Parasitology Research (2019) 118:2877–2883
https://doi.org/10.1007/s00436-019-06423-6

HELMINTHOLOGY - ORIGINAL PAPER

The importance of anthelmintic efficacy monitoring: results of an outreach effort

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Received: 21 April 2019 / Accepted: 7 August 2019 / Published online: 17 August 2019

Abstract

Anthelmintic resistance in equine cyathostomin parasites is widespread. A surveillance-based parasite control program using fecal egg counts (FECs) and fecal egg count reduction tests (FECRTs) to decrease anthelmintic use and monitor treatment efficacy is recommended. The purpose of this study was to examine shifts in equine parasite control program management practices via a short course presented by the Penn State Extension, and to highlight how data collected from these programs is useful for monitoring anthelmintic efficacy on a large scale. Horse owners were enrolled after participating in a short course and filled out questionnaire surveys about their parasite management programs pre and post study, horse information, and farm information. FECs were performed at three time points, and horses above a 300 strongyle eggs per gram cut-off were treated with pyrantel pamoate, fenbendazole, or ivermectin. Two weeks post-treatment, FECRTs were performed to determine treatment efficacy, which included 29 farms with 513 individual treatments. Prior to the study, only 30.6% of farms used FECs, but after the study, 97.3% of farms said they would use FECs in the future. Horses were given an average of 4.1 anthelmintic treatments per year before the study, and post study 89.2% of farms were able to reduce the number of anthelmintic treatments used. Fenbendazole was effective on zero farms, pyrantel pamoate on 7.4% of farms, and ivermectin on 92.9% of farms. This outreach project helped generate information about anthelmintic efficacy levels, causing a shift in practices on participating farms, and collected useful anthelmintic resistance data.

Keywords Horses · Strongyle · Fecal egg count · Anthelmintic resistance · Education

Introduction

Strongyle parasites are found in equids worldwide and are the main focus of modern anthelmintic treatment programs for adult domestic horses. Large strongyles, such as the highly pathogenic *Strongylus vulgaris*, are no longer commonly found in domestic horses (Herd 1990; Nielsen et al. 2012). This has led the focus of anthelmintic treatment programs to shift to the less pathogenic but more common and abundant cyathostomin parasites (Kaplan and Nielsen 2010; Nielsen et al. 2019). Mass emergence of cyathostomin larvae from mucosal cysts in the large intestine can cause larval cyathostominosis, a disease characterized by watery diarrhea, dehydration, and ventral edema that has a case-fatality rate of around 50% (Reid et al. 1995; Love et al. 1999; Peregrine et al. 2006).

Traditional anthelmintic treatment programs utilized fixed intervals for treatment while rotating drug class, which led to widespread overuse of anthelmintics that has been associated with emergence of anthelmintic resistance in cyathostomin parasites (Biggin et al. 1999; Kaplan et al. 2004; Peregrine et al. 2014; Smith et al. 2015). Benzimidazole and pyrimidine resistance have been reported all over the world in addition to several reports of shortened egg reappearance periods for both ivermectin and moxidectin (Traversa et al. 2009; Rossano et al. 2010; Peregrine et al. 2014; Kumar et al. 2016; Bellaw et al. 2018). The current status of anthelmintic efficacy in domestic horses is concerning to both scientists and horse owners, particularly because no new drug classes or modes of action have been introduced since ivermectin in the 1980s.

Section Editor: Hiroshi Sato

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Due to the lack of new drugs, it is absolutely essential to monitor current trends in anthelmintic resistance to keep those in the equine industry informed as well as preserve current anthelmintics for as long as possible via surveillance-based methods (Kaplan and Nielsen et al. 2010).

Anthelmintic resistance occurs on a population level, which in equine parasites typically means individual farm populations; however, regional studies of multiple populations can help indicate overall efficacy for different drug classes. This can be particularly useful for horse owners and veterinarians in a region or state for developing proper anthelmintic treatment programs. Pennsylvania ranks number eight in the USA for total number of horses, and the equine industry has an economic impact of $1.7 billion, providing over 43,000 jobs in the state (American Horse Council Foundation 2018). Despite the large impact of horses on the state economy, limited studies regarding anthelmintic efficacy have been conducted in Pennsylvania. One study on the Mid-Atlantic region of the USA included Pennsylvania, but data was not analyzed down to the state level (Smith et al. 2015). The National Animal Health Monitoring System (NAHMS) Equine 2015–2016 study also included Pennsylvania, but the state was included as part of the Northeast region for data analysis, so no state-specific information could be determined (Nielsen et al. 2018b). There have been no studies on the state of Pennsylvania alone or analyzing state data separately conducted regarding anthelmintic efficacy.

The American Association of Equine Practitioners (AAEP) recommends using fecal egg counts (FECs) for surveillance-based anthelmintic treatment programs, where only those horses above an arbitrary cut-off—typically 200 eggs per gram (EPG)—are treated (Nielsen et al. 2019). Additionally, another FEC can be performed 2 weeks post-treatment for use in a fecal egg count reduction test (FECRT), where percent reduction in egg shedding is calculated in order to determine treatment efficacy (Nielsen et al. 2019). While egg shedding does not correlate to worm burden, egg shedding indicates the presence of adult parasites, and reducing the number of eggs shed reduces infection pressure on the pasture (Nielsen et al. 2010). The FEC and FECRT are useful tools for both monitoring individual anthelmintic treatment programs, as well as overall surveillance of anthelmintic efficacy.

Outside of scientific publication, parasitologists and veterinarians use magazines, equine websites, and the AAEP guidelines to encourage widespread use of FEC for reduction of anthelmintic use in horses (Nielsen et al. 2019). Despite these efforts to educate the general horse-owning public about the importance of using FEC and FECRT in parasite management programs, these practices are not commonly used. A recent study in rural Kentucky indicated only 10.6% of horses in the study had FECs performed as a part of their routine anthelmintic treatment programs, and another study showed that only 30.4% of participating Kentucky thoroughbred farms used FECs as part of their program (Robert et al. 2015; Scare et al. 2018). The NAHMS Equine 2015–2016 study found that 25.3% of farms had utilized FECs in the past 5 years, and only 4.2% had ever used FECRT on their farms (Nielsen et al. 2018a). A multi-national study also found that few horse owners in Germany, Austria, and the Netherlands used FECs (Becher et al. 2018). This low overall usage of FECs in parasite control programs indicates the importance of outreach programs for both veterinarians and horse owners so that anthelmintics currently on the market can be preserved for as long as possible. Not only can these outreach programs help encourage a shift in attitude and perceptions for parasite control programs, but they can also provide valuable data regarding anthelmintic efficacy.

The aims of this study were to (1) show how outreach programs can change attitudes towards FEC, (2) determine treatment efficacy for three major equine anthelmintic products (ivermectin, pyrantel pamoate, and fenbendazole) at both individual farm and state levels, (3) conduct a survey regarding different parasite control program practices, and (4) examine epidemiological factors associated with anthelmintic treatment efficacy.

Materials and methods

Enrollment of participants

This study took place March–October 2015 and March–October 2016 as part of a comprehensive parasite management short course titled “Managing Equine Parasites Using a Whole Farm Approach.” The course included topics such as parasite biology, how to conduct FECs, anthelmintic classes, parasite management, and pasture management. The course was offered at six different locations statewide. Farm and horse owners were required to attend this course in order to be enrolled in the study, where they then filled out a questionnaire about their previous parasite control knowledge and horse information sheets.

Questionnaires

The questionnaires investigated information on individual horses, such as age, sex, breed, use, weight (as determined by the owner with a weight tape), deworming history, and colic history. Information on the farms was also collected, such as farm type, farm size, pasture size, number of anthelmintics used, FEC use, and manure management practices. A follow-up questionnaire was also sent to participants to investigate reduction in anthelmintic use, use of FEC post study, and changes in pasture management. All information for this study was collected and utilized for research purposes with

written consent of the participants, and an informed consent form was signed by all participants.

Anthelmintic administration and fecal egg count reduction test

All FECs and FECRTs were conducted using the modified McMaster method outlined in the AAEP guidelines and only identified strongyle eggs (Nielsen et al. 2019; Nielsen and Reinemeyer 2018). Anthelmintics were provided to owners for horses with a FEC above 300 EPG. FECs were conducted at three time points: March/April, June/July, and September/October. For practical reasons, only farms that had at least three horses, instead of the recommended six horses per farm, in the > 300 EPG category participated in the FECRT portion of the study (Nielsen et al. 2019). Owners returned for FECRTs 2 weeks post-treatment for every anthelmintic provided. The first anthelmintic given to owners was pyrantel pamoate (Strongid ®, Zoetis Services LLC, Parsippany, NJ; Exodus, Bimeda Inc., Dublin, Ireland), and then fenbendazole (Safe-Guard ®, Merck Animal Health, Madison, NJ; Panacur ®, Merck Animal Health, Madison, NJ), and finally ivermectin (DuraMectin™, Durvet, Inc., Blue Springs, MO; Zimecterin Gold®, Merial Limited, Duluth, Georgia). Any horses that did not have an egg count reduction of 100% after treatment with pyrantel pamoate and fenbendazole were given ivermectin, and an additional FECRT was performed to ensure efficacy. Weight determination in pounds using a weight tape, anthelmintic dose in accordance with product labeling, and oral administration of anthelmintic treatment were all completed by study participants.

Statistical analysis

All statistical analyses were performed in SAS software for Microsoft version 9.4 (SAS Institute Inc., Cary, NC, 2012) and IBM SPSS Statistics for Microsoft, Version 24.0 (IBM Corporation, Armonk, NY, 2016). Effect of epidemiological factors on FEC was performed via two steps, and epidemiological factors included in both models were age (< 1 year, 1 to < 5 years, 5 to < 20 years, 20 to < 30 years, 30+ years), sex (male or female), breed (Arabian, thoroughbred, quarter horse, donkey/mule, other), weight (90.7 to < 226.8 kg, 226.8 to < 453.6 kg, 453.6 to < 544.3 kg, 544.3+ kg), total horses on the farm (< 5, 5 to < 10, 10 to < 30, 30+), farm type (boarding/lesson, breeding, private), removed manure from pastures (yes or no), spread manure on pasture (yes or no), total farm size (< 4.0 ha, 4.0 to < 20.2 ha, 20.2+ ha), and pasture size (< 0.4 ha, 0.4–0.8 ha, > 0.8–2.0 ha, > 2.0–4.0 ha, > 4.0 ha). First, the probability of producing a negative (no eggs seen) count versus a non-negative count was modeled via binary logistic regression of the epidemiological factors. In order to account for within-subject correlation, a random effect for horse was utilized. Next, a negative binomial regression was fit on the positive counts to determine which factors produce higher FEC. Any epidemiological factor with more than two categories that produced significant values was analyzed with a post hoc contrast using least square means.

Anthelmintic efficacies and the effect of year and grazing versus non-grazing season (May 1–September 30 for grazing season) on percent reduction were compared using *t* tests. A Pearson correlation was also used to determine correlation between percent reduction and anthelmintic used. All results were interpreted at the $p = 0.05$ significance level.

Results

Questionnaires

There were 221 total people that attended the course, and 62 farms filled out the farm survey portion with enough data to be included for analysis. These farms had an average number of 9.7 horses per farm and an average size of 5.4 ha. Out of these farms, 37.1% removed manure from pastures and 19.4% spread manure on pasture. There were 19 (30.6%) farms that used FECs prior to the study, and out of those, six (31.6%) removed manure from pastures and three (15.8%) spread manure on pasture. Out of the 43 (69.3%) farms that did not use FECs, horses were given an average of 4.1 anthelmintic treatments per year, ranging from twice per year to eight times per year.

Over the course of the study, there was an average decrease of 23.6 treatments per farm and 2.4 treatments per horse, when the FEC system was introduced (Fig. 1). There were 273 horses that participated in all FEC time points. These horses were treated an average of 1.1 times during the study (Fig. 2), which is significantly lower than the number of treatments given to horses on farms that did not use FECs ($p = 0.0002$).

Owners and managers from 37 horse farms responded to the post-study survey. Overall, 89.2% were able to reduce the number of anthelmintics used on their farm and 97.3% planned on using FEC on their farms in the future. Additionally, 73.0% of farms took steps to prevent grazing near areas with a large amount of manure, and 46.0% stopped harrowing to disrupt and spread fecal piles in pastures or restricted harrowing to later in the fall. Full results of the post-study survey are presented in Table 1.

Fecal egg count and fecal egg count reduction test

Overall, there were 56 farms that participated in the FEC portion of the study. Out of those, 49 (87.5%) had at least one anthelmintic treatment and 7 (12.5%) did not have any horses receiving anthelmintic treatment. Farms were next screened for those that had at least three individual treatments with

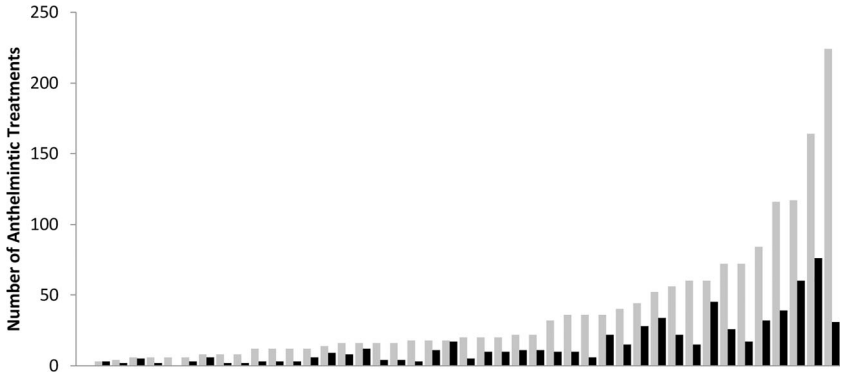

Fig. 1 Number of anthelmintic treatments used per farm prior to the study (gray) and after the study (black). The average number of treatments prior to the study was 37.3 per farm, and after the study it was 14.6 treatments per farm

any of the three drugs used in the study. With this taken into consideration, there were 29 farms that had at least one drug with three or more data points (Table 2).

There were a total of 513 individual treatments throughout the study, with 29.6% of those being ivermectin, 41.7% pyrantel pamoate, and 28.7% fenbendazole. There was significant correlation between drug class given to the horse and percent reduction ($p = 0.0002$). Compared with ivermectin, fenbendazole and pyrantel pamoate ($p \leq 0.0001$) produced lower percent reduction (Fig. 3). Fenbendazole and pyrantel pamoate produced similar mean percent reductions ($p = 0.812$). Year did not have a significant effect on percent reduction, but grazing versus non-grazing season did ($p = 0.003$).

Epidemiological factors such as age, sex, breed, weight, spreading manure on pasture, pasture size, and total farm size did not have any significant effect on FEC. Horses in the 1 to < 5-year-old age group were more likely to produce negative counts than horses in the 5 to < 20-year-old and 20 to < 30-year-old age groups ($p = 0.0052$, $p = 0.0007$), and farms with 10 to < 30 horses were more likely to produce negative counts than those with 30 or more horses ($p = 0.0203$). Horses on farms that removed manure from pastures were significantly more likely to produce negative counts than those that were on farms that did not remove manure from pastures ($p = 0.0127$). Breeding farms and farms with 5 to < 10 horses were more likely to produce higher counts ($p = 0.0092$, $p = 0.0007$).

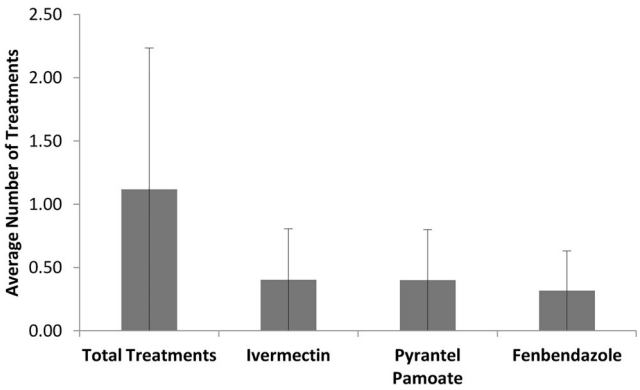

Fig. 2 Average number of anthelmintic treatments per horse over the course of the study. The average total number of treatments per horse ($n = 273$) was 1.1, ivermectin 0.4, pyrantel pamoate 0.4, and fenbendazole 0.3. Error bars represent standard deviation

Discussion

This is the first study to evaluate the effect of an outreach program teaching horse owners the value of FEC monitoring on their farms. Furthermore, the study provided useful information about the efficacy of three commonly used equine anthelmintics in the state of Pennsylvania. Out of the 56 farms evaluated, only 30.6% utilized FEC in their parasite control program at the beginning of the study, but after the short course, 97.3% of 37 respondents indicated that they would use FECs for new horses and check drug efficacy using FECRTs. This indicates a strong attitude shift in response to education on parasite management practices. While it would be ideal if FECs were used for all horses 3–4 times every year, using FECs for new horses to place them into a shedding category can also help reduce the number of anthelmintic treatments applied on farms (Nielsen et al. 2019). During this study, there was a decrease of 2.4 treatments per horse, and at the end of the study, 89.2% of farms reported a decrease in the number of anthelmintics used. Even though no follow-up studies have conducted to determine actual application of the programs, a change in attitude and willingness is still a step in the right direction. Outreach programs could be very beneficial in other states, and even countries, to help encourage even more of a shift towards using FECs and surveillance-based parasite control programs that can help reduce anthelmintic use and hopefully slow down the spread of anthelmintic resistance.

Interestingly, horses in the younger age range were more likely to produce negative counts than older horses, although there was no effect of age on production of higher FEC results. Typically, FEC decreases as a horse

Table 1 Combined results for post-study surveys from 2015 and 2016. Values are reported as percentages with the number of farms in parenthesis where $n = 37$

	Yes	No	N/A
	Reported as % (number) where $n = 37$		
As a result of participating in the project were you able to…			
Identify the high shedders of small strongyle eggs on your farm?	100 (37)	0 (0)	0 (0)
Identify the low shedders that had good immunity against small strongyles?	97.3 (36)	2.7 (1)	0 (0)
Determine the effectiveness of the dewormers used on your farm?	91.9 (34)	2.7 (1)	5.4 (2)
Reduce your use of dewormers?	89.2 (33)	8.1 (3)	2.7 (1)
Reduce your fear of small strongyle parasites which often leads to over deworming?	94.6 (35)	5.4 (2)	0 (0)
Increase your confidence in using fecal egg count (surveillance-based) deworming practices?	100 (37)	0 (0)	0 (0)
As a result of participating in this project do you now or do you plan to…			
Conduct fecal egg counts on new horses?	97.3 (36)	2.7 (1)	0 (0)
Conduct pre and post deworming egg counts on new horses using products that are still working on your farm to be sure the new horse does not have resistant parasites?	97.3 (36)	2.7 (1)	0 (0)
As a result of attending the educational program, please list any other farm practices that you have adopted to reduce exposure to parasites.			
Took steps to improve my pastures so horses do not graze near manured areas	73.0 (27)	27.0 (10)	0 (0)
Removed manure from the pastures	32.4 (12)	59.5 (22)	8.1 (3)
Stop harrowing pastures or harrow only in late fall	46.0 (17)	21.6 (8)	32.4 (12)

ages until it reaches geriatric ages, when it may start exhibiting higher counts (Adams et al. 2015; Levy et al. 2015; Kornaś et al. 2015). Farms with a moderate number of horses were also more likely to have horses with negative counts, whereas farms with fewer horses and those used for breeding were more likely to produce higher counts. While overall farm size and pasture size were indicated in the study, stocking density of each pasture is unknown. A relatively large number of horses on a small pasture increases density (horses per hectare), which can in turn affect infection pressure on the pasture. Additionally, farms that removed manure from pastures were more likely to have horses that produced negative counts. Farm management practices have been shown to affect FEC, and in particular one large scale study in the UK with donkeys indicated that feces removal significantly reduces egg shedding (Corbett et al. 2014). Grazing season also had a significant effect on percent reduction, but this result is likely skewed because all of the fenbendazole treatments took place in the grazing season.

One previous study including Pennsylvania in anthelmintic efficacy analysis indicated pyrimidine/benzimidazole was only effective for 21.4% of operations (Nielsen et al. 2018b). Another study that included Pennsylvania indicated mean efficacies of 33.3% and 68.6% for fenbendazole and pyrantel pamoate, respectively (Smith et al. 2015). Neither study separated analyses into state-specific information by farm, but these results are consistent with those found in this study. Fenbendazole resistance was indicated on 94.4% of farms, and pyrantel pamoate resistance was indicated on 66.7% of farms, with the former drug exhibiting acceptable efficacy (36.6%) on zero farms and the latter (66.9%) on only 7.4% of farms. This paints a stark picture for anthelmintic resistance in the state, suggesting that fenbendazole resistance is widespread and pyrantel pamoate resistance is highly prevalent. Currently, only macrocyclic lactones, specifically ivermectin evaluated in this study, are the only drugs exhibiting very little resistance, with a single farm in this study indicating reduced efficacy.

Table 2 Number of farms in each of the resistance categories based on American Association of Equine Practitioners (Nielsen et al. 2016) guidelines. Values reported as number of farms with percentage in parenthesis

	None	Susceptible	Suspected	Resistant	Total number of farms
	Number of farms (%)				
Fenbendazole	0 (0)	0 (0)	1 (5.6)	17 (94.4)	18
Ivermectin	13 (92.9)	1 (7.1)	0 (0)	0 (0)	14
Pyrantel pamoate	2 (7.4)	4 (14.8)	3 (11.1)	18 (66.7)	27

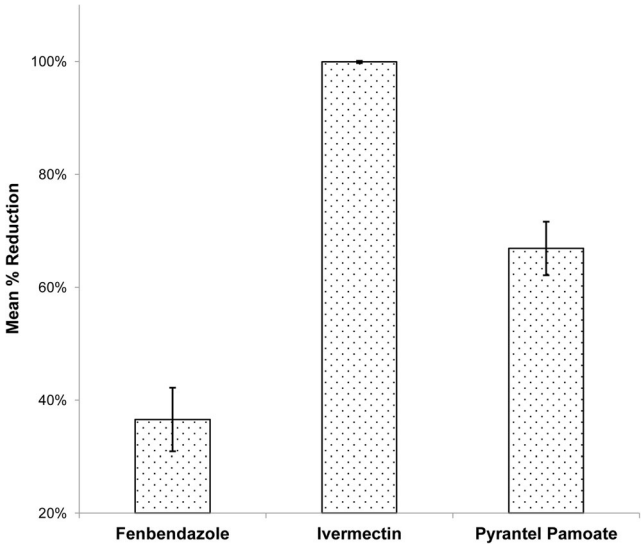

Fig. 3 Mean percent reduction for each drug used in the study. Fenbendazole and pyrantel pamoate produced significantly lower percent reductions than ivermectin ($p \leq 0.0001$). Error bars represent 95% confidence interval

While some epidemiological factors may play a role in the probability of horses on a farm producing either negative or high FEC values, management practices such as removal of feces and utilization of a proper parasite control program are essential. This study indicates a need for vigilance when determining which anthelmintic products veterinarians and owners use for their horses. Fenbendazole and pyrantel pamoate products most likely will not work for parasite control on many farms, leaving the macrocyclic lactones—ivermectin and moxidectin—as the products of choice. While resistance to these drugs is not yet prevalent, proper use of the drugs via selective treatment using FEC and continued monitoring of efficacy with the FECRT are important for slowing down the process of equine gastrointestinal parasites developing resistance.

Finally, this study highlights the importance of outreach programs and educational courses for parasite control programs and helping stymie the spread of anthelmintic resistance. The outreach program and short course provided an opportunity for the data collection that was used for this study, which in turn allowed the status of anthelmintic resistance in Pennsylvania to be studied. This program clearly helped increase awareness and willingness to use FECs, which in turn helps reduce the number of anthelmintic treatments administered on horse farms. Participants even reported lower numbers of anthelmintics being used on their farms after the study. Reducing anthelmintic use could help slow the development of anthelmintic resistance to macrocyclic lactones, the only drug class still generally effective against cyathostomins. This extension outreach program and educational short course provide an example that other states and countries can follow to help spread knowledge and increase information regarding anthelmintic resistance in equine parasites.

Acknowledgments The authors would like to thank Eric Roemmele for all of his statistical help, and the farm owners who participated in this study for their dedication and enthusiasm.

Funding information Funding for this study was provided by Penn State Extension–College of Agricultural Sciences and through a USDA Sustainable Agriculture Research and Education grant.

Compliance with ethical standards

All information for this study was collected and utilized for research purposes with written consent of the participants, and an informed consent form was signed by all participants.

Conflict of interest The authors declare that they have no conflicts of interest.

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