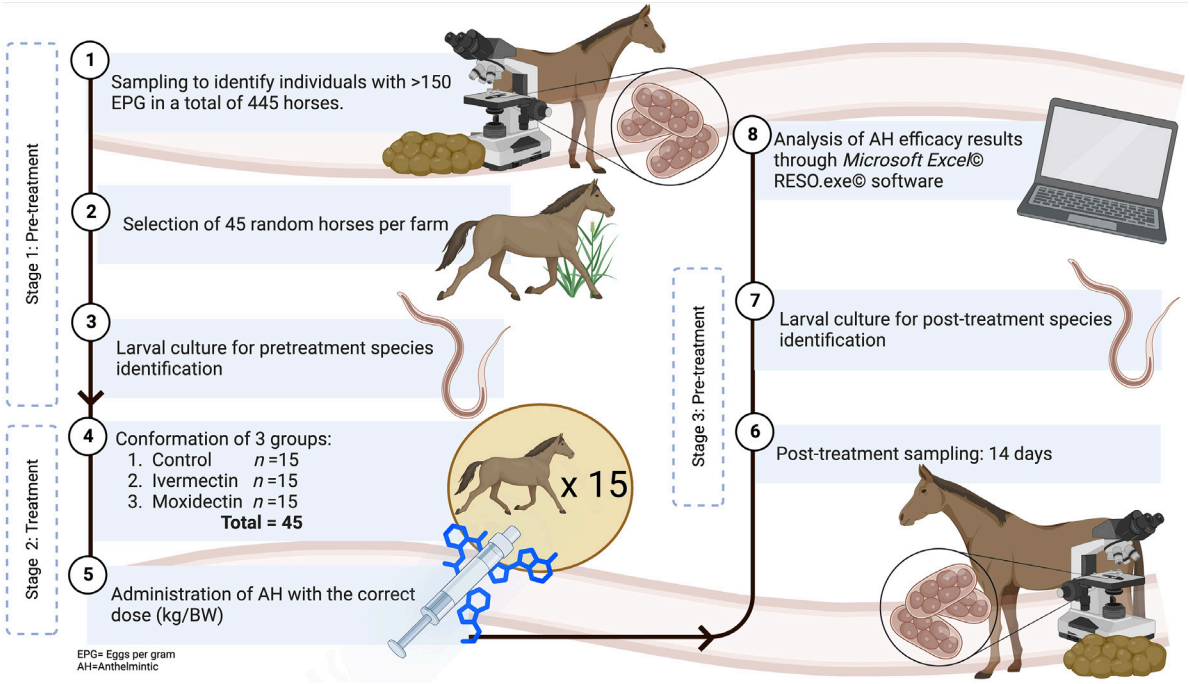

Figure 1. Diagram of the Faecal Egg Count Reduction Test (FECRT) methodology applied on each farm (Created with BioRender.com).

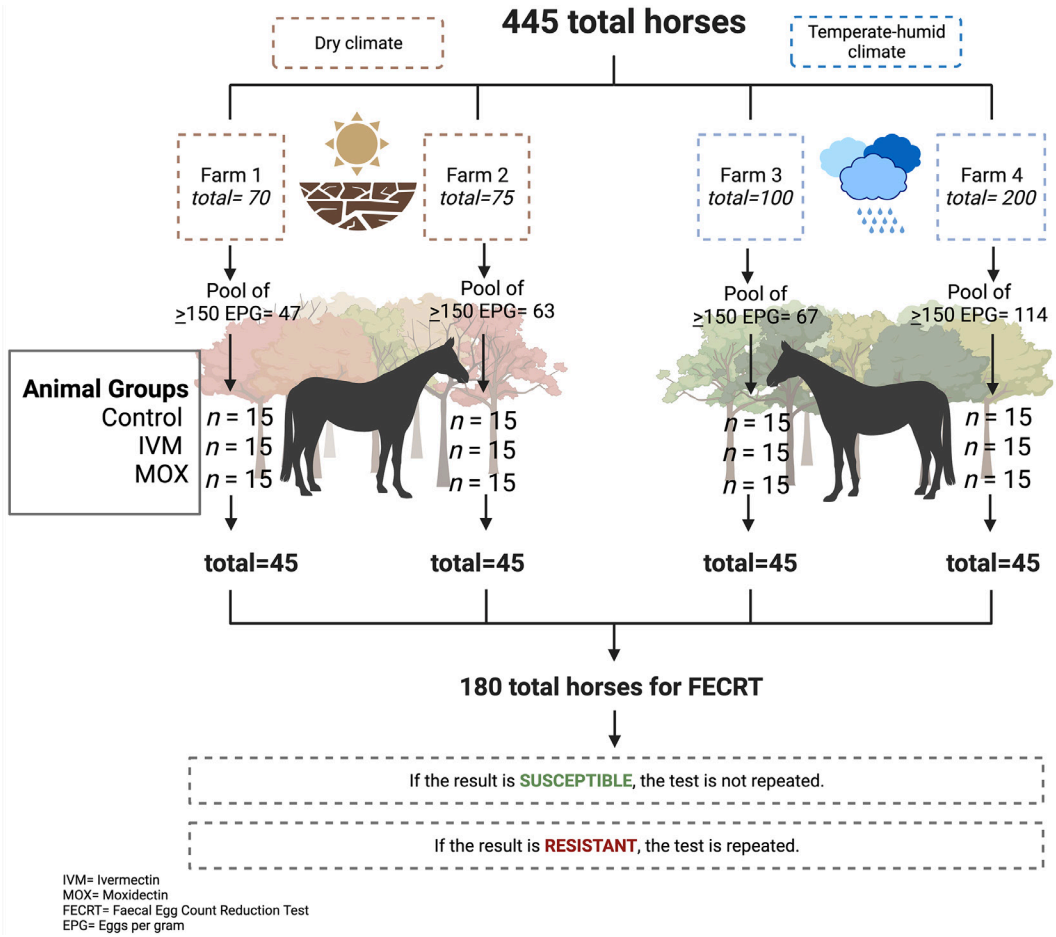

Figure 2. Progressive sampling scheme, the selection of animals, and distribution of experimental groups by farm and region (Created with BioRender.com).

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Table 2. Animals that met the selection criteria of ≥ 150 EPG on each farm and from which they were selected for stage 2

Farm	Total horse population	<150EPG	≥150EPG	Population selected for FECRT
1	70	23	47	45
2	75	12	63	45
3	100	33	67	45
4	200	81	114	45
Total	**445**			**180**

FECRT= Faecal egg count reduction test
EPG= Eggs per gram

Data analysis

The data obtained were analyzed using the program RESO.exe©; CSIRO, 1993, Animal Health Division of Microsoft Excel©. Data were considered RESISTANT when the percentage of reduction was < 95 %.[23]

Results

Questionnaire

Basic information about the farms (location and climate), total horse population, individual horse information (breed and intended use), and deworming program (frequency, type of AH used, and main criteria) was gathered from horse-owners and farm veterinarians (Table 1).

Faecal egg count reduction test

The results of stage 1 performed in the total population (n = 445) are shown in Table 2. The percentage of horses that had ≥ 150 EPG were farm 1, 67.1 % (47/70); farm 2, 84 % (63/75); farm 3, 67 % (67/100), and farm 4, 57 % (114 /200). The population for the next stage of the study was randomLy selected (45 horses per farm).

The effectiveness of IVM and MOX against strongylid-type nematode populations is shown in Table 3 and Figure 3. Farms 1, 2, and 4 showed 100 % efficacy for both MLs, indicating that the nematodes were susceptible to IVM and MOX molecules. The arithmetic mean of the EPG released pre- and post-treatment, showed the effectiveness of both MLs only in farms 1, 2, and 4 (Figure 4). A lack of efficacy due to parasite resistance (93 % for IVM and 87 % for MOX) was observed on farm 3. Therefore, six months after the first test, a second FECRT was performed on farm 3 (3b) (Table and Figure 3) according to the American Association of Equine Practitioners (AAEP) guidelines.[24]

No horse on farm 3 received any anthelmintic treatment during that 6-month period while starting the second trial. With the same initial population (100 horses) at that moment 72 animals met the same inclusion criteria for testing (≥ 150 EPG). RandomLy, 15 horses were treated with oral IVM 1.87 %; 15 horses with oral MOX 2 % at the same initial dosages, and the third group of 15 horses was the non-treatment control group (Figure 3). The effectiveness of 100 % for IVM and 94 % for MOX was then observed (Table 3, Figures 3 and 4).

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Table 3. Ivermectin and moxidectin effectiveness in four horse-breeding farms in different ecological regions

Farm		Ivermectin 1.87 %			Moxidectin 2 %		
		Effectiveness	LCI	UCI	Effectiveness	LCI	UCI
1		100 %	100	100	100 %	97	100
2		99 %	98	100	100 %	100	100
3	3a	93 %	52	99	87 %	12	97
	3b	100 %§	100	100	94 %§	84	100
4		100 %	100	100	100 %	100	100

§Resistance corroboration after six months
LCI = Lower confidence interval
UCI = Upper confidence interval

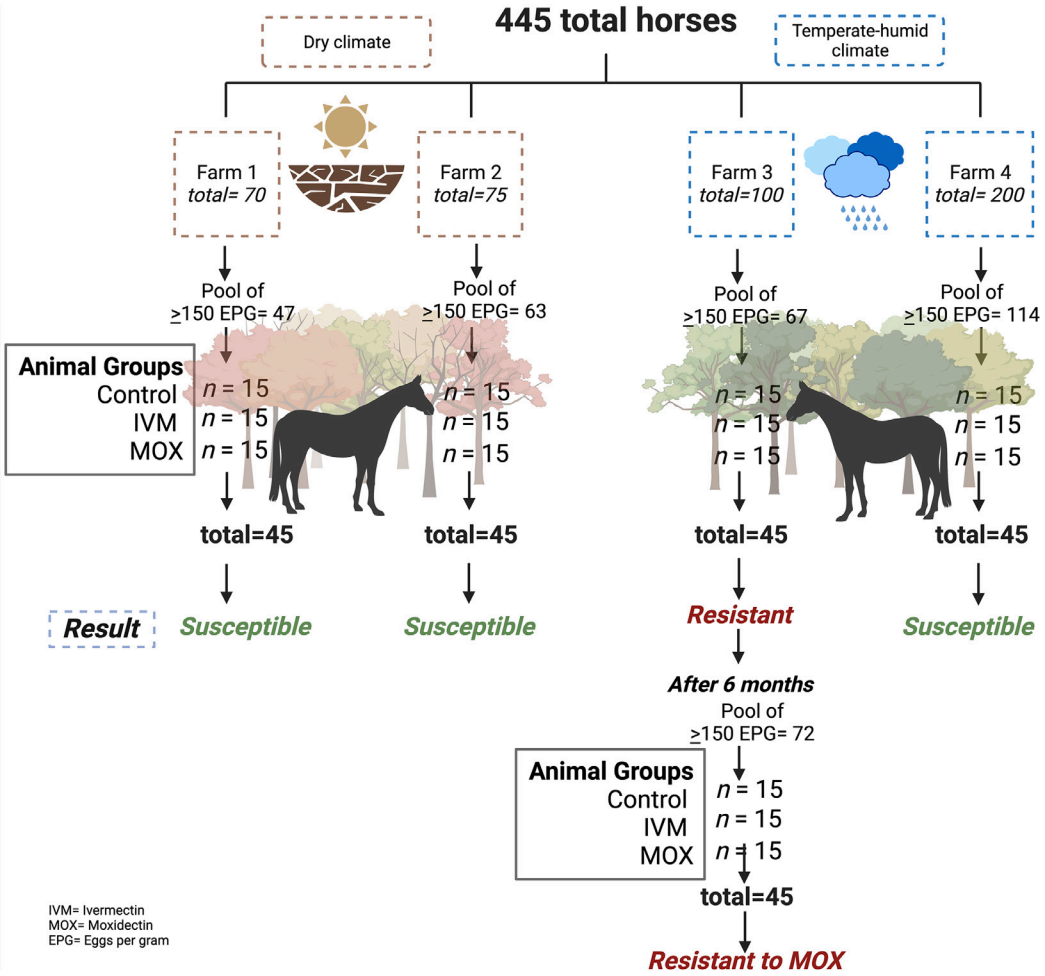

Figure 3. Diagram of Faecal Egg Count Reduction Test (FECRT) results showing susceptibility and resistance to ivermectin and moxidectin in farms one to 4 (Created with BioRender.com).

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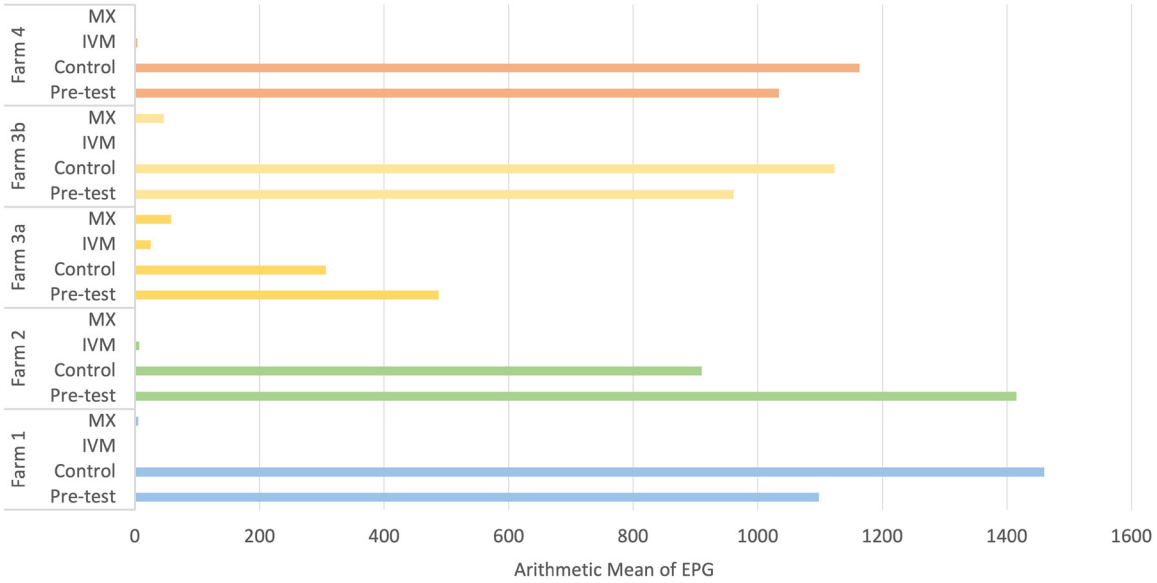

	Farm 1				Farm 2				Farm 3a				Farm 3b				Farm 4			
	Pre-test	Control	IVM	MX	Pre-test	Control	IVM	MX	Pre-test	Control	IVM	MX	Pre-test	Control	IVM	MX	Pre-test	Control	IVM	MX
Mean	1098	1460	0	5	1416	910	7	0	488	306	25	58	961	1123	0	47	1034	1163	3	0
SD	1090	1444	0	16	1090	1037	18	0	379	276	66	97	788	817	0	117	588	828	13	0
Max	4100	4900	0	50	4150	3250	50	0	1450	1150	250	250	2950	2450	0	450	2800	3000	50	0
Min	150	50	0	0	150	0	0	0	150	0	0	0	150	150	0	0	150	200	0	0
Median	650	1375	0	0	1150	200	0	0	325	275	0	25	700	1150	0	0	950	950	0	0

IVM: ivermectin; MOX: moxidectin.

Figure 4. Pre- and post-treatment arithmetic mean of eggs per gram (EPG) from four horse-breeding farms.

Larval culture

In both regions (dry and temperate-humid climate), 2 400 L3 (600 per farm) were identified, and 100 % of these larvae were of the cyathostomin group (non-migratory strongyles) in the pre-treatment and post-treatment samples. The morphological characteristics found were sheathed larvae with long and acute larval tails and 6–8 intestinal cells. No larvae with 18 or more than 20 intestinal cells or morphology suggestive of migratory strongylids, such as *Strongylus* species, were found.

Discussion

Results from this study show that the AHR phenomenon in the central and central-western regions of Mexico occurs mainly due to non-migratory strongylid populations. Only larvae of the cyathostomin group or non-migratory strongylids were found in all four farms. Migratory strongylids need a prepatent period of at least six months, and when deworming is frequent (three to four times a year), the development of the larvae to an adult stage is negatively affected, probably due to the high susceptibility of these nematodes to ML, as mentioned by Grice et al.[24] In addition, we found low or no prevalence of migratory strongylids such as *Strongylus* spp., which coincides with previous studies.[7, 25]

Equine cyathostomosis could be caused by at least 53 different species, and approximately 11–15 species have a higher prevalence in the cecum and large colon.[26] Due to the abundance and richness of cyathostomins, AH efficacy tests

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may not consider this a significant factor, as shown in this study. FECRT is a field test, not a molecular or serological test. It has not been possible to distinguish between species of cyathostomins in equines through coproculture, and therefore, the resistance that each of their species may present is not detected. This can be evidenced in nematode species of ruminants. All these studies were initiated in these animal species and not in equines.

The results obtained are limited because we cannot distinguish between several cyathostomin species. However, it is the test recommended by international organizations.[4, 6, 10, 16] Serology, molecular biology, and proteomics technologies need to be developed and implemented for the identification of these species in horses.[6, 19, 27] In addition, factors such as the age of the horses, the time of the year related to early or late L3, cyathostomins hypobiosis processes, hosting types, feeding practices, and group randomization procedures, must be considered, as mentioned by Nielsen et al.[28] The inadequate management of farm 3 while administering ML without any established criteria or pretreatment diagnosis resulted in the lack of effectiveness of MOX. The continuous use of IVM and MOX molecules resulted in a greater number of resistant strongyles-type nematodes (Figure 4).

The effectiveness of IVM (93% in the first test and 100% in the second) (Table 3 and Figure 3) highlights the existence of horses with parasite resistance to this endectocide (Figure 4). the confidence intervals of both samples [CI 52–99 (3a) and CI 12-97 (3b)] indicate that only a couple of animals were excreting resistant nematodes and must be treated as potential high shedders. ML can continue to be used in this farm if strategies to preserve both molecules are implemented. In addition, to avoid high shedders spreading resistant parasites in the pasture, a resistance management strategy (refuge) could be implemented.

This strategy involves deliberately allowing the survival of cyathostomin populations that have not been recently exposed to any treatment. The progeny of unselected parasites provides a source of susceptible nematodes that can dilute resistant nematodes that survive AH, thereby reducing the rate of AHR development. In farm 3, two fecal samples were obtained six months apart; therefore, animals were grazing in different areas when each sample was obtained. This would explain why in the first FECRT, the results showed lower and upper limits pointing toward IVM resistance (Table 3 and Figure 3), whereas in the second FECRT, the animals were in a different pasture that probably contained IVM-susceptible nematodes.

A similar phenomenon probably occurred during the MOX test (3b), with the only exception being that although the effectiveness of the molecule showed a slight increase, most of the nematode resistance prevailed (Table 3 and Figure 4). In this sense, the appearance of the impending AHR is a factor that should lead to immediate action to change deworming practices, with strategic and selective deworming being a possible solution to the problem.[29] Since the use of AH remains the irreplaceable method in terms of efficacy and practicality, every horse farm should first monitor the need for treatment and subsequently its effectiveness by monthly coproparasitological analysis.

Macrocyclic lactone resistance to cyathostomins was first reported in 2005.[30] Since then, multiple cases of this resistance have been reported in Italy,[31, 32] France,[14] Germany,[32] England[32] and Lithuania.[14] In Latin America, ML effectiveness studies have been conducted mainly in Brazil[12, 30] and Mexico.[33] Canever et al.[12] evaluated the effectiveness of IVM and MOX against cyathostomins

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and found levels of 5-65 % and 16 %, respectively. Rosado-Aguilar et al.[33] reported 60 % resistance to IVM in five horse farms located in the southern region of Mexico in 2014. To the best of our knowledge, no other AHR studies have been published in Mexico.

We believe that the AHR phenomenon could be an ongoing health problem in several farms in Mexico mainly due to misuse and overuse of ML because of the ease of acquiring deworming products by horse owners without a veterinary prescription and its frequent administration to treat any type of parasites without a proper diagnosis. This information is fundamental to limiting the negative impact on animal health and welfare and constitutes the first step for the rational use of AH. In central and central-western Mexico horse breeding farms, it is necessary to implement integrated parasite management (IPM), which is a non-renewable and effective resource to amplify the effectiveness of ML. To achieve this objective, it is necessary to include actions such as: 1) strategic deworming based on coproparasitoscopic diagnosis, 2) improvement of feeding management practices, 3) manure management in the pasture to reduce the source of contamination, and 4) biological control methods, such as pathogenic fungi, bacteria, and mites or entomopathogenic nematodes.[6, 34, 35] IPM is an approach to a strategy that may change consciousness toward horse breeding farms under sustainable tools.

Conclusions

The present study demonstrates high levels of effectiveness of IVM and MOX in treating horses against cyathostomins; however, we report an increasing and impending resistance to MOX in one farm. IPM of nematodes is mandatory for prolonging the effectiveness of ML in the region.

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Conflicts of interest

The authors of the present paper declare that we have no actual or potential conflict of interest that could inappropriately influence the work presented.

Author contributions

Conceptualization: C Martínez-Ortiz-de-Montellano, RI Rodríguez-Vivas.
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Formal analysis: C Martínez-Ortiz-de-Montellano, G Galicia-Velázquez.
Funding acquisition: C Martínez-Ortiz-de-Montellano.
Investigation: C Martínez-Ortiz-de-Montellano, G Galicia-Velázquez.
Methodology: C Martínez-Ortiz-de-Montellano, RI Rodriguez-Vivas, JA Rosado- Aguilar.
Project administration: C Martínez-Ortiz-de-Montellano.
Resources: C Martínez-Ortiz-de-Montellano.
Software: C Martínez-Ortiz-de-Montellano.
Supervision: C Martínez-Ortiz-de-Montellano, RI Rodriguez-Vivas, JA Rosado-Aguilar.
Validation: RI Rodriguez-Vivas.
Visualization: Rodriguez-Vivas, JA Rosado-Aguilar.
Writing-original draft: G Galicia-Velázquez.
Writing-review and editing: C Martínez-Ortiz-de-Montellano, RI Rodriguez-Vivas, JA Rosado-Aguilar, G Galicia-Velázquez.

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