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Analysis of strongyle egg shedding consistency in horses and factors that affect it

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Abstract

Strongyle egg shedding consistency in horses and factors affecting consistency were investigated. Faecal samples were collected from 26 equine populations over one grazing season. Samples were collected on four 'screening' occasions (S1–S4) and FEC performed using a sensitive method (to 1 eggs per gram (epg) egg detection limit). On each screening occasion, FEC were assigned an egg shedding category: 1 (<50 epg) to 7 (>500 epg); and a treatment category: <200 epg (no treatment) or ≥ 200 epg (treatment). Rank changes in shedding and treatment categories between S1 and subsequent screening occasions were calculated. Factors affecting the likelihood of an individual changing shedding or treatment category were assessed using multivariable logistic regression of FEC data from horses that had not received anthelmintic during the study. In total, 573 horses were sampled at S1, 468 at S2, 417 at S3 and 83 at S4. Results showed that between S1 and S4, 73.5% (61/83) horses remained in the same egg shedding category and 94.0% (78/83) in the same treatment category. For horses that did not receive anthelmintic (n=304), 90.4% (225/304) remained in the same egg shedding category. Horses under 5 years-old were more likely to change egg shedding (odds ratio, OR 3.3, 95% CI: 1.22-8.46) and treatment (OR 2.8, 95% CI: 1.1-6.3) categories compared to older horses. These results suggest a high level of consistency in strongyle egg shedding in individuals within one season, and withholding anthelmintics from horses with negative or low (i.e. <50 epg) FEC does not appear to be associated with significant increases in egg shedding.

Keywords: Horse, Cyathostomins, Strongyle egg shedding, Targeted anthelmintic treatment

1. Introduction

Anthelmintic resistant nematodes pose a threat to equine health and welfare. Targeting anthelmintic treatments to individual horses based on levels of nematode egg shedding is proposed to achieve control, whilst reducing anthelmintic use and selection for resistance. Helminth parasites are typically overdispersed in their hosts: i.e. relatively few individuals within populations are infected with the majority of the associated parasite population [1-4]. Strongyle nematodes in the large intestine are the most important equine parasites and have a high prevalence [5]. Overdispersion has been demonstrated for strongyle faecal egg counts (FEC) in horses, with most individuals shedding relatively low numbers of eggs [6-8]. This overdispersion underpins the rationale behind targeted anthelmintic treatment strategies [5, 9-10], whereby only horses identified as shedding above a specific threshold of eggs (typically ≥200 eggs per gram (epg)) are recommended for treatment. Several studies have shown consistency in strongyle egg shedding patterns in individuals over time [7, 11-14], which could further enhance efficient targeting of anthelmintic treatments and reduce the required frequency of FEC testing. The aim here was to investigate strongyle egg shedding consistency in populations of horses in the UK that followed a targeted anthelmintic treatment programme, with a focus on horses identified as low strongyle egg shedders (<50 epg), to examine whether non-treatment of these individuals was associated with increases in egg shedding levels over time. This study also analysed factors associated with shedding consistency in individuals within these populations. A longitudinal cohort study design was used.

2. Materials and methods

2.1. Study population

During 2010–2012, 573 horses from 26 equine holdings (yards) in Scotland and England were recruited (see *supplementary data*). The number of individual's resident on each yard ranged from 7-72 (median, 20 horses). Most horses (i.e. those on 19 yards) received moxidectin prior to the start of the study, although all had received a macrocyclic lactone (ML) within the six months prior to the start of the study (n=573). On three yards, information on which anthelmintic was last administered was not supplied. Most horses were resident at livery yards (n=23 yards). The remainder comprised of a non-Thoroughbred stud farm, a sport horse yard and a rescue/welfare sanctuary. In total, 573 horses were screened at S1, 468 horses at S2 and 417 at S3. A total of 83 horses on three yards were screened at S4. Each horse was assigned an age category. The age distribution was: foal (<2 years; n=26), youngster (≥2 and <5 years; n=68), adult (≥5 and < 18 years; n=418) and geriatric (≥18 years; n=61).

All horses included in this study had access to grazing, were at pasture during the study and had been treated with a ML anthelmintic within the previous 6 months. Horses were at pasture for a minimum of eight hours per day, and were grazed on the same pastures for the duration of the trial (i.e. returned to the same pasture after sampling and treatment). Yards with a minimum of 10 horses were included and all horses were subject to the same anthelmintic treatment regimen (Section 2.2). Each yard was supplied with a questionnaire to provide information on the demographics (i.e. yard function, number of horses etc.), anthelmintic usage (frequency of treatment, last product used, type of deworming programme followed) and general management

practices. Yard managers were asked to supply the age of the horses included in the study, but not specific details on breed and sex. On all yards, the manager was the point of contact and was responsible for completion of the questionnaire, coordinating sample collection and postage, and administering anthelmintic treatments.

2.2. Targeted treatment protocol

Horses that were previously treated with anthelmintic were sampled after a minimum period of 18 weeks had elapsed after administration of moxidectin, or 14 weeks for ivermectin. The first sample (Screen 1, S1, n=573) was collected between February and March. Horses with FEC ≥200 epg were treated with pyrantel embonate following the manufacturer's instructions (Strongid-P™[a] at a dose rate of 19mg/kg bodyweight). All horses were FEC screened 8-10 weeks later: this was based on a 6-week strongyle egg reappearance period (ERP) for pyrantel embonate [15, 16], plus an additional two weeks (Screen 2, S2; May/June, n=468). Horses with FEC ≥ 200 epg at S2 were treated with ivermectin (Eqvalan® oral paste for horses[b]; 0.2mg/kg). All horses were screened 10–12 weeks later based on an ivermectin strongyle ERP of 8-10 weeks [15], plus two additional weeks (Screen 3, S3; August/September, n=417). At this point, horses with a FEC of ≥200 epg were treated with moxidectin (Equest®[c]; 0.4 mg/kg). On some yards a fourth screen (S4; October/December, n=83) was performed on horses that had not received anthelmintic treatment following S3.

2.3. Sample collection and faecal egg count methodology

Samples were collected from freshly passed faeces and placed into individually labeled zip-lock bags. Yard managers were provided with instructions on how to collect, store and post the samples, and were asked to collect at least three boli from

freshly voided faeces and to place these into the bag, expelling air before sealing. The samples were sent immediately to the Moredun Research Institute and stored at approximately 4˚C. All samples were processed within 4 days of excretion to reduce the effect of egg degradation [17]. A modification of the salt flotation method (1.2 specific gravity) with a detection limit down to 1 epg was used [18]. All samples were analysed in duplicate by taking two 10 ml aliquots from a 100 ml dilution of a well-mixed 10 g sub-sample and an average taken to estimate the epg count.

2.4. Data analysis

2.4.1. Egg shedding and anthelmintic treatment categories

Each FEC data point on each screening occasion (S1, S2, S3, S4) was assigned a shedding category: 1 (0–49 epg); 2 (50–99 epg); 3 (100–199 epg); 4 (200–299 epg); 5 (300–399 epg); 6 (400–499 epg) and 7 (>500 epg). Each FEC data point was also assigned a treatment category: < 200 epg (0, no treatment) or ≥ 200 epg (1, treatment).

2.4.2. Egg shedding consistency

To assess shedding consistency, the rank change in shedding category between S1 and S2, S1 and S3 and S1 and S4 was calculated. For example, if the category was ranked as 1 for S1 and 6 for S2, the rank change would be 5. Conversely, if the category was measured as 6 for S1 and 3 for S3, the rank change would be -3. If the category remained the same, rank change assigned was 0.

2.4.3. Anthelmintic treatment consistency

Treatment category consistency was determined across S1 and S2, S1 and S3 and S1 and S4. If an individual's FEC changed from < 200 epg to ≥ 200 epg between two sampling points, the rank assigned was 1. If it changed from ≥ 200 epg to < 200 epg, it was assigned as -1, and if it remained above or below the 200 epg threshold, was assigned 0.

2.4.4. Egg shedding and treatment consistency analysis

For each sampling occasion, the number and percentage of horses in each shedding and treatment category were calculated. Further, the number and percentage of horses that were in each rank change category of shedding (-6 to 6) and treatment (-1, 0 or 1) between S1 and each subsequent screening occasion was calculated. To test if there was a significant difference (p<0.05) in the proportion of horses falling into each shedding and treatment category between screening occasions, a binomial test was performed using the prop.test function in RStudio. All analyses were performed in RStudio, version 2.15.1 (The R Foundation for Statistical Computing, 2012). The analysis that was applied to the anthelmintic-treated horses was applied to horses that had not received anthelmintic during the study to assess if no treatment was associated with lower shedding consistency and rising FEC over time. All horses included in this analysis had FEC < 200 epg at S1.

2.4.5. Factors affecting egg shedding and anthelmintic treatment consistency

The effect of age, last anthelmintic administered and number of weeks after the expected ERP of each anthelmintic on the likelihood of a horse changing shedding or

157 treatment category between S1 and S3 was assessed using multivariable logistic
158 regression. Factors included in the initial model are shown in Table 1. Regression
159 analysis was performed using the GLM function in R (RStudio, version 2.15.1 (The R
160 Foundation for Statistical Computing, 2012)), specifying the family as binomial,
161 linked to logit transformation, g, where P is the probability of a horse changing
162 shedding or treatment category, β_i is the model (slope) coefficient and X_i is the
163 explanatory variable (Equation 1).

164 $$g = \ln[P/(1-P)] = \beta_0 + \beta_1 X_0 + ... + \beta_k X_k$$

165 Equation 1

166 The probability of a change in egg shedding and treatment category was estimated
167 using Equation 2.

168 $$P=\exp[\beta_0 + \beta_1 X_0 + ... + \beta_k X_k]/(1+\exp[\beta_0 + \beta_1 X_0 + ... + \beta_k X_k])$$

169 Equation 2

170 Regression models were initially populated with all potential explanatory variables
171 (age category, last treatment, time in weeks since expected ERP elapsed, Table 1),
172 then variables with the highest, non-significant p-values removed in a stepwise
173 process until a model with only significant terms remained. The effect of removing
174 factors from the model was evaluated using log-likelihood ratio tests (LRT) [19, 20].
175 *P*-values (Wald) of ≤ 0.05 indicated factors that had a significant influence on
176 changing shedding or treatment category in the final model. The Hosmer-Lemeshow
177 test [21] was used to assess overall model fit using the 'ResourceSelection' package
178 [22].
179

3. Results

3.1. Consistency of strongyle egg shedding and treatment with anthelmintic

At S1, 70.0% (401/573) of horses were shedding less than 50 epg (Figure 1a). This level of shedding was not significantly different at S2 (65.0%, 304/468) and S3 (66.2%, 276/417), with the proportions in shedding categories 2 and 3 also not significantly different compared to S1. The percentage of horses in category 7 was low compared to the percentage of horses in category 1: for example, 5.8% (33/573) at S1 and 0.0% (0/83) at S4. On each occasion, the percentage of horses in the 'no treatment' category ranged from 83.6% (479/573) at S1 to 91.6% (76/83) at S4 (Figure 1b).

3.2. Egg shedding and treatment consistency

Most horses remained in the same egg shedding and treatment category over time (Figure 2a). From S1 to S2, 61.5% horses (288/468) remained in the same shedding category; from S1-S3, 58.3% (243/417), and from S1-S4, 73.5% (61/83). Between S1 and S2, 16.6% (77/468) horses moved to a lower shedding category and 22.0% (103/468) to a higher shedding category. Between S1 and S3, 23.7% (99/417) moved into a lower shedding category and 18.0% (75/417) moved into a higher shedding category. Between S1 and S4, 14.5% (12/83) horses moved into a lower shedding category and 8.3% (10/83) individuals moved to a higher shedding category. Between S1 and S2, S1 and S3 and S1 and S4, 81.8% (383/468), 82.7% (345/417) and 94.0% (78/83) horses remained in the same treatment category, respectively (Figure 2b). The egg shedding consistency in a sub-group that did not receive anthelmintic during the entire study (n=304) was investigated. The proportion of

horses shedding < 50 epg at S1 was 97.3% (296/304), at S2, 84.9% (258/304) and at S3, 85.5% (213/249). The change in proportion of horses shedding <50 epg was consistent throughout the study (p>0.05). Between S1 and S2, 92.1% (280/304) horses remained in the same shedding category, 0.3% (1/304) were in a lower shedding category and 7.9% (23/304) in a higher shedding category. Between S1 and S3, the percentage of horses that remained in the same shedding category was 90.4% (225/304) and the percentage of horses that were in a higher shedding category was 9.6% (24/304).

3.3. Factors associated with changing strongyle egg shedding category or treatment category over time

Only horses (n=346) for which information on age and last anthelmintic treatment were available were included in these analyses. Factors affecting the likelihood of a horse changing egg shedding category were investigated (Model 1). In the final model, age (young horses ≥2 - <5 years) and last treatment with moxidectin were identified as significant explanatory variables (Table 2). Young horses (≥2 - < 5 years) were more likely to change strongyle egg shedding category compared to foals (<2 years), adult horses (≥5 - <18 years) and geriatric horses (≥18 years, OR=3.3, 95% CI=1.22 – 8.46, p=0.02), while horses that had received moxidectin at the last anthelmintic treatment were less likely to change strongyle egg shedding category compared to those that received ivermectin or pyrantel (OR=0.15, 95% CI=0.05-0.17, p<0.0001). Factors affecting the likelihood of a horse changing treatment category between S1 and S3 were also investigated (Model 2). In the final model (Table 2), young horses (≥2 - < 5 years) were more likely to change treatment category compared to foals (<2 years), adult horses (≥5 - <18 years) and geriatric horses (≥18

230 years, OR=2.8, 95% CI = 1.1-6.3, p=0.03) and horses that received moxidectin as
231 their last treatment were significantly less likely to change treatment group compared
232 to horses that received ivermectin or pyrantel (OR=0.15, 95% CI=0.1-0.4, p <0.0001).
233

234 **4. Discussion**

235 Knowledge that horses shed strongyle eggs at consistent levels over time can help
236 underpin evidence-based targeted treatment control programmes [23]. Currently,
237 FEC-directed targeted treatment programmes are recommended for adult horses,
238 whereby individuals are screened for strongyle egg shedding every 4-6 weeks during
239 the grazing season, and those excreting ≥200 epg treated with anthelmintic [10]. Here,
240 strongyle egg shedding in horses, analysed over three to four sampling occasions
241 within a grazing season, was found to be consistent. These results agree with those of
242 previous studies which sampled horses over longer periods (1–3 years) [12-14] and
243 shorter periods [11]. Each of these studies reported a high level of shedding
244 consistency in horses sampled from the general population. One study reported that, if
245 the first two FEC were 0 epg, there was an 82% probability that the third FEC would
246 be 0 epg and a 91% chance that it would be <200 epg. Additionally, these authors
247 found that if the first two counts were <200 epg, there was an 84% chance that the
248 next FEC would be <200 epg, and if the first two FEC were ≥200 epg, there was a
249 59% probability that the next FEC would be ≥200 epg [12]. This latter study
250 demonstrated that egg shedding was consistent over a longer sampling period
251 compared to that of the current one, particularly in horses that were measured as
252 shedding 0 epg at the start. In a later survey [13], which followed a similar sampling
253 time frame to ours, horses were treated with anthelmintic when the FEC was
254 measured as ≥250 epg and only data from horses that did not receive anthelmintic

were analysed. The authors reported that if the first two FEC were 0 epg, there was a 62% probability that the maximum FEC of the next seven samples would be 0 epg, and if the first two FEC were 0 epg, there was an 88% probability that the maximum FEC of the next seven counts would be <200 epg. The authors concluded that, for individual horses, the magnitude of the initial FEC was significantly positively correlated to the maximal FEC of the subsequent eight counts [13]. In a further study, the repeatability of strongyle egg counts was assessed in naturally infected horses [14]. In that study, samples were collected over nine consecutive months, and analysed using a McMaster method with an egg count detection limit of 20 epg. These authors defined repeatability as the variance between horses divided by the total variance, meaning that a value of 0 indicates no consistency in FEC and a value of 1 indicates perfect consistency. Using raw egg counts (i.e. the number of actual eggs counted before applying the multiplication factor), the within-horse repeatability was 0.52 in all horses and 0.53 when horses that had received treatment were excluded from the analysis [14]. Despite each of the studies described following different counting methodology and distinctive statistical analyses, all demonstrate that strongyle egg shedding in horses was consistent over short and longer sampling time-frames, especially in low egg shedding categories, indicating that such horses tend to excrete low numbers of eggs in the absence of anthelmintic treatment, over a prolonged period. In contrast to these reports, one study examined strongyle egg shedding consistency in ponies managed for conservation purposes, which remained largely untreated with anthelmintic over several years, and found that shedding consistency at individual level was generally weak [7]. In that study, FEC data were analysed using general additive mixed models to estimate repeatability of FEC at individual level and to test for differences in mean FEC amongst populations and age

280 classes. Climate and season were found to exert a significant effect on FECs
281 measured in individuals in populations that did not receive anthelmintic and a strong
282 interaction was identified between age and climate. The lack of individual consistency
283 observed by Wood et al. (2013) compared with other studies [11-14] could be due to
284 the length of time over which the data were collected, the FEC method used, the
285 absence of anthelmintic treatments, the type of statistical analysis undertaken, or the
286 nature of and level of exposure to parasite infection.
287 Past studies investigating strongyle egg shedding consistency focused on estimates of
288 strongyle FEC using McMaster methods with egg detection limits (dl) between 20
289 and 50 epg [7, 11-14]. In the current study, a more sensitive count method was used.
290 FEC generated using McMaster methods tend to generate higher epg estimates and
291 greater variance. In addition, using a FEC method with a higher egg dl (i.e.
292 multiplication factor), the methodology will be less sensitive to relatively small
293 changes in egg abundance, and larger multiplication factors will artificially inflate
294 variance [6, 24]. This may potentially lead to lower observed consistency between
295 egg counts from the same individual over time because of the greater degree of
296 artefactual variation in FEC. On the other hand, the higher number of false negative
297 FEC arising from the limited dl of traditional McMaster methods could artificially
298 increase apparent consistency of 0 measured epg over time in low-shedding horses. In
299 the present study, measures were taken to ensure samples were collected, stored and
300 processed in a way to minimise egg degradation [17], to ensure that a representative
301 sample was collected to reduce egg clumping in faeces [10], and to minimise the
302 effects of rounding error by using a very sensitive FEC method. However, the way in
303 which the samples were collected and handled on each yard on each sampling
304 occasion could have impacted the consistency of the results.

Here, egg shedding consistency was higher in adult and geriatric horses compared to youngsters. This lower level of consistency in FEC shedding in younger horses could be related to lower immunity [25] compared with older horses, such that variation in parasite challenge is less buffered and feeds through to downstream variation in FEC.

In the current study, horses that were treated with moxidectin prior to the start of the study were less likely to change shedding or treatment category compared to those that had been treated with ivermectin or pyrantel. A likely reason for this is that shedding would be lower in horses previously treated with moxidectin due to its persistent effect against parasites *in vivo* [26]. Moxidectin has an elimination half-life of 23.11 days compared to ivermectin (4.25 days) and pyrantel (13.43 hours) [26]. This means that parasites are exposed to active anthelmintic for longer periods; hence the greater strongyle ERP observed after moxidectin treatment [26]. Moxidectin exhibits higher larvicidal activity compared to the other two anthelmintics. particularly against mucosal larvae [26-28]. This will also affect the strongyle ERP observed after moxidectin administration compared to other equine anthelmintics [26, 29].

Recruitment of yards was non-random, being through veterinary practices and the BHS website. Nevertheless, there was heterogeneity in the yards recruited, which is likely to have provided a fairly representative sample of the UK equine population, outside breeding establishments, which have a younger age profile [6]. As such, further research is required to assess consistency of strongyle egg shedding in populations on breeding farms.

It should be noted that the FEC data collected here were obtained over a single grazing season, and the effect of season and climate were not accounted for, both of which can impact on strongyle egg shedding patterns [7]. Furthermore, the effects of

management practices such as removal of faeces from pasture were not investigated, which has been shown to significantly reduce FEC in donkeys that grazed pasture where dung was removed twice-weekly by reducing larval populations on pasture [30]. Long-term studies investigating strongyle egg shedding patterns taking account of season, climate and management practices are warranted as these factors impact the intensity of larval contamination on pasture, which will in turn affect the egg shedding intensity downstream. This will help to better inform FEC-directed targeted anthelmintic treatment programmes, and to understand the appropriate frequency of FEC testing.

In conclusion, egg shedding and treatment status at individual level were found to be relatively consistent, especially in adult horses, regardless of whether or not they had been treated with anthelmintic, indicating that horses with negative or low FEC (<50 epg) initially were significantly more likely to have low FEC on subsequent occasions, and horses with a high FEC (≥200 epg) were significantly more likely to have a high FEC on subsequent occasions. Further, an adult horse not requiring anthelmintic treatment initially (based on a 200 epg threshold), would be less likely to require treatment on subsequent occasions in that season. These findings would suggest that the majority of adult horses are able to regulate their strongyle burden, leading to a maintained low FEC status. There is a lack of published information on long term patterns of strongyle egg shedding in equine populations, especially in horses managed under FEC-directed anthelmintic treatment programmes; this should be assessed in future, particularly in horses that are not receiving regular anthelmintic treatments.

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Manufacturer's details

[a]Strongid-P™ paste, Elanco Animal Health, Basingstoke, Hampshire, UK.

[b]Eqvalan® oral paste for horses, Merial Animal Health, Harlow, Essex, UK.

[c]Equest® Oral Gel, Zoetis UK Limited, Tadworth, Surrey, UK.

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447

448 **Tables**

449 **Table 1.** Variables included in the initial logistic regression model

Variable	Responses
Age category	Foal (<2 years), youngster (≥2 and <5 years), adult (≥5 and < 18 years) and geriatric (≥18 years).
Last anthelmintic class administered	IVM, MOX, PYR
Time since expected *ERP elapsed	Weeks

Notes: *Egg reappearance period (ERP); ivermectin (IVM); moxidectin (MOX), pyrantel (PYR)

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455 **Table 2.** Factors significantly affecting the odds of a horse changing egg shedding
456 category (Model 1) and treatment category (Model 2) between sampling occasions S1
457 and S3 as assessed by logistic regression. For each significant variable, the logit
458 coefficient, the standard error (SE), the odds ratio (OR) and associated 95%
459 confidence intervals (CI), and the significance ($p<0.05$) are presented.

Model (Fit[1])	Significant variable	Factor	Logit coefficient	(SE)	OR (95% CI)	*p (Wald)*
1 (0.11)			1.44	0.27	Na	<0.0001
	Age	Youngsters (2-5 years)	1.20	0.55	3.3 (1.22 – 8.46)	0.02 (LRT)
	Last treatment	MOX	-1.90	0.31	0.15 (0.05-0.17)	<0.0001
2 (0.38)			1.48	0.22	Na	<0.0001
	Age	Youngsters (2-5 years)	1.04	0.47	2.8 (1.1 - 6.3)	0.028 (LRT)
	Last treatment	MOX	-1.89	0.61	0.15 (0.1 – 0.4)	<0.0001

Notes: [1] Model fit was assessed using the Hosmer and Lemeshow test. MOX = moxidectin, SE=standard error, OR = odds ratio, Na= not applicable; LRT = likelihood ratio test.

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461 **Figure Captions**

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463 **Figure 1.** The percentage of horses that fell into each strongyle egg shedding category (1-7)
464 on each screening occasion (S1-S4), with category 1 (<49 eggs per gram (epg) represented by
465 the darkest shade of grey and subsequent categories in lighter shades (A). The percentage of
466 horses that were either shedding <200 epg (Category 0, dark grey) or ≥200 epg (Category 2,
467 light grey) as measured by faecal egg count (FEC) on each screening occasion (B). The width
468 of the bars is proportional to the number of observations per screening occasion. No statistical
469 differences (p<0.05) in proportions between screening occasions as determined by the
470 binomial test were observed.

471

472 **Figure 2**. The rank change in egg shedding category and treatment category between
473 sampling occasions (S1 and S2, S1 and S3 and S1 and S4). The percentage of horses either
474 remained in the same egg shedding category (0), increased egg shedding category (1 to 6) or
475 decreased egg shedding category (-1 to -6), with a rank change in -6 categories represented by
476 the darkest shade of grey and subsequent ascending categories in lighter shades (A). The
477 percentage of horses that were either remained in the same treatment category (0, mid-grey),
478 increased in treatment category (1, light grey) or decreased in treatment category (-1, dark
479 grey) (B) as measured by faecal egg count (FEC) on each screening occasion. The width of
480 the bars is proportional to the number of observations per screening occasion. There were no
481 statistical differences (p<0.05) in proportions between screening occasions as determined by
482 the binomial test.

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