**Strongyle egg reappearance period after moxidectin treatment and its relationship with management factors in UK equine populations**

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**Abstract**

Parasitic nematodes, particularly cyathostomins, are ubiquitous in grazing horses world-wide. Considerable burdens of cyathostomin larvae can encyst in the large intestinal wall. The most recommended treatment against these pathogenic stages is moxidectin. Information is required on how effective moxidectin is against cyathostomin populations in different regions. The objectives here were to determine the efficacy of moxidectin treatment and estimate the strongyle egg reappearance period (ERP) after treatment in several equine populations, to confirm the type of strongyle nematodes present and to identify other (i.e. management) factors associated with shortened ERP. Eight yards were recruited and moxidectin in combination with praziquantel administered to all horses (n=261). Faecal egg count (FEC) analysis was performed at weeks 0, 2, 6, 10 and 12 after treatment to determine efficacy and ERP. The ERP was estimated using two previously published methods. Morphological identification of cultured third stage larvae from the sample population was compared to a *Strongylus vulgaris*-specific end-point PCR to examine the presence of *S. vulgaris* in samples before and after treatment. Strongyle egg shedding patterns were also compared to worm management practices at each site. At 2 weeks post-treatment, moxidectin was highly effective (faecal egg count reduction range, 99.9-100%). The strongyle ERP ranged from 6 weeks to > 12 weeks depending on the calculation method applied. Only cyathostomin larvae were detected by morphological identification. The results from the coprocultures and PCR showed that *S. vulgaris* was absent before and after treatment. Analysis revealed that regular faecal removal from pasture was associated with lower average FEC and lower prevalence of egg shedding.

*Keywords: moxidectin, equines, strongyles, egg reappearance period*

1. **Introduction**

Cyathostomins are ubiquitous in equid populations worldwide. Large numbers of cyathostomin larvae can encyst in the large intestinal wall of horses that graze contaminated pasture (Matthews, 2008). In northern temperate areas, cyathostomin larvae usually encyst in late autumn or winter and when they emerge from the gut wall in large numbers, can cause a fatal typhlocolitis known as larval cyathostominosis (Giles et al., 1985). The treatment of choice against the encysted stages is a single dose of moxidectin or a five-day course of fenbendazole. Given the high levels of fenbendazole resistance in cyathostomins in developed regions (Matthews, 2014), the former treatment is frequently recommended (Reinemeyer et al., 2015).

A recent review (Matthews, 2014) summarized the many reports of anthelmintic resistance in cyathostomins, classified as either a low faecal egg count reduction (FECR) ~ 2 weeks after therapy or a shortened strongyle egg reappearance period (ERP). Particularly, resistance in cyathostomins has been reported using the faecal egg count reduction test (FECRT) for fenbendazole (Lester et al., 2013; Stratford et al., 2014), pyrantel (Traversa et al., 2009; Stratford et al., 2014) and ivermectin (Traversa et al., 2009). Reduced efficacy of moxidectin, measured as a reduced ERP, considered to be an early indicator of anthelmintic resistance in nematode populations (Sangster, 2001), has been observed in geographically-widespread populations (Rossano et al., 2010; Lyons et al., 2011; Geurden et al., 2014; Relf et al., 2014; van Doorn et al., 2014). Furthermore, failure of ivermectin and moxidectin to adequately control cyathostomins up to 28 days after treatment was reported in Brazil (Molento et al., 2008).

The current recommendations for adult horses in several countries (including the UK and USA) are to administer a cyathostomin larvicidal treatment (usually moxidectin) in late autumn/early winter in the northern hemisphere, as there is no diagnostic test to detect these stages (Matthews, 2008; AAEP, 2013). Should moxidectin resistance levels worsen, there will be no chemical options left for treatment of encysted larvae, as no new anthelmintic classes are under development in the short to medium term. The aim here was to determine efficacy of moxidectin and the strongyle ERP after treatment in adult equine populations based in the UK. The last UK study that examined strongyle ERP after moxidectin treatment contained limited numbers of horses, all of which were yearlings or foals (Relf et al., 2014). Immature horses tend to have higher strongyle egg output and worm burdens than adult horses, probably because of higher susceptibility to infection due to a lack of acquired immunity (Smith, 1978; Love and Duncan, 1992; Relf et al., 2013). As a result, observed strongyle ERPs after anthelmintic treatment tends to be shorter in immature horses (Rossano et al., 2010). Hence, ERP analyses of young populations of horses could lead to false positive assumptions for drug failure or emerging anthelmintic resistance.

Large strongyle species (for example, *Strongylus vulgaris*) can cause serious disease if not adequately controlled (Nielsen et al., 2016). A recent Danish study indicated a rise in *S. vulgaris* prevalence where targeted therapy based on faecal egg count (FEC) results was delivered over several years (Nielsen et al., 2012). For this reason, the study here also included examination of the presence of large and small strongyles in samples from the population and compared traditional morphological analysis after coproculture with an *S. vulgaris*-specific end-point PCR using DNA extracted from parasite eggs (Bracken et al., 2012). Finally, the potential impact of management parameters (for example, faecal removal from pasture, deworming protocol followed) on population FEC levels and ERP was explored.

1. **Materials and Methods**
   1. **Participating premises**

Premises that participated (n=8) were selected via an online questionnaire previously distributed (April-July 2015, Tzelos et al., unpublished). This questionnaire was promoted via social media (mainly Facebook1). In addition, 384 equine practice email addresses were obtained from the British Equine Veterinary Association website2. An email detailing the study background and an online link to the survey were distributed to the practices inviting them to promote the survey to clients via websites, social media and/or newsletters. A direct email was also sent to 518 premises, comprising riding schools and livery yards, listed on the British Horse Society website3. Respondents (n=652) to the original survey were asked whether they would be willing to participate in further parasitological studies. Yard owners with ≥20 permanent equine residents that were willing to participate (n=41), were then contacted. Eight agreed to take part in the current study. Six were located in England, one in Wales and one in Scotland. Demographic details and the survey answers for each yard are presented in Table 1 and in Supplementary Table 1, respectively.

* 1. **Sample collection and analysis**

Horse owners/managers were asked to collect faecal samples from all horses residing on each premise to examine efficacy of moxidectin and the strongyle ERP after treatment. Treatment was administered by horse owners (n=2), the manager of the premise (n=5) or a member of staff (n=1) and the dose determined by visual estimation of weight (n=1), scales (n=4) or weigh tape (n=3). Samples were taken at week 0 (before treatment), week 2 post-treatment (pt; for the FECRT), and weeks 6, 10 and 12 pt (for ERP). Participants were sent detailed sampling instructions, the methodology being based on previous protocols to ensure that an adequate representation of sample was obtained from each horse (Lester et al., 2013). Samples were posted to Moredun Research Institute under regulations of the Royal Mail, UK, and stored at 4°C upon arrival. All samples were processed on the day of arrival and within 2-3 days of collection. A modification of the salt (NaCl) flotation method (Christie and Jackson, 1982) was used for the FEC analysis (Bartley and Elsheika, 2011). This method can detect as few as 1 egg per gram (EPG) of faeces. Coprocultures were performed on all samples that were positive for strongyle eggs. Positive samples from each yard were pooled (~10 g of faeces per sample) and eggs cultured to third stage larvae (L3) to examine the presence of small and/or large strongyles using enumeration and morphology of intestinal cells (Thienpont et al., 1986). Tail length was not examined. In addition, approximately 10 g of faeces per positive sample from each premise were used to recover eggs, which were pooled and stored in 100% ethanol at -20°C (Bartley et al., 2003) until DNA extraction.

* 1. ***Strongylus vulgaris*-specific end-point PCR**

In addition to morphological identification of larvae, the presence of *S. vulgaris* eggs in pre- and post-treatment samples was examined using a specific end-point PCR (Bracken et al., 2012). Parasite egg DNA was extracted from pooled samples and used as described previously (Nielsen et al., 2008; Bracken et al., 2012). The house-keeping gene used for verifying integrity of each DNA preparation was the rDNA region spanning the ITS-2 (Gasser and Monti, 1997). *S. vulgaris* DNA extracted from adult worms (Kentucky, USA, isolate), was used as a positive control and DNA, extracted from eggs obtained from faecal samples from a UK horse population not previously treated with macrocyclic lactones (Wood et al., 2013), was used as an additional control. This population had a history of large strongyle infection detected by morphological identification of L3 and a history of *S. vulgaris*-associated disease (Matthews and Tzelos, unpublished).

* 1. **FECRT and ERP analyses**

Microsoft® Office Excel® 2010 was used for data recording and analysis. Arithmetic group mean FEC for week 0 and week 2 were used to estimate FECR for each yard. The FECR was calculated using the following formula, based on the guidelines recommended by the WAAVP (Coles et al., 1992).

There are no clearly defined cut-off values for determination of anthelmintic efficacy in horses (Vidyashankar et al., 2012). Here, previously published recommendations were followed, i.e. an arithmetic mean FECR of >95% (Kaplan and Nielsen, 2010) with 95% lower confidence limits (LCL) were calculated to provide an accurate indication of the range of the data (Vidyashankar et al., 2007). Non-parametric bootstrapping was used to estimate confidence intervals for FECR. Observed individual FEC were resampled with replacement from each of the pre- and post-treatment arrays, and FECR re-calculated. The upper and lower 2.5-percentiles of 10,000 simulations were taken as the 95% confident limits of FECR (Efron, 1979). PopTools software4 (CSIRO, Australia) was used for bootstrapping. A LCL of 90% was selected such that resistance would be indicated if the % mean FECR was below 95% and the LCL was below 90%. If either the % mean FECR *or* the LCL was below the cut-off values, anthelmintic resistance would be suggested. The cut-off values reflect the original efficacy in anthelmintic-sensitive strongyle populations of macrocyclic lactone anthelmintics when first registered (Xiao et al., 1994). There is no agreed consensus as to how to calculate/interpret post-treatment strongyle ERP data in horses. The current recommended ERP or treatment interval, as indicated on the UK Veterinary Medicines Directorate’s product information database5, for moxidectin (EquestTM oral gel, 18,92 mg/g, oral gel for horses and ponies, Zoetis) is 90 days (~12.8 weeks). Here, faecal samples were collected at weeks 6, 10 and 12 following treatment. Once samples from all periods were analysed, the following approaches were employed to calculate ERP: Method 1: ERP defined as the week of the first positive strongyle FEC after treatment (Little et al., 2003); Method 2: defined as the week in which the group arithmetic mean FEC exceeded 10% of the group arithmetic mean FEC of week 0 (AAEP, 2013). .

* 1. **The impact of management measures on strongyle egg shedding patterns**

Mean group FECs from each premise were used to compare FEC levels and various management practices cited by the respondents. This was performed separately for every time-point analysed. Minitab® 17 was used for statistical analysis. The fit of the data to a normal distribution was assessed using the Anderson-Darling test. The non-parametric Kruskal-Wallis Test was used to compare the mean FEC of horses on premises grouped as follows: interval treatment protocol (n=3) *versus* targeted treatment protocol (n=5); faecal removal from pasture (n=5) *versus* no faecal removal from pasture (n=3); livery (n=5) *versus* other types of premise (multipurpose, stud farm, private; n=3). Spearman’s rank correlation was used to examine whether FEC levels were related to the number of equines per premise. A P-value <0.05 was considered significant.

1. **Results**
   1. **Moxidectin efficacy and strongyle ERP after moxidectin treatment**

All parasite eggs observed belonged to strongyle genera; neither *Parascaris equorum* nor *Anoplocephala perfoliata* eggs were observed. The mean percentage FECR (Table 2) on all yards was > 95% (range: 99.9–100%) and the 95% LCL was > 90% (range: 99.7–100%). The strongyle ERP recorded using Method 1 was 6 weeks for all premises and using Method 2, ranged from 6 to > 12 weeks (Table 3). Some owners/managers, after consulting with their anthelmintic prescriber, administered moxidectin to the high shedding horses (i.e. those with a FEC of >200 EPG) at week 10 pt, two weeks after which the FECs of all of these individuals were zero. This was the reason for the increase in mean % FECR for week 12 at premises 5, 6 and 8 (Table 2).

* 1. **Prevalence of strongyle parasite species detected before and after treatment**

Morphological identification of the strongyle larvae demonstrated that only small strongyle species were present before and after moxidectin treatment on all premises. The PCR results also demonstrated that there were no *S. vulgaris* present at all time points (Supplementary Figure 1).

* 1. **Impact of demographic and management factors on strongyle egg shedding levels**

The mean group FECs from premises that removed faeces from pasture (Premises 1, 5, 6, 7, 8) were compared with mean group FECs from premises that did not (Premises 2, 3, 4). The median, 25th and 75th interpercentile range and range of mean FEC for the different time points in premises grouped according to pasture management protocol are shown in Figure 1. The Anderson-Darling normality test showed that the data were not normally distributed (P<0.05) for week 2 pt and week 6 pt, so the non-parametric Kruskal-Wallis Test was employed to compare FEC levels between the two groups. There was a significant difference in median FEC at weeks 10 and 12 pt (P=0.025). There was no significant difference at weeks 0, 2 or 6 pt. A significant difference in FEC levels was also observed at 6 weeks pt when premises were grouped according to deworming protocol (P=0.025), with higher median counts in premises that followed targeted (n=5) as opposed to interval (n=3) treatment protocols. All premises that followed interval treatment regimens removed faeces from pasture. However, within the ‘targeted treatment’ group, there were two premises that removed faeces from pasture and three that did not. The Kruskal-Wallis test showed that there was no significant difference between ‘targeted treatment’ premises that removed (n=2) and did not remove (n=3) faeces; however, FEC levels were higher on premises that did not remove faeces regularly (median=6.632) than those that did (median=3.056). There was no significant difference in FEC levels at any time point when livery (n=5) were compared to other types (n=3) of premise and Spearman’s rank correlation showed that there was no significant relationship between FEC level and number of equines present.

1. **Discussion**

This study examined the effect of moxidectin on strongyle egg shedding in predominantly adult horse populations in the UK. Moxidectin administration proved to be effective in all populations in reducing FECs at 2 weeks pt; however, a shortened ERP was observed in all groups (at 6 weeks after treatment; Method 1) or on seven premises (at 6 or 10 weeks after treatment; Method 2). The treatments were administered by the respondents and so the authors cannot be certain that the correct dose was always administered. Nevertheless, all horses (with one exception at Premise 3) exhibited 0 epg two weeks after treatment. *S. vulgaris* was not observed in the coprocultures nor in the *S. vulgaris*-specific end-point PCR. Larval identification analysis showed that on all premises, parasites recovered before and after treatment were small strongyles (cyathostomins). This was not surprising since all premises had used a macrocyclic lactone compound in 2015. On premises where faeces were removed from pasture, mean FEC levels were lower and fewer animals positive for FEC compared to sites where faeces were not removed. Indeed, there was a significant difference in mean FEC at 10 and 12 weeks after treatment, with higher egg shedding detected at sites where faeces were not routinely removed. There was also a significant difference in mean FEC levels when premises were grouped according to deworming protocol, with those following targeted deworming having higher FEC levels at week 6 pt than interval treatment premises. The type of premise (livery *versus* other type) and the number of resident horses per premise had no relationship with FEC level.

Similar high efficacy of moxidectin has been reported in previous studies in the UK (Lester et al., 2013; Relf et al., 2014; Stratford et al., 2014) and elsewhere (Rossano et al., 2010; Lyons et al., 2011; Geurden et al., 2014; van Doorn et al., 2014). All identified high efficacy of moxidectin 2 weeks after treatment, but a shortened strongyle ERP. Cyathostomin resistance to moxidectin, identified as an inability to reduce mean FEC by >95% of Day 0 FEC has been reported in Brazil (Molento et al., 2008). Particularly, it is mentioned that “Of greatest concern is the failure of all the products tested to give adequate control of cyathostomins up to 28 days after treatment” (Molento et al., 2008). To date, there have been no other published reports of cyathostomin resistance to moxidectin. Current recommendations regarding the ERP/treatment interval for moxidectin (EquestTM oral gel, Zoetis) is 90 days in the UK5 and other European countries (for example Greece6, Spain7, Germany8). The quoted ERP in Australia is at least 14 weeks9, whilst in the USA, product information10 states that “one administration suppresses strongyle egg production for 84 days”. The most recent UK study on stud farms reported an ERP of 4 weeks for Method 1 and >6, 8 and 9 weeks for Method 2, respectively, on each farm (Relf et al., 2014). In this previous study, all horses for which a shortened ERP was reported were yearlings or foals. Two other studies in Europe reported a 6-week ERP based on Method 2 (Geurden et al., 2014; van Doorn et al., 2014). Geurden et al. (2014) examined efficacy and ERP after moxidectin treatment at 32 sites in Belgium, Italy and the Netherlands (12, 10 and 10, respectively); however, the age range was not reported. It is known that young animals are more likely to have higher levels of strongyle egg shedding than adult horses (Boersema et al., 1996). Thus, mean group FEC levels in young animals after treatment might reach 10% of Day 0 mean group FEC faster compared to adults. Similarly, in a US-based study, a shortened strongyle ERP was observed in yearlings after moxidectin treatment (Rossano et al., 2010). In the USA, strongyle ERPs of 5 and 6 weeks were reported using Methods 1 and 2, respectively (Rossano et al., 2010). Earlier studies showed that previous exposure to cyathostomins affects strongyle FEC output (Smith, 1978; Love and Duncan, 1992). Generally lower FECs were observed in animals previously exposed to cyathostomins (as is usually the case for adults), than in cyathostomin-naïve animals (as is usually the case for immature horses) (Love and Duncan, 1992). Previous exposure reduces susceptibility to infection and leads to slower development of the worms in the host (Smith, 1978; Love and Duncan, 1992). Thus far, only one published study has reported a shortened strongyle ERP in adult horses after moxidectin treatment (van Doorn et al., 2014). In this Dutch study, which included only 13 horses (age range 4 months – 4 years), strongyle ERP after moxidectin treatment was 6-8 weeks using Method 2. Nevertheless, the numbers of immature and adult horses treated were not reported (van Doorn et al., 2014). In the current study, out of a total of 261 horses, only two were under 2 years-old (Premises 4 and 7) and, with the exception of one site (Premise 1), a shortened ERP was observed after treatment. Keeping the age of the animals in mind, the results here are of concern, because the strongyle ERP reported is similar to the previous studies performed in populations of immature horses.

When moxidectin was first introduced, studies indicated that FECs in treated horses were suppressed for >12 weeks, and up to 22 weeks, after treatment (Demeulenaere et al., 1997; DiPietro et al., 1997; Boersema et al., 1998). Further evaluation showed a prolonged persistence of moxidectin, and the compound could be detected in plasma up to 75 days after administration (Perez et al., 1999). Prolonged persistence of moxidectin could lead to sub-therapeutic exposure of the nematodes to anthelmintic over time, theoretically increasing selection pressure for resistance. It is most likely that the strongyle eggs first observed here after anthelmintic administration were derived from female adult worms that developed from mucosal larval stages that survived treatment, as opposed to eggs derived from newly-acquired infections post treatment; this is because of the prolonged pre-patent period likely to occur in previously-exposed adult horses (Love and Duncan, 1992; Chapman et al., 2002). Likewise, eggs are unlikely to have come from adult female worms surviving treatment, as in that case the FECs would be expected to persist at 2 weeks pt, which was not the case here. If the eggs observed after treatment do originate from cyathostomins that are at the early stages of developing resistance, it is essential that best practice be implemented in control programmes. Central to this, is the application of improved pasture hygiene to reduce contamination and, in so doing, break the parasite life cycle to reduce infection level. In addition to reducing stocking density and implementing rotational grazing, dung removal is recommended (Corbett et al., 2014). This should be implemented at a frequency that does not enable translation of L3 from faeces onto grass. While dependent on climate, the current recommendation in temperate areas is to remove faeces at least twice-weekly (Corbett et al., 2014). This practice has had variable uptake across different regions. A study in Sweden nearly a decade ago reported a lack of implementation, with only 6% of respondents stating that they removed faeces from pasture (Lind et al., 2007). A recent study in the UK indicated better uptake, with 395 out of 492 (~80%) respondents stating that they undertook dung removal (Easton et al., 2016). In one study, pasture hygiene was not identified as a risk factor for infection level; however, the authors believed that the procedure was not being performed properly (Hinney et al., 2011). It was also demonstrated that monthly removal from pastures in South Africa had no impact on donkey FEC levels and more frequent removal was indicated (Matthee et al., 2002). In other studies in Germany and Austria, the application of pasture hygiene has been associated with reduced FEC in horses (Becher et al., 2010). Faecal removal has the potential to increase grazing area by eliminating the separation of pasture into roughs and lawns (Herd, 1986). In the current study, on five premises, faeces were regularly removed from pasture and on three they were not. This enabled a basic comparison in relation to ERP after treatment. Despite a small sample size, a significant difference was observed between the two groups (removal *versus* no removal) at weeks 10 and 12 after treatment (P=0.025), with higher FEC levels being observed at premises that did not practise removal. This could result from a higher level of infection, due to poor pasture hygiene conditions. Consequently, higher larval burdens might accumulate in horses. Larvicidal efficacy of moxidectin has been estimated to be 63.6% and 85.2% against encysted L3 (EL3) and late L3/L4 (LL3/L4) encysted cyathostomins, respectively (Reinemeyer et al., 2015). As a result, the number larvae surviving treatment, and subsequently laying eggs, could be higher in horses grazing in pastures with poor hygiene conditions. Furthermore, premises that followed targeted deworming had significantly higher FEC levels at week 6 pt than those that followed an interval protocol. A potential explanation for this is that three out of five ‘targeted treatment’ premises did not remove faeces from pasture, whilst all ‘interval treatment’ premises did. FEC levels were higher on the ‘targeted treatment’ premises that did not remove faeces from pasture; however, the low sample size did not permit statistical examination of interactions between targeted treatment and faecal removal. The analysis suggests that targeted treatment protocols should be combined with faecal removal from pasture. This is because, if FEC analysis is not performed at regular intervals, there is a risk that considerable numbers of eggs could be shed, and when faeces are not removed, these hatch to larvae which translate to pasture. Faecal removal is not the only parameter that affects pasture contamination; this is also affected by stocking density and climate. For this reason, targeted treatment protocols must encompass high levels of general management, particularly when high proportions of young horses are present.

**5. Conclusions**

In conclusion, a shortened moxidectin ERP was observed on seven out of eight sites, in a primarily adult horse population with more than 20 animals per site. This is the first time that this age group and sample size have been studied in this context. Preliminary evidence suggested a relationship between the implementation of pasture hygiene methods and FEC after treatment; where faeces were removed, there were fewer FEC-positive horses and significantly lower mean egg shedding levels at certain time points after moxidectin administration compared to premises where faeces were not removed.

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Table 1. Details of the participating premises. Location of the premise, type of premise; number, age range and median age of permanent residents; type of deworming protocol implemented before the study on each premise (interval *versus* targeted treatment protocol); and, whether faeces were removed regularly from pastures on each premise.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Reference number (location) | Type of premise | Number of permanent residents (age range; median) | Previous type of deworming protocol | Faeces removed from pasture? (frequency) |
| 1  (South-East England) | Multipurpose | 31 (3-29; 11) | Interval treatment | Yes  (daily) |
| 2  (South Wales) | Livery | 35 (4-24; 11) | Targeted treatment | No |
| 3  (South Scotland) | Livery | 46 (4-27; 13) | Targeted treatment | No |
| 4  (Central England) | Livery | 20 (1-41; 14) | Targeted treatment | No |
| 5  (South-East England) | Livery | 22 (4-30; 15.5) | Targeted treatment | Yes  (every 2-7 days) |
| 6  (Northern England) | Livery | 23 (4-25; 12) | Interval treatment | Yes  (every 2-7 days) |
| 7  (South-west England) | Stud farm | 61 (1-21; 10) | Interval treatment | Yes  (every 2-7 days) |
| 8  (Central England) | Private | 23 (7-29; 16) | Targeted treatment | Yes  (every 2-7 days) |

Table 2. Faecal egg count (FEC) data from all premises and time points. Detailed are the group arithmetic mean rounded to the nearest integer in eggs per gram (EPG), and the maximum individual EPG score; the number of positive horses (EPG>0) and the % of yard population; the arithmetic mean faecal egg count reduction (FECR; %) compared to week 0; and, the 95% lower confidence limits (LCL; %) observed at each site for all time points.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Premise (n horses tested) | Time point  (week) | Mean EPG (max) | Number of strongyle FEC - positive horses (% of yard population) | Mean % FECR | 95% LCL |
| 1 (n=31) | 0 | 97 (1044) | 17 (55%) | - | - |
| 2 | 0 (0) | 0 | 100 | 100 |
| 6 | 0 (1) | 4 (13%) | 99.9 | 99.7 |
| 10 | 3 (36) | 10 (32%) | 96.8 | 93.6 |
| 12 | 5 (84) | 10 (32%) | 95.3 | 89 |
| 2 (n=35) | 0 | 98 (477) | 25 (71%) | - | - |
| 2 | 0 (0) | 0 | 100 | 100 |
| 6 | 13 (180) | 11 (31%) | 86.3 | 70.4 |
| 10 | 38 (342) | 19 (54%) | 60.9 | 31.7 |
| 12 | 27 (219) | 17 (49%) | 72.2 | 52.6 |
| 3 (n=46) | 0 | 83 (774) | 39 (85%) | - | - |
| 2 | 0 (4) | 1 (2%) | 99.9 | 99.7 |
| 6 | 2 (20) | 14 (30%) | 97.9 | 96.2 |
| 10 | 20 (126) | 36 (78%) | 75.7 | 65.1 |
| 12 | 46 (237) | 35 (76%) | 44.5 | 21.5 |
| 4 (n=20) | 0 | 199 (882) | 16 (80%) | - | - |
| 2 | 0 (0) | 0 | 100 | 100 |
| 6 | 7 (78) | 5 (25%) | 96.7 | 92 |
| 10 | 36 (174) | 15 (75%) | 82 | 67.8 |
| 12 | 32 (198) | 15 (75%) | 83.7 | 69.2 |
| 5 (n=22) | 0 | 78 (693) | 11 (50%) | - | - |
| 2 | 0 (0) | 0 | 100 | 100 |
| 6 | 1 (20) | 1 (5%) | 98.5 | 95.7 |
| 10 | 18 (288) | 3 (14%) | 77.4 | 34 |
| 12 | 1 (12) | 2 (9%) | 98.9 | 97 |
| 6 (n=23) | 0 | 17 (108) | 13 (57%) | - | - |
| 2 | 0 (0) | 0 | 100 | 100 |
| 6 | 1 (12) | 4 (17%) | 95.6 | 88.5 |
| 10 | 10 (150) | 7 (30%) | 40 | -59.3 |
| 12 | 4 (39) | 6 (26%) | 76.7 | 46.6 |
| 7 (n=61) | 0 | 157 (999) | 57 (93%) | - | - |
| 2 | 0 (0) | 0 | 100 | 100 |
| 6 | 1 (30) | 10 (16%) | 99.3 | 98.5 |
| 10 | 19 (225) | 28 (46%) | 87.7 | 79 |
| 12 | 26 (531) | 24 (39%) | 83.2 | 65.8 |
| 8 (n=23) | 0 | 47 (810) | 7 (30%) | - | - |
| 2 | 0 (0) | 0 | 100 | 100 |
| 6 | 5 (111) | 5 (22%) | 89.4 | 68.8 |
| 10 | 15 (324) | 7 (30%) | 69 | 8.3 |
| 12 | 1 (10) | 3 (13%) | 98.7 | 96.5 |

Table 3. Strongyle egg reappearance period after moxidectin treatment, calculated using two different methods as detailed in Section 2. 4.

|  |  |  |
| --- | --- | --- |
| Premise | ERP - Method 1 | ERP - Method 2 |
| 1 | 6 weeks | >12 weeks |
| 2 | 6 weeks | 6 weeks |
| 3 | 6 weeks | 10 weeks |
| 4 | 6 weeks | 10 weeks |
| 5 | 6 weeks | 10 weeks |
| 6 | 6 weeks | 10 weeks |
| 7 | 6 weeks | 10 weeks |
| 8 | 6 weeks | 6 weeks |

Figure captions

Figure 1. Median (circle within box), the 25th and 75th interpercentile range (box) and the range of the mean FECs (whiskers) at the different time points in premises grouped according to the pasture management protocol (faecal removal [YES, Y] *versus* no removal [No, N]).