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Research paper

Anthelmintic efficacy of single active and combination products against commonly occurring parasites in foals

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ABSTRACT

Parasite control in foals is complicated by the concurrent presence of biologically diverse parasites with differing levels of anthelmintic resistance. Several combination anthelmintic products are available for use in horses, but information on their efficacies against important equine parasites is scarce. Two trials were performed in New Zealand during 2008 and 2011 on four different farms with substantially different anthelmintic treatment histories. The first trial evaluated the efficacy of an ivermectin/praziquantel/oxibendazole combination, a single active oxibendazole, and a single-active macrocyclic lactone (ML) in 49 foals located on three farms. The second trial evaluated two combination anthelmintic products and three single-active ML products and enrolled a total of 110 foals on three farms. Foals in the second trial were allocated to one of six anthelmintic treatment groups; oxfendazole/pyrantel embonate, pyrantel embonate/ivermectin/praziquantel, ivermectin/praziquantel, abamectin/praziquantel, moxidectin/praziquantel, and a placebo-treated control. In both trials, foals were monitored monthly prior to treatment, and fecal egg counts (FECs) of *Parascaris* spp., strongylid, and *Strongyloides westeri* were determined. A "rolling enrolment" process was implemented whereby foals were systematically allocated to a treatment group and treated with the corresponding anthelmintic following the first appearance of *Parascaris* spp. eggs in the faeces. A generalised linear model was used to evaluate the effect of farm and treatment on Day14 FEC (ln) for each parasite. Three different FECR calculation methods were employed as follows; i) FECR(T) pre and post treatment ii) FECR (C) in the treated group compared with control, and iii) FECR (P) pre- and post- treatment in the treated and control groups. Across both trials, treatment with ML single active products failed to achieve > 95% reduction in *Parascaris* spp. FEC on two of three farms. The pyrantel embonate/oxfendazole and ivermectin/ praziquantel/oxibendazole combinations demonstrated full efficacy against *Parascaris* spp. This is in contrast to the anti-strongylid efficacies determined, where the pyrantel embonate/oxfendazole combination and single active oxibendazole had reduced efficacy on one farm, while the macrocyclic lactones generally had good efficacy. *Strongyloides* egg counts were sporadic in both trials, and allowed limited insight into anthelmintic efficacy. The study illustrated the importance of keeping an untreated or placebo-treated control group in studies evaluating anti-*Parascaris* efficacy and it demonstrated the utility of a rolling enrolment procedure, where foals are enrolled over the course of a defined period of time. Furthermore, the study demonstrated the value of a farm specific FECR monitoring programme and the complexity of parasite control in foals, where combination anthelmintic products can be employed to target multiple species of parasites.

1. Introduction

Parasite control in juvenile horses is complicated by the presence of a variety of different parasites, increasing levels of anthelmintic resistance within these, and the susceptible status of the young horse (Reinemeyer and Nielsen, 2017). Foals are exposed to infection with *Strongyloides westeri*, *Parascaris* spp., and cyathostomin nematodes and each requires different considerations for treatment and control (Reinemeyer and Nielsen, 2017). The equine threadworm, *S. westeri*, is transmitted lactogenically to the foals and is the first nematode to reach

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patency in the intestinal tract. It was historically susceptible to ivermectin (Ludwig et al., 1983), oxibendazole, and fenbendazole treatment (Drudge et al., 1981a, 1981b), but there is a lack of studies documenting the current status of anthelmintic efficacy against this parasite. The most important parasite in foals is *Parascaris* spp., for which resistance to the macrocyclic lactones is now widely reported across the world, and case reports suggesting loss of fenbendazole and pyrantel efficacy now exist (Armstrong et al., 2014; Nielsen, 2016). The *Parascaris* spp. are most often accompanied by cyathostomin parasites, which are reported widely resistant to benzimidazole and pyrantel products (Peregrine et al., 2014). Furthermore, reduced egg reappearance periods following ivermectin and moxidectin treatment have been associated with apparent resistance at the luminal fourth larval (L4) stage (Bellaw et al., 2018; Lyons et al., 2009, 2010).

Traditional approaches for parasite control in foals have been based on regular anthelmintic therapy often administered at frequent intervals (Bolwell et al., 2015; Fritzen et al., 2010; Hinney et al., 2011; Relf et al., 2012; Robert et al., 2015). However, widespread resistance to the macrocyclic lactone (ML) anthelmintics in *Parascaris* spp., and to the benzimidazole and pyrimidine classes in cyathostomins has complicated this approach because, whichever of these actives is used, its efficacy against at least one class of nematodes is likely to be compromised. The use of combination deworming products, *i.e.* products containing multiple drug classes with overlapping spectra of activity, offers a potentially easy and useful solution to this dilemma.

Combination deworming products are available for equine use in several countries, and are used extra-label in others, but there is little publically available data on their efficacy. In contrast, there has been extensive research on the use and benefits of combination dewormers against parasites of ruminants (Bartram et al., 2012; Geary et al., 2012). Equine studies have documented an additive anthelmintic efficacy against cyathostomin parasites when pyrantel pamoate and oxibendazole were administered concurrently (Kaplan et al., 2014; Scare et al., 2018), but another study suggested that the combination was unlikely to be sustainable in a situation where resistance is already well established against both active ingredients (Scare et al., 2018). To date, a few studies have reported treatment efficacy of anthelmintic combinations against parasites of foals (Bishop et al., 2014; Lyons et al., 2016; Wilkes et al., 2017). The purpose of this study was to assess the efficacy of commercially available combinations of oxibendazole/ivermectin, oxfendazole/ivermectin, and pyrantel embonate/oxfendazole as well as single active products containing oxibendazole and the macrocyclic lactones ivermectin, abamectin and moxidectin against *Parascaris* spp., strongylid and *Strongyloides westeri* parasites in foals.

2. Materials and methods

2.1. Experimental design

Two trials were performed in the North Island of New Zealand in 2008 (Trial 1, n = 49 foals) and in 2011 (Trial 2, n = 110 foals). Both trials were prospective, blinded controlled studies and both sourced foals of different breeds from three farms in three regions, Auckland (Standardbreds), Waikato (Thoroughbreds) and Gisborne (Warmbloods). For Trial 1, ethics clearance was provided through the Ancare Scientific Ltd Animal Ethics Committee.

Trial inspection was carried out by an Ancare Scientific Animal Ethics representative on 11 April 2008. Trial 2 was carried out with approval from PharmVet Solutions (Aucland, New Zealand) Animal Ethics Committee under protocol number 211. Trial 1 included four treatment groups: ivermectin/praziquantel/oxibendazole (IPO), oxibendazole (OXI), ivermectin/praziquantel (IP), and an untreated control group. Trial 2 included the following six treatment groups: pyrantel embonate/oxfendazole (PEO), pyrantel embonate/ivermectin/praziquantel (PEIP), ivermectin/praziquantel (IP), abamectin/praziquantel (AP), moxidectin/praziquantel (MP), and a placebo-treated control.

This study was performed with written consent from each stud and in accordance with the code of ethical conduct under the New Zealand Animal Welfare Act 1999 and monitored by PharmVet Solutions (Auckland, New Zealand).

2.2. Farm treatment history

A total of four farms were enrolled in the studies described herein. The farms in Auckland and Gisborne were the same farms in both trials, whereas the Waikato farm was different between Trials 1 (W1) and 2 (W2). Each farm had different drenching regimens in the 12 months prior to entry into the study. The Waikato farms (W1, W2) routinely drenched all of their stock with an ivermectin-praziquantel product every 6 weeks, and the Gisborne farm had not drenched their mares and foals for over two years. The Auckland farm had been using an ivermectin-praziquantel product routinely prior to Trial 1 and an ivermectin-oxfendazole combination in their mares and foals every six weeks from 10 weeks after foaling prior to Trial 2. For inclusion in the trial, the mares were not drenched within four weeks before foaling and neither the mares nor foals were drenched from date of foaling until they were allocated to a treatment in the study.

2.3. Treatment groups

The products evaluated are all registered for use in horses in New Zealand. In Trial 1 the treatment groups included i) IPO: 0.2 mg/kg ivermectin, 10 mg/kg oxibendazole, 2.5 mg/kg praziquantel (N = 12, Triumph, Merial Ancare, Auckland New Zealand), ii) OXI: 10 mg/kg oxibendazole (N = 11, Oximinth, Virbac, Milperra, Australia), iii) IP: 0.2 mg/kg ivermectin, 2.5 mg/kg praziquantel (N = 11, Parade, Merial Ancare, Auckland New Zealand), and iv) an untreated control group (N = 15).

In Trial 2 the treatment groups included i) PEO: 13.2 mg/kg pyrantel embonate and 10 mg/kg oxfendazole (N = 20, Strategy T, Virbac, Milperra, Australia), ii) PEIP: 13.2 mg/kg pyrantel embonate, 0.2 mg/kg ivermectin, 1.5 mg/kg praziquantel (N = 20, Equimax Elevation, Virbac, Milperra, Australia), iii) IP: 0.2 mg/kg ivermectin, 1.5 mg/kg praziquantel (N = 17, Equimax LV, Virbac, Milperra, Australia), iv) AP: 0.2 mg/kg abamectin, 2.5 mg/kg praziquantel (N = 17, Genesis, Merial, North Ryde, Australia), v) MP: 0.4 mg/kg moxidectin, 2.5 mg/kg praziquantel (N = 13, Equest Plus Tape, Pfizer, West Ryde, Australia), and a placebo, non-hazardous water-based control paste (N = 23, Virbac, Milperra, Australia).

2.4. Blinding

The study investigator, horse owners, stud managers and laboratory technicians were blinded to treatments as the barrel of each tube of drench was obscured with a trial label indicating a code for the treatment and the dose required per kilogram of animal body weight as per the manufacturer's label directions.

2.5. Treatment schedule

A rolling enrolment approach was implemented in both trials. Individual faecal egg count (FEC) analysis was performed at 4, 8 and 12 weeks of age and then every two weeks until the foals returned a positive *Parascaris* spp. FEC greater than 50 eggs per gram (EPG). Within four days of receiving confirmation of *Parascaris* spp. infection, each foal was allocated to one treatment group on treatment day (Day 0) in a set sequential order as follows: Trial 1 i) IPO, ii) OXI, iii) IP, and iv) untreated control. Post treatment FECs in Trial 1were determined on Day 14. In Trial 2 the foals were systematically allocated to treatment group in the following order i) PEO, ii) PEIP, iii) IP, iv) AP, v) MP, and vi) placebo-treated control, irrespective of their farm of origin. Post-treatment FECs in Trial 2 were determined at Days 14, 28 and 56 post

treatment.

2.6. Foal enrolment

In Trial 1, a total of 49 foals were screened and 35 returned a positive *Parascaris* spp. FEC. In Trial 2, a total of 127 foals were screened and 106 returned a positive *Parascaris* spp. FEC. For inclusion in the study, all foals remained on their property of origin for the duration of the study, and none of the foals were treated with anthelmintics prior to the study. Foal weights were estimated by use of a weigh-band (The Coburn Company Inc, Whitewater, WI, USA) within 96 h prior to treatment. Based on the foal weight, the treatment dose was rounded up on average by 32 ± 23 kg (mean ± SD) for all treatments to avoid under-dosing and to minimise variation between treatment groups due to differences in the gradations on each test product syringe.

2.7. Withdrawal criteria

Any foals in Trial 2 with a positive FEC for *Parascaris* spp. after treatment were withdrawn from subsequent analysis and administered an alternative anthelmintic to remove the residual infection. Any foals not sampled for faeces on both Day 0 and Day 14 were also withdrawn from the analysis. Foals were monitored daily by stud personnel and could be withdrawn from the study if they were in poor health or required veterinary care.

2.8. Faecal egg counts

Faecal samples were analysed at a commercial diagnostic laboratory (Gribbles Veterinary Pathology, Hamilton, New Zealand) using a modified McMaster counting technique to determine egg counts with a detection limit of 50 EPG (Gibson, 1965; Stafford et al., 1994). The flotation medium was a saturated salt solution with a specific gravity of 1.20. The laboratory differentiated the FEC results into three categories – *Parascaris* spp., strongylids, and *S. westeri* based on the distinctive appearance of the parasite eggs (Reinemeyer and Nielsen, 2017).

2.9. Statistical analyses

2.9.1. Faecal egg count reduction test

The arithmetic means were calculated for FEC on Day 0 and at Day 14. Three fecal egg count reduction test (FECRT) analyses were calculated as follows:

i) The FECRT(T) was calculated for the pre- and post-treatment samples in the same treatment group (T) as follows:

$$FECRT\,(T) = 100 \times \left(1 - \left(\frac{T14}{T0}\right)\right),$$

where T0 and T14 are the individual FECs at Day 0 (T0) and Day 14 (T14) (Kaplan et al., 2004). The group mean FECR% (T) was then calculated with 95% confidence intervals.

ii) The FECRT(C) was calculated using the arithmetic group mean of a treated group at Day 14 post-treatment and compared with the placebo-treated control group (C) as follows:

$$FECRT\,(C) = 100 \times \left(1 - \left(\frac{T14}{C14}\right)\right),$$

where T14 and C14 are the arithmetic mean FEC of the treatment group (T14) and control group (C14) at Day 14 post-treatment (Coles et al., 1992).

iii) FECRT(P) was calculated using the Presidente formula (Presidente, 1985) as follows:

$$FECRT\,(P) = 100 \times (1 - \left(\left(\frac{T14}{T0}\right) \times \left(\frac{C0}{C14}\right)\right),$$

Where T0 and C0 are the arithmetic group mean faecal egg counts pre-treatment for the treated and placebo-treated groups, respectively. Similarly, T14 and C14 are the group mean egg counts at 14 days post treatment.

2.9.2. General linear model analysis

The FEC data were log-transformed to achieve normal distribution of residuals. The multivariate, general linear model (GLM, Minitab 17) was used to assess the effect of farm and treatment on the lnFEC for *Parascaris* spp. and strongylids. The multivariate models were restricted to data from Day 0 and 14 since those time periods contained all the data, prior to withdrawals due to elevation in FEC. The statistical model incorporated a fixed effect for farm and a random effect for horse ID to adjust for the effects of these factors. Marginal means for FEC for each combination of treatment and week were presented in the log-transformed scale and as back-transformed means.

Pair-wise comparisons were conducted using standard error terms derived from the multivariable model to assess the same specific treatment group comparisons. Results were presented as Benjamini-Hochberg adjusted p-values to minimize the risk of type 1 statistical error associated with multiple comparisons.

2.9.3. Bayesian MCMC procedure

A Bayesian Markov chain Monte Carlo (MCMC) procedure was performed using Trial 2 data to determine the probability of FEC reduction > 90% for this data using the freely available web interface http://shiny.math.uzh.ch/user/furrer/shinyas/shiny-eggCounts/ visited on 8 August 2018. This software is based on the R package (Torgerson et al., 2014; Wang, 2018). Data from all three farms were combined for this analysis and the paired model was selected for pre- and post-treatment analysis within an animal and without zero-inflation as described by Pena-Espinoza (Pena-Espinoza et al., 2016).

3. Results

The mean age of the foals at treatment was 16.8 weeks (range 11–22 weeks) and their average bodyweight on Day 0 at time of treatment in Trial 1 was 203 kg (range 140–255 kg) and in Trial 2 was 233 kg (range 145–361 kg).

3.1. Effect of farm

In both trials, at Day 14 there was an overall significant effect of "farm" on the lnFEC for *Parascaris* spp. ($P < 0.0001$), but not for strongylid egg counts. In both trials, the Gisborne farm had significantly lower ascarid FECs post treatment compared with the other two farms, and in Trial 2, all three farms were statistically different from each other ($P < 0.0001$).

3.2. Parascaris spp

In Trial 1, the only treatment that resulted in > 95% reduction on all three farms regardless of FECR calculation method was OXI alone or in combination with ivermectin (IPO) (Table 1). On one farm (Gisborne), the IP combination was 100% effective in its reduction of FEC as determined by all three FECR calculation methods. In Trial 2, the only treatment that resulted in > 95% reduction on all three farms regardless of FECR calculation method was the PEO combination. Reduced efficacy of PEIP was observed on one farm (A, Table 2) by all FECR calculation methods. On two of the farms (A, W2), all three of the ML-praziquantel combination products (IP, AP, MP) failed to achieve a 95% reduction in *Parascaris* spp. (Table 2), while 100% reductions were achieved on the third farm (G), with the exception of a single foal treated with IP.

In Trial 2, the Day 14 mean *Parascaris* spp. FEC for the placebo group was significantly higher than PEO and PEIP treated groups

Table 1
Ascarid and strongylid faecal egg count reduction data from the three farms in Trial 1 with three different methods of calculating the faecal egg count reduction (FECR).

Farm			Faecal egg counts		Faecal egg count reduction		
	Treatment[a] *Parascaris* spp.	N	Pre	Post	FECR(T)[b]	FECR(C)[c]	FECR(P)[d]
Auckland	IPO	1	1,050	0	100.0% [-]	100.0%	100.0%
(A)	OXI	2	775.0	0.0	100.0% [-]	100.0%	100.0%
	IP	1	300.0	1,050.0	0.0% [-]	−500.0%	−800.0%
	Placebo	2	450.0	175.0	56.3% [49.0–63.5]	–	–
Gisborne	IPO	4	850.0	0.0	100.0% [-]	100.0%	100.0%
(G)	OXI	5	580.0	0.0	100.0% [-]	100.0%	100.0%
	IP	3	266.7	0.0	100.0% [-]	100.0%	100.0%
	Placebo	3	2916.7	850	54.1% [18.6–89.6]	–	–
Waikato	IPO	3	2,883.3	0.0	100.0% [-]	100.0%	100.0%
(W1)	OXI	3	2,033.3	33.3	95.6% [86.9–104.2]	98.8%	98.9%
	IP	3	2,700	3,783.3	0.0% [0.0–37.1]	−38.2%	3.4%
	Placebo	4	1887.5	2737.5	0.0% [0.0–27.4]	–	–
Strongylids							Strongylids
Auckland	IPO	0	–	–	–	–	–
(A)	OXI	2	150.0	50.0	66.7% [1.3–132.0]	86.7%	64.4%
	IP	2	350.0	0.0	100.0% [-]	100.0%	100.0%
	Placebo	4	400.0	375.0	0.0% [0.0–9.7]	–	–
Gisborne	IPO	8	1,050.0	0.0	100.0% [-]	100.0%	100.0%
(G)	OXI	5	2,170.3	30.0	98.7% [95.9–101.4]	98.4%	99.0%
	IP	6	975.0	0.0	100.0% [-]	100.0%	100.0%
	Placebo	6	1,308.3	1,883.3	0.0% [0.0–240.4]	–	–
Waikato	IPO	1	1,000.0	0.0	100.0% [-]	100.0%	100.0%
(W1)	OXI	1	2,950.0	250.0	91.5% [-]	75.6%	99.2%
	IP	2	225.0	0.0	100.0% [-]	100.0%	100.0%
	Placebo	2	100.0	1,025.0	0.0% [0.0–888.0]	–	–

[a] IPO: ivermectin, praziquantel and oxibendazole, OXI: oxibendazole, IP: ivermectin and praziquantel.
[b] (1-(post/pre))*100%.
[c] $(1-(post_{treated}/post_{placebo}))*100\%$.
[d] $(1-((post_{treated}/pre_{treated})*(pre_{placebo}/post_{placebo})))*100\%$.

($P < 0.001$), but was not different from the IP, AP or MP treated groups. Furthermore, the PEO groups had significantly lower Day 14 FECs than PEIP treated groups ($P = 0.013$) and both the PEO and PEIP treated groups were significantly lower than the IP, AP, and MP treated groups ($P < 0.001$).

In Trial 2, the Bayesian hierarchical model estimated that the PEO treatment had an overall FECR of 91% with a 95% uncertainty interval of 90–93, resulting in a 100% probability that the FECR was > 90% for this population. For all other treatment groups, there was a 0% probability that the FECR exceeded 90%.

3.3. Strongylids

Generally all three macrocyclic lactones reduced strongylid FECs by 100% with the exception of AP on the Waikato (W2) farm in Trial 2 (Table 2). This reduced efficacy observed in this group was due to the strongylid FEC of one of four foals increasing from 650 EPG pre-treatment to 2300 EPG post-treatment. The egg counts of the other three foals in this group were all reduced to 0 EPG. The OXI product in Trial 1 failed to achieve > 95% FECR on two farms (A, W1), but was > 95% on the third farm (Table 1), while the other treatments containing ivermectin were 100% effective. In Trial 2, the PEO product was 100% effective in reducing the strongylid FEC on one farm (W2), but < 95% effective on the remaining two farms.

In Trial 2, the Day 14 mean FEC was significantly higher for the placebo-treated group than all other groups ($P < 0.001$), while no other treatment comparisons were statistically significant.

The Bayesian hierarchical model estimated that the MP combinations had an overall FECR of 100% with a 95% uncertainty interval of 99–100, resulting in 100% probability that the FECR was > 90% for this population. The PEO treatment group had a 64.5% probability that the probability that the reduction in the strongylid FEC was greater than 90%.

3.4. Strongyloides westeri

In Trial 1, seven foals from two farms were *S. westeri* FEC positive pre-treatment. All of these foals were negative for *S. westeri* FEC on Day 14 after treatment. However, on Day 14 after treatment, three other foals were positive for *S. westeri* FEC. In Trial 2, 22 foals were *S. westeri* FEC positive on two of the farms (Table 3). In 15 of the treated foals, all treatments elicited a 100% reduction with the exception of one foal in the IP treated group whose FEC decreased from 100 to 50 EPG. In the placebo control group there was a 90% reduction in the *S. westeri* FEC after treatment.

3.5. Egg reappearance following pyrantel embonate/oxfendazole treatment

In Trial 2, a total of 55 foals were withdrawn from the study in Week 4 after treatment due to *Parascaris* spp. FECs exceeding 50 EPG. These foals were spread across five treatment groups, but none occurred in the PEO group. At Week 8 after treatment, an additional 12 foals were withdrawn across all treatment groups for the same reason. Due to the large number of foals withdrawn from the macrocyclic lactone treatment groups, the results determined at four and eight weeks post treatment were considered to be biased for these groups and were therefore not statistically analysed.

Following treatment with PEO, the mean *Parascaris* spp. and *S. westeri* FECs were reduced to below pre-treatment levels for the duration of the eight-week trial period (Fig. 1). The mean strongylid FEC was initially reduced to below pre-treatment levels, but increased above those levels by eight weeks post-treatment (Fig. 1).

Table 2
Ascarid and strongylid faecal egg count reduction data from the three farms in Trial 2 with three different methods of calculating the faecal egg count reduction (FECR.

Farm			Faecal egg counts		Faecal egg count reduction		
	Treatment[a] *Parascaris* spp.	N	Pre	Post	FECR(T)[b]	FECR(C)[c]	FECR(P)[d]
Auckland	PEO	7	6,035.7	7.1	97.1% [91.5–102.7]	99.6%	100.0%
(A)	PEIP	5	1,200.0	1,040.0	52.5% [9.5–95.5]	48.5%	80.9%
	IP	5	1,760.0	1,620.0	35.0% [3.0–66.9]	19.8%	79.8%
	AP	5	1,530.0	2,670.0	1.2% [−1.2–3.6]	0.0%	61.6%
	MP	5	320.0	1,120.0	10.0% [−3.1–23.1]	44.5%	23.1%
	Placebo	8	443.8	2,018.8	0.0% [-]	–	–
Gisborne	PEO	2	7,500.0	0.0	100.0% [-]	100.0%	100.0%
(G)	PEIP	2	3,125.0	0.0	100.0% [-]	100.0%	100.0%
	IP	1	400	50	87.5% [-]	94.4%	75.0%
	AP	1	2,200	0	100.0% [-]	100.0%	100.0%
	MP	2	875.0	0.0	100.0% [-]	100.0%	100.0%
	Placebo	1	450	900	0.0% [-]	–	–
Waikato	PEO	11	277.3	0.0	100.0% [-]	100.0%	100.0%
(W2)	PEIP	13	346.2	7.7	99.2% [97.6–100.8]	98.9%	99.1%
	IP	11	140.9	186.4	29.1% [1.95–56.2]	72.9%	49.4%
	AP	10	185.0	205.0	34.2% [8.58–59.8]	70.2%	57.6%
	MP	6	283.3	133.3	60.4% [29.77–91.0]	80.6%	82.0%
	Placebo	11	263.6	688.6	22.7% [−1.51–47.0]	–	–
	Strongylids						
Auckland	PEO	4	387.5	150	75.0% [26.0–124.0]	76.3%	84.7%
(A)	PEIP	4	350.0	12.5	96.4% [89.4–103.4]	98.0%	98.6%
	IP	4	150.0	0.0	100.0% [-]	100.0%	100.0%
	AP	2	1,025	0.0	100.0% [-]	100.0%	100.0%
	MP	3	66.7	0.0	100.0% [-]	100.0%	100.0%
	Placebo	6	250.0	633.3	10.0% [−9.6–29.6]	–	–
Gisborne	PEO	2	2,150.0	50.0	94.7% [92.2–102.5]	94.1%	98.4%
(G)	PEIP	2	475.0	0.0	100.0% [-]	100.0%	100.0%
	IP	1	1,250	0	100.0% [-]	100.0%	100.0%
	AP	2	1,450.0	0.0	100.0% [-]	100.0%	100.0%
	MP	2	500.0	0.0	100.0% [-]	100.0%	100.0%
	Placebo	2	600.0	850.0	27.3% [−26.18–80.7]	–	–
Waikato	PEO	5	450.0	0.0	100.0% [-]	100.0%	100.0%
(W2)	PEIP	5	140.0	0.0	100.0% [-]	100.0%	100.0%
	IP	6	325.0	0.0	100.0% [-]	100.0%	100.0%
	AP	4	250.0	575.0	75.0% [26.0–124.0]	−64.3%	−80.7%
	MP	2	100.0	0.0	100.0% [-]	100.0%	100.0%
	Placebo	4	275.0	350.0	52.7% [9.6–95.8]	–	–

[a] PEO: pyrantel embonate and oxfendazole, PEIP: pyrantel embonate, ivermectin, and praziquantel, IP: ivermectin and praziquantel, AP: abamectin and praziquantel, MP: moxidectin and praziquantel.
[b] (1-(post/pre))*100%.
[c] (1-($post_{treated}$/$post_{placebo}$))*100%.
[d] (1-(($post_{treated}$/$pre_{treated}$)*($pre_{placebo}$/$post_{placebo}$)))*100%.

4. Discussion

This study provided some unique and useful information about the anthelmintic efficacy of several commercially available combination products against *Parascaris* spp., strongylids, and *S. westeri* parasites in foals. Furthermore, the study allowed demonstration of several important study design elements for assessing anti-*Parascaris* efficacy of anthelmintic products: the rolling enrolment model, and provision of

Table 3
Strongyloides westeri faecal egg count reduction data in Trial 2. Two farms (Auckland and Waikato) had foals positive for this parasite pre-treatment, and treatment groups were combined across these.

	Number of foals (N)		Faecal egg counts		Faecal egg count reduction		
Treatment[a]	Auckland	Waikato	Pre	Post	FECR(T)[b]	FECR(C)[c]	FECR(P)[d]
PEO	2	2	1,100.0	0.0	100.0% [-]	100.0%	100.0%
PEIP	2	2	525.0	0.0	100.0% [-]	100.0%	100.0%
IP	2	1	2,500.0	16.7	83.3% [50.7–116.0]	66.7%	95.3%
AP	1	3	850.0	0.0	100.0% [-]	100.0%	100.0%
MP	0	1	200	0	100.0% [-]	100.0%	100.0%
Placebo	0	6	350.0	50.0	90.0% [70.4–109.6]	–	–

[a] PEO: pyrantel embonate and oxfendazole, PEIP: pyrantel embonate, ivermectin, and praziquantel, IP: ivermectin and praziquantel, AP: abamectin and praziquantel, MP: moxidectin and praziquantel.
[b] (1-(post/pre))*100%.
[c] (1-($post_{treated}$/$post_{placebo}$))*100%.
[d] (1-(($post_{treated}$/$pre_{treated}$)*($pre_{placebo}$/$post_{placebo}$)))*100%.

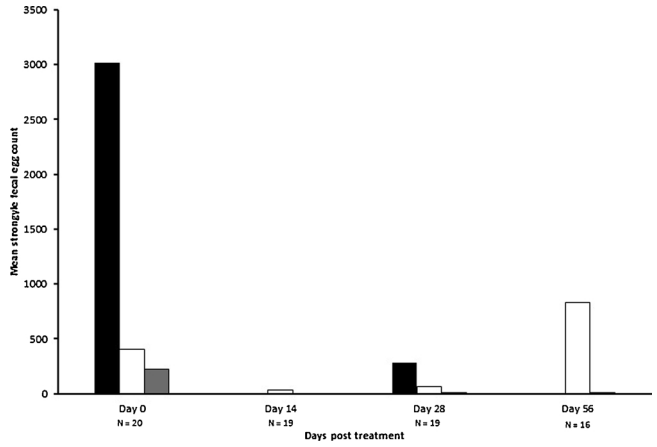

Fig. 1. Mean faecal egg counts following treatment with the pyrantel embonate-oxfendazole (PEO) combination. Arithmetic mean faecal egg counts (± sem) for *Parascaris* spp. (black), strongylids (white) and *Strongyloides westeri* (grey).

untreated or placebo-treated control groups.

Macrocyclic lactone resistance in *Parascaris* spp. to ivermectin in Trial 1 and to all three ML actives (ivermectin, abamectin and moxidectin) in Trial 2 was demonstrated in the Auckland and Waikato farms (Tables 1 and 2). It is interesting to note that the Gisborne farm, which generally exhibited the highest treatment efficacies across both trials, also had the least intensive anthelmintic treatment history. The apparently reduced ivermectin efficacy observed on this farm against *Parascaris* spp. in Trial 2 was observed in a single foal with 50 EPG in the post-treatment sample, and may, thus, not reflect reduced efficacy. These observations illustrate the point that treatment history can impact anthelmintic efficacy on a given farm, and that farms with little or no anthelmintic resistance still exist. The reduced efficacy of the PEIP combination on the Auckland farm suggested the presence of emerging resistance to pyrantel embonate as well as in *Parascaris* spp. A few cases of pyrantel-resistant *Parascaris* spp. populations have been reported in recent years, including in Australia (Armstrong et al., 2014), so it appears plausible that resistance could occur simultaneously to both the pyrimidine and macrocyclic lactone classes, but this has yet to be reported.

The efficacy of the benzimidazole combinations against *Parascaris* spp. demonstrated the practical benefit of using combinations when the resistance status of parasites on a property is unknown *i.e.,* there is greater likelihood that the treatment will be effective. In Trial 1, OXI alone was highly efficacious against *Parascaris* spp. on all farms. In both Trial 1 and 2, the combinations containing a benzimidazole (IPO or PEO) were efficacious against *Parascaris* spp. on all three farms. In contrast, both OXI and PEO demonstrated reduced efficacy against strongyles on one farm. Similarly, products containing MLs had good efficacy against strongylid parasites in both trials, but evidence of ML resistance in *Parascaris* spp. was noted in both trials. Thus, combinations containing BZ and ML products may offer advantages in foals co-infected with strongylids and ascarids, as single active products are unlikely to have full efficacy against both parasite categories. How using such combination products over time may affect further resistance development remains to be investigated.

The egg reappearance period (ERP) for *Parascaris* spp. following anthelmintic treatment should be interpreted with the greatest caution as foals are known to eliminate these parasites at about 5–7 months of age (Fabiani et al., 2016). Unfortunately in Trial 2, the FEC data in the placebo treated group were unavailable for comparison with the treated groups as the foals with positive FEC were withdrawn from the trial after Day 14 to satisfy stud management criteria. As mentioned, the foals enrolled in the present study, were approximately 4 months old at the day of treatment, which means that patent *Parascaris* spp. infections were unlikely to remain well established two months later. Thus, in general, ERP data are only meaningful for strongylid parasites.

The strongylid efficacy data were much as expected given the current global resistance situation involving these parasites (Peregrine et al., 2014). Reduced efficacy of the OXI alone and the PEO combination was observed on the Auckland farm, while ML efficacies were high against these parasites. The observed reduced efficacy of AP on the Waikato farm (W2) was based on a single increasing egg count post-treatment, and it is possible that the foal in question did not consume the full dose.

The *S. westeri* data were sparse and only allowed limited efficacy estimates (Table 3). There is no evidence suggesting development of anthelmintic resistance in this parasite, and macrocyclic lactones have been reported to be particularly efficacious (Costa et al., 1998; Felippelli et al., 2015). The observed reduced efficacy of IP in this study was again due to a single foal not reducing its egg count to 0 EPG, and it should be noted that the placebo product was 90.0% efficacious. *Strongyloides westeri* is sporadic and inconsistent in occurrence in this age group, which makes the parasite less suitable for FECRT testing. Certainly, an untreated control group is required for interpreting the data.

While untreated or placebo-treated control groups are normally included in FECRT studies conducted with livestock animals (Coles et al., 1992), equine anthelmintic efficacy studies are usually designed without control groups and make use of the FECR(T) calculation method (Vidyashankar et al., 2012). The main reason for this is the limited number of FEC positive animals present on a given farm, and the reluctance among owners and managers to leave groups untreated. While this approach appears to work well with strongylid FEC data, which has been described to be consistent within individual horses over time (Nielsen et al., 2006; Carstensen et al., 2013), *Parascaris* spp. and *S. westeri* FEC data may be very different. As mentioned above, equine *Parascaris* spp. occurrence, abundance, and FEC levels are largely dictated by foal age with a clear peak at 4–5 months of age, before the host immune response starts eliminating the parasites (Fabiani et al., 2016). This means that depending on where a given foal is relative to this *Parascaris* spp. peak, its *Parascaris* spp. FEC may be either naturally increasing or declining over the 14-day period used to conduct the FECRT. Without a control group, there is no way of accounting for this pattern. Similarly, *S. westeri* FECs in this age group were found to be of very sporadic occurrence, where it is unlikely to remain consistently positive over a 14-day period as illustrated by the reduction observed in the control group (Table 3). Taken together, there are strong arguments for the value of an untreated or placebo-treated control group for evaluating treatment efficacy against *Parascaris* spp. The Presidente formula (FECR(P)) used here appears appropriate for calculating FECR results given that it accounts for changes occurring in the control group. It is worth noticing that it generally returned higher anti-*Parascaris* spp. efficacy estimates than the two other calculation methods used because of the general increase in *Parascaris* spp. FEC in the control groups (Tables 1 and 2).

The rolling enrolment of foals over the course of the study may be particularly useful for *Parascaris* spp. treatment efficacy studies. With the limited number of foals typically available on many horse farms, this approach is likely to increase the number of foals eligible for the study. Foals are born over the course of several months during late winter, spring, and early summer, so a single treatment time point will generally mean that some foals will be too young and others too old to harbour patent ascarid infections. The rolling enrolment scheme can help ensure that foals are enrolled within the aforementioned ascarid peak period (Fabiani et al., 2016), and, thus, the study design used here can help account for age as a confounder. Rolling enrolment requires multiple visits to each farm over the course of a four-month period, so it is more labour-intensive than the classic FECRT approach, but it can improve the quality of the data generated, and should be recommended

for future equine ascarid FECRT studies.

In conclusion, this study found evidence of resistance to three different macrocyclic lactone anthelmintics in *Parascaris* spp., and only the oxibendazole alone or in combination with an ML and the pyrantel embonate/oxfendazole combination demonstrated adequate *Parascaris* spp. FEC reduction on all farms. This contrasted with the anti-strongylid efficacy data, where the benzimidazoles, in the absence of a ML, failed to achieve a satisfactory reduction in FEC on two farms in each trial. The efficacy data also appeared to reflect differences in anthelmintic treatment intensity on the four studied farms, with the least treated farm exhibiting the highest efficacy levels. Furthermore, the data illustrated the importance of control groups for studies evaluating anthelmintic treatment efficacy against non-strongylid nematodes in horses, and it demonstrated the utility of a rolling enrolment study design, although the study was still limited by small group sizes. Taken together, this study illustrated the importance of evaluating anthelmintic efficacy of combination anthelmintic products, and how parasite control can be particularly challenging in foals, given the very different parasite categories present at any one time.

Conflict of interest

The authors declare no conflicts of interest.

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