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The efficacy of Ivermectin against strongyles in yearlings on Thoroughbred breeding farms in New Zealand

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Highlights

- Faecal egg count reduction tests used to assess efficacy of ivermectin against strongyles in yearling Thoroughbreds
- 113/117 yearlings had a faecal egg count of zero seven days after ivermectin treatment
- On three out of six stud farms there was a suggestion of shortened egg reappearance periods.
- Shortened egg reappearance period could indicate a decline in ivermectin efficacy against the luminal stages.

Abstract

Against a global background of increasing anthelmintic resistance in parasites and a domestic over-reliance on anthelmintics to control gastrointestinal parasites, little is currently known about the resistance status of equine parasites in New Zealand. The objective of the study was to investigate the efficacy of ivermectin in yearling Thoroughbreds. Data were collected from six stud farms from November 2014 to January 2015. Yearlings were selected based on not being treated with anthelmintics for a minimum of six weeks previously and on having a preliminary screening strongyle faecal egg count (FEC) of 25 eggs per gram (EPG) of faeces or greater. On Day 0, faeces were collected and yearlings were treated with ivermectin (a minimum of 200µg/kg). On Days 7, 14 and 21 faeces were again collected from the yearlings, with follow-up samples take on either Day 28, 35 or 42 depending on the stud farm. In total, six stud farms and 117 yearlings (median 23 yearlings; range 5 to 27 yearlings per stud) were eligible for the study. The mean FEC on Day 0 was 609.6 EPG (range 100 to 2000). The majority of horses (113/117) had zero egg counts 7-days after treatment. One and five horses had positive FEC 14 and 21 days' post-treatment, respectively. The efficacy of ivermectin ranged from 99.8% to 100% on Day 7 to between 98.5% and 100% on Day 21. On one stud, the efficacy on day 14 was 94.4%. There was reduced efficacy (<90%) on three studs 28 to 42 days' post-treatment suggesting shortened egg reappearance period (ERP). On one stud farm (n=7), egg counts remained zero up to 42 days' post-treatment. The FECRT observed could be interpreted

as consistent with the continued efficacy of ivermectin in the egg-laying adult stages. However, shortened ERP after ivermectin treatment is a concern and current practices for parasite control in horses in New Zealand are arguably not sustainable.

Keywords: horse, strongyle, anthelmintic resistance, faecal egg count reduction test (FECRT).

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1.1 Introduction

For the last 40 years, horses have been treated regularly with anthelmintics for the purpose of controlling gastrointestinal parasites and to reduce reinfection by keeping the numbers of infective (L3) parasites on pasture to a minimum (Nielsen et al., 2014a). The frequent treatment of horses and lack of refugia have selected for parasites that are resistant to anthelmintics (Kaplan and Nielsen, 2010; Nielsen et al., 2014a; Reinemeyer, 2012; Traversa et al., 2012). Consequently, there have been reports of anthelmintic resistance in important parasites of horses; the cyathostomin species and *Parascaris equorum*, to the commonly used families of anthelmintics (Kaplan and Nielsen, 2010; Nielsen et al., 2014a; Reinemeyer, 2012; Traversa et al., 2012).

Currently, the most widely used method for determining if an anthelmintic treatment is effective is the faecal egg count reduction test (FECRT) (Coles et al., 1992; Coles et al., 2006; Kaplan and Nielsen, 2010). The method for the test is simple and can be applied to all anthelmintic classes available (Kaplan and Nielsen, 2010). A number of recent studies investigating anthelmintic resistance in horses have utilised the FECRT as the primary method for identifying resistance (Garcia et al., 2013; Kaplan et al., 2004; Morris and Colgan, 2012; Stratford et al., 2014; Traversa et al., 2009). The method involves sampling on day zero before treatment and again 7 and/or 14 days post treatment; an effective anthelmintic treatment will result in a reduction in egg count following treatment by greater than 95% (Coles et al., 2006; Kaplan and Nielsen, 2010; Traversa et al., 2009). Screening faecal egg counts (FEC) may be used before the initial treatment to ensure that the egg counts are high enough for treatment, as it can be hard to interpret the results in horses with low FEC (Kaplan et al., 2004).

Despite the fact that surveillance based control strategies have been advocated to reduce reliance on anthelmintics and to delay the further development of resistance (Kaplan and Vidyashankar, 2012; Nielsen et al., 2014a), studies in New Zealand have identified a systemic overuse of anthelmintics in the racing industry (Bolwell et al., 2015; Rosanowski et al., 2016). In these studies,

the most commonly used anthelmintics belonged to the macrocyclic lactone family; ivermectin, abamectin and moxidectin. Ivermectin resistance has been reported in breeding horses in the United Kingdom (Relf et al., 2014) and there is at least one report of resistance in the cyathostomins in New Zealand (Scott et al., 2015). Ivermectin resistance in *Parascaris equorum* in young horses has been identified (Bishop et al., 2014; Nielsen et al., 2014b). While few studies have identified overt cyathostomin resistance using FECRT, some studies have identified reduced egg reappearance period (ERP) (Larsen et al., 2011; Molento et al., 2012; Relf et al., 2014). In other studies, ERP following ivermectin treatment decreased to four weeks (Lyons et al., 2008; Stratford et al., 2011; von Samson-Himmelstjerna et al., 2007). It has been identified that while ivermectin treatment was effective against adult strongyles, luminal stages (L4) that were not all removed by treatment had subsequently matured and were able to produce eggs (Lyons et al., 2009; Lyons et al., 2008). Consequently, ERP has been used as one of the early indicators of anthelmintic resistance in cyathostomins.

The target population for this study focused on one of the largest sectors (Thoroughbred breeding and racing) of the equine industry in New Zealand (Bolwell et al., Submitted). There is a regional concentration in commercial Thoroughbred breeding and training around the Waikato and South Auckland areas and significant seasonal movement of horses between stud farms, with a higher frequency of movements on stud farms with more horses (Bolwell et al., Submitted; Rosanowski et al., 2013). As a result, the management practices of horses on stud farms, particularly weanlings and yearlings, are reported to be fairly homogenous Rogers (Rogers et al., 2007). Given the temperate climate of New Zealand, breeding and youngstock are managed at pasture all year round, although toward the end of their first year, Thoroughbred yearlings are prepared for sale and subsequent racing (Bolwell et al., 2012). A pasture based system, combined with high stocking densities on commercial stud farms (Rogers et al., 2007), results in a longer exposure time when infestation may be possible. Furthermore, yearlings and youngstock have previously been identified as being frequently treated on stud farms (Bolwell et al., 2015; Rogers et al., 2007; Stowers et al., 2009).

These characteristics of the breeding industry provide an opportunity to monitor parasite control in what may be considered a sentinel population most likely to be susceptible to development of resistance. The clinical signs of gastrointestinal parasites impact on the body condition score of the horse, which has been shown to have impact on the value of horses at sales (Pagan et al., 2006).

While anthelmintic resistance has been reported elsewhere, to date few studies have examined the efficacy of ivermectin against cyathostomin in New Zealand. Therefore, the objective of this study was to investigate the efficacy of ivermectin against cyathostomin in yearling Thoroughbred horses, as determined through FECRT.

1.2 Materials and methods

1.2.1 Study population and data collection

In November 2014, six commercial Thoroughbred stud farms in the Waikato region of New Zealand were recruited. The inclusion criteria required stud masters to have at least 10 Thoroughbred yearlings undergoing preparation for yearling sales at the end of January 2015 and to have the horses available for FEC testing, treatment and follow-up before the sales. Yearlings selected for the study could not have been treated with anthelmintic products in the six weeks prior to preliminary screening, or 12 weeks prior to preliminary screening for horses treated with moxidectin. A short face-to-face interview regarding parasite control practices on the stud farm, with specific reference to the yearlings, was conducted with the stud master prior to preliminary screening. One stud master (Farm 5) had expressed concerns regarding shortened ERP following anthelmintic treatment in yearlings prepared for sale in the previous year.

Preliminary screening of yearlings was undertaken at least seven days prior to the start of the trial and horses with a FEC of 25 eggs per gram of faeces (EPG) or greater on this sampling occasion were included in the study. On Day 0, faeces were collected from eligible horses, followed by treatment with ivermectin (Eqvalan[®], Merial Ancare, New Zealand) at a minimum of the standard recommended

dose rate of 200 micrograms per kilogram bodyweight overseen by a veterinarian contracted to the project or the stud farm's own veterinarian. Follow-up collections of faeces were conducted on Days 7, 14 and 21 and on one further occasion, on either Day 28, 35 or 42 after treatment.

On FEC sampling occasions, at least 10 grams of freshly voided faeces were collected from several faecal balls from each animal. All faecal samples were collected and stored in airtight containers at 4°C until processing. All samples were processed by New Zealand Veterinary Pathology (NZVP) using a standard faecal floatation technique (modified McMasters technique). To increase the minimum detectable positive egg count from 50 EPG to 25 EPG, the weight of faeces was doubled, from 1.7 grams to 3.4 grams per sample. To this, 25.5 mls of saturated saline at a specific gravity of between 1.19 to 1.25 g/ml was combined with faeces and samples of the mixture pipetted into two chambers of a McMasters slide. Eggs were identified as either strongyle or *P. equorum*. All samples were processed within 24 hours of collection.

1.2.2 Statistical analysis

Only horses with a FEC of between 100 and 2000 EPG on Day 0 were included in the statistical analysis. Faecal egg count reduction tests (FECRT) were calculated compared to Day 0 counts for each stud farm for all sampling occasions after treatment, using the guidelines recommended by the World Association for the Advancement of Veterinary Parasitology (Coles et al., 2006). Confidence limits (CL) were calculated using the non-parametric bootstrapping methods, with 10,000 iterations (Torgerson et al., 2014). Resistance to ivermectin was defined as a percentage reduction in FEC of less than 95% and lower confidence limit of less than 90% (Coles et al., 1992; Coles et al., 2006). If only one of these criteria were met, resistance was suspected on the stud farm. For each stud farm, ERP was reported as the first week when the percentage reduction in FEC fell below 90% compared to the Day 0 FEC (von Samson-Himmelstjerna et al., 2007), excluding any horses with a positive FEC on Day 7 or 14. All analyses of FEC and FECRT were conducted using R version 3.2.2 and the package eggCounts (Torgerson et al., 2014).

1.3 Results

1.3.1 FEC, FECRT and ERP

In total, 193 yearlings on six stud farms underwent preliminary screening; 62.6% (124/198) were colts. The median age at the start of the trial was 14 months (range 12 to 16 months). In total, 148 yearlings had a positive FEC (>25 EPG) at preliminary screening and were included in the study and subsequently treated with ivermectin on Day 0. Prior to treatment, the average FEC was 574.8 EPG (range 0 to 7,550). Despite horses being selected based on a preliminary positive FEC, 5% (2/39), 33% (4/12), 17% (2/12) and 10% (3/30) yearlings from Stud 2, 3, 4, and 5, respectively, did not have positive FEC at Day 0. In total, 117 horses were eligible for inclusion in the study based on Day 0 FEC and the average FEC for eligible horses was 609.6 EPG (range 100 to 2000). On four of the studs, horses were dosed at the minimum dose rate based on measured weight or estimated by weight tape and on Stud 5 all horses were treated at a 600 kg dose rate. Stud 6 discontinued the study after Day 7 and Stud 1 discontinued after Day 21. Follow-up samples were collected on Day 28 and Day 35 on one stud each and Day 42 for two further studs. Table 1 summarises the number of yearlings treated and the stud FEC for each sampling day during the study period.

The results of FECRT are summarised in Table 2. The majority of horses (113/117; 97%) had zero egg counts 7-days after treatment. One and five horses had positive FEC 14 and 21 days post-treatment, respectively. On Stud Five one horse had a positive FEC on Day 7, 14 and 21. On day 21, 13% (3/23) of horses had a positive FEC, with 25 EPG each. The efficacy of ivermectin on Stud 5 on day 14 was 94.4% (CL 80.9 to 100). On all other studs the efficacy of ivermectin ranged from 99.8% to 100% on Day 7 to between 98.5% and 100% on Day 21. On Stud 3, the efficacy of ivermectin on Day 28 was 79.6% (CL 35.1 to 94.4). On Stud 2, the efficacy of ivermectin on Day 35 was 34.6% (CL 0 to 81.0), with three of the four horses tested having a positive FEC (250 to 775 EPG). For Stud 4 and Stud 5, the efficacy of ivermectin on Day 42 was 100% (CL 100 to 100) and 65.8% (CL 45.7 to 82.8),

respectively. On Stud 5, 63% (14/22) of horses with a negative FEC on Days 7 and 14 had a positive FEC on Day 42. By Day 42, Stud 4 did not have any horses with a positive FEC.

1.3.2 Parasite control practices

All stud masters provided information regarding the most recent anthelmintic product used to treat yearlings, one stud master provided information for 6 months of treatments and two stud masters provided information on all treatments in the previous year, providing details on a total of 18 treatment occasions. In all treatments, products included two or more families of anthelmintics. The most commonly used anthelmintic family was the macrocyclic lactones (14/18; Abamectin n=8), followed by praziquantel (12/18), benzimidazoles (8/18; oxfendazole n=5) and the tetrahydropyrimidines (5/18; pyrantel n=3). In two of the three complete treatment records, the anthelmintic products used were rotated between anthelmintic families at least once. The parasite control strategies used by the stud masters are summarised in Table 3. On the stud farm treating yearlings based on FEC results, the strategy included treating yearlings a minimum of twice per year. In the cohort of yearlings included in the study, horses were treated six-weekly, based on FEC results. On some stud farms, harrowing, cross-grazing and paddock rotation was used. On all stud farms, faeces were not collected from paddocks where yearlings were kept.

1.4 Discussion

The FECRT results of this preliminary study showed continued efficacy of ivermectin against the egg-laying adult stages, but the potential for strongyle resistance to ivermectin based on shortened ERP was identified. Yearlings and youngstock have previously been identified as some of the most frequently treated animals on stud farms, with anthelmintic treatment on intervals of less than 6 weekly or up to 15 times per year (Bolwell et al., 2015). If resistant parasites were present, it would be likely that they would be present in this group. Yearlings are often managed more intensively than other horses on the stud farm, with periods of stabling or yarding prior to sale (Bolwell et al., 2010). This change to intensive management provides an opportunity for regular monitoring and

sample collection from yearlings. However, the commercial nature of yearling preparation and the increased labour demands during this time make on-farm sample collection challenging, which did result in some loss to follow-up in the current study. However, the management practices and number of yearlings on farm were considered to be representative of a wider population of commercial Thoroughbred farms (n=40).

Recent studies have reported shortened ERP for ivermectin of only four to five weeks (von Samson-Himmelstjerna et al., 2007), with the normal ERP for ivermectin reported as 8 to 14 weeks (Molento et al., 2012). Shortened ERP were observed on three studs in the current study when FEC were conducted 28 to 42 days after treatment. Studies have identified reduced ERP as one of the early indicators of anthelmintic resistance in cyathostomins (Stratford et al., 2011; von Samson-Himmelstjerna et al., 2007). A shortened ERP is important as declining efficacy against the larval nematodes of horses is likely to presage overt resistance to ivermectin in the adult stages. Additionally, for stud masters attempting to implement targeted treatment regimens, where treatment is given only when FEC exceed a certain threshold, the reduced ERP may mean that some horses may actually be treated more frequently since they have egg counts high enough to trigger retreatment after as little as 4 to 6 weeks. The more frequent treatment of horses was already occurring on at least one of the stud farms involved in the current study.

While one stud farm met a definition for anthelmintic resistance (Coles et al., 2006), with treatment with ivermectin failing to reduce the EPG of faecal samples on Day 7 and Day 14 by more than 95% and the lower CL of reduction being less than 90%, it is unclear whether this was due to parasites that were resistant to ivermectin or horses not receiving a full therapeutic dose. The low efficacy of ivermectin observed 14-days after treatment on Stud 5 was the result of continued shedding of eggs by only one animal. It is possible that this animal may not have been adequately treated, despite yearlings on this stud farm being dosed at rates appropriate for 600 kg horses (approximately 1.5 times appropriate dosing) and no reports of dosing failure in these horses. This finding is similar to a

previous report, where two out of 13 horses had post treatment egg counts of <90% of the pre-treatment FEC (Kaplan et al., 2004). When the efficacy of ivermectin was retested on this farm, with horses treated with either full and half dose, efficacy was high (FECRT of >99.99%) (Vidyashankar et al., 2012). Without follow-up retreatment on this stud farm, this result only raises the suspicion of anthelmintic resistance.

Even though widespread inefficacy has not been identified in the current study, the current practices for parasite control in breeding and race horses in New Zealand are arguably not sustainable (Bolwell et al., 2015). Furthermore, identification of shortened ERP on more than one stud farm should be a cause for concern, given its link with reduced efficacy in luminal larval stages of cyathostomins (Lyons et al., 2009; Molento et al., 2012). This study has indicated that we may be on the road to overt resistance in the Thoroughbred industry in New Zealand, but further work across more equine properties, in different groups of horses and using other families of anthelmintics should be conducted.

In the future, greater use may need to be made of targeted programs whereby animals are treated with anthelmintic only after a significant level of egg shedding has been demonstrated (Kaplan et al., 2004). The use of this strategy will help protect the efficacy of existing anthelmintics. However, as demonstrated here the targeted strategy may not be as effective at slowing anthelmintic resistance if resistance is already developing and egg reappearance periods have significantly shortened. After treatment with abamectin and morantel, 70% of horses sampled 4 weeks after treatment had egg counts in excess of the treatment threshold (150 EPG or more) for treatment and required retreatment (Scott et al., 2016). Future work is required to elucidate the efficacy of all families of anthelmintics being used in horses in New Zealand.

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1.6 Conflict of interest

The authors report no conflict of interest.

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Table 1: Number of yearlings treated with ivermectin and the faecal egg count (FEC) on Days 0, 7, 14, 21, 28, 35 and 42 of the study. Data collected from yearlings with a minimum preliminary test FEC of 25 eggs per gram from six Thoroughbred stud farms in the Waikato region of New Zealand (n=117).

Stud	Number of yearlings (Number positive at pre-screen)	Mean Day 0 FEC (range)	Mean Day 7 FEC (range)	Mean Day 14 FEC (range)	Mean Day 21 FEC (range)	Mean Day 28 FEC (range)	Mean Day 35 FEC (range)	Mean Day 42 FEC (range)
Stud One	27 (30)	759.3 (225 - 1800)	1.9 (0 - 50)	0 (0 - 0) ^a	1 (0 - 25) ^a			
Stud Two	33 (39)	784.1 (100 - 2000)	1.5 (0 - 25)	1.5 (0 - 125)	0 (0 - 275)		331.3 (0 - 775) ^b	
Stud Three	5 (12)	685 (150 - 1950)	0 (0 - 25)	0 (0 - 25)	10 (0 - 50)	140 (0 - 250)		
Stud Four	7 (12)	550 (100 - 1075)	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)			0 (0 - 0)
Stud Five	23 (30)	308.7 (100 - 700)	65.2 (0 - 1500)	17.4 (0 - 400)	3.3 (0 - 125)			105.4 (0 - 500)
Stud Six	23 (25)	570.7 (100 - 1900)	0 (0 - 0)					

^a n=26

^b n=4

Table 2: Faecal egg count reduction test (FECRT) results for Days 7 to 21 for yearlings on six Thoroughbred stud farms in the Waikato treated with ivermectin. Data collected from yearlings with a faecal egg count on Day 0 of between 100 and 2000 eggs per gram from six Thoroughbred stud farms in the Waikato region of New Zealand (n=117).

Stud Farm	Number of yearlings	Day 7 FECRT (95% confidence limit)	Day 14 FECRT (95% confidence limit)	Day 21 FECRT (95% confidence limit)
Stud One	27	99.8 (99.1 - 100)	100 (100 - 100)	99.9 (99.6 - 100) ^a
Stud Two	33	99.8 (99.4 - 100)	99.8 (99.4 - 100)	100 (100 - 100)
Stud Three	5	100 (100 - 100)	100 (100 - 100)	98.5 (89.5 - 100)
Stud Four	7	100 (100 - 100)	100 (100 - 100)	100 (100 - 100)
Stud Five	23	78.9 (31 - 100)	94.4 (80.9 - 100)	98.9 (97.8 - 100)
Stud Six	22	100 (100 - 100)		

^a n=26

Table 3: Parasite control practices used by stud masters to control gastrointestinal parasites in yearling Thoroughbreds. Data collected from a survey of six Thoroughbred stud farms in the Waikato region of New Zealand.

Stud Farm	Anthelmintic			Yearling paddocks		
	Treatment strategy	Treatment interval	Dose rate calculated	Harrowed	Rotated	Cross-grazed
Stud One	Interval	6 to 8 weekly	Scales	A few times a year	A few times a year	Monthly
Stud Two	Interval	6 to 8 weekly	Scales	Never	A few times a year	A few times a year
Stud Three	Interval and Targeted	As required ^a	Tape	Never	Fortnightly	Monthly
Stud Four	Interval	5 weekly	Estimate	A few times a year	A few times a year	Monthly/Yearly
Stud Five	Targeted	Minimum twice per year ^b	Estimate and scales	A few times a year	Monthly	A few times a year
Stud Six	Worming product interval	<6 weekly	Estimate	Monthly	Monthly	Fortnightly

^a Only the previous anthelmintic treatment date was available from this stud

^b On this stud farm yearlings had been treated every 6 weeks for the previous 6 months