



## Research paper

# The application of faecal egg count results and statistical inference for clinical decision making in foals

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## ABSTRACT

This study investigated the impact of variability in *Parascaris* spp. and strongyle faecal egg counts (FEC) from foals on treatment decision-making and detection of a patent infection. A single faecal sample was collected once daily for three days from 53 foals and a FEC was performed on three separate portions of each sample (total of nine egg counts per foal). Differences in the decision to administer an anthelmintic using the results of a single count ( $C_1$ ), the mean of three ( $\bar{X}_{1-3}$ ) or nine counts ( $\bar{X}_{1-9}$ ) and the upper 5% confidence limit of the gamma confidence interval (CI) of the estimate of the distribution mean ( $\mu$ ) from three ( $UCL_{1-3}$ ) and nine counts ( $UCL_{1-9}$ ) were determined for a range of egg count thresholds. The  $UCL_{1-9}$  was used as the best estimate of  $\mu$ , hypothesis testing for treatment and the comparison of treatment decision-making using  $C_1$ ,  $\bar{X}_{1-3}$ ,  $\bar{X}_{1-9}$  and  $UCL_{1-3}$ . The results of this study demonstrated that a point estimate ( $C_1$  or  $\bar{X}_{1-3}$ ) was of limited value for estimating the distribution mean of egg counts in faeces and there was overall poor agreement in treatment decision-making for individual foals using  $C_1$  compared with  $UCL_{1-9}$ . Of the foals with  $C_1$  of zero eggs per gram, 54% and 47% had *Parascaris* and strongyle eggs in subsequent counts, respectively. The egg density in faeces is inhomogeneous, resulting in considerable variability in egg count results for an individual foal: between faecal piles, different portions of a faecal pile and days. The use of the negative binomial distribution CI for  $\mu$  takes this variability into account and is recommended for use when interpreting FEC data from horses.

## 1. Introduction

The faecal egg count (FEC) is the most commonly used method for quantifying the nematode burden of horses (Lester and Matthews, 2014) and may be used for diagnosis, surveillance to identify high egg shedders and faecal egg count reduction testing (FECRT) for detection of anthelmintic resistance (AR). A single FEC result is commonly used to estimate the egg distribution mean in a horse and make an assessment of the parasite burden in that animal (Wilkes et al., 2016). While many authors have reported high variability in egg count data (Denwood et al., 2012; Mes, 2003; Vidyashankar et al., 2012; Warnick, 1992) and previous studies have presented the percentage of variation at different levels (faecal pile/ball/portion) (Carstensen et al., 2013; Denwood et al., 2012), the impact of this variability on decision-making in clinical practice has not been reported.

*Parascaris equorum* and *P. univalens* are ascarids of foals and yearlings (Nielsen et al., 2014b). Large numbers of *Parascaris* worms can result in small intestinal impaction, and occasionally, intestinal rupture (Southwood et al., 1998). Ascarid infections are commonly first encountered at 3–5 months of age and are uncommon in horses older than

18 months of age as the majority of horses acquire immunity to *Parascaris* and egg shedding ceases (Clayton and Duncan, 1979). However, foals can exhibit biphasic ascarid egg shedding, with an initial peak at 3–4 months and a second peak at 8–10 months of age (Donoghue et al., 2015; Fabiani et al., 2016), and obtaining egg counts from weanlings and yearlings to monitor for ascarid infections may be advisable (Reinemeyer and Nielsen, 2017). At this age there may also be concurrent infection with strongyles. Co-infection represents a challenge for effective parasite management as a single anthelmintic class may not be effective against both parasite groups, highlighting the requirement for the use of regular faecal examinations to determine the presence of ascarids and strongyles and efficacy of anthelmintics against these parasites to direct appropriate control regimens (Erskine et al., 2016). Parasite control in foals is a poorly defined balance of lessening anthelmintic use and risk of AR with preservation of animal health and welfare and requires further elucidation of parasite epidemiology (Kaplan and Nielsen, 2010; Leathwick et al., 2016). Recent investigations of parasite dynamics in foals have described differences in progression of cyathostomin infection in comparison to mature horses (Nielsen and Lyons, 2017), infection by large strongyles at an

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early age (Fabiani et al., 2016) and biphasic peaks in *Parascaris* infection (Donoghue et al., 2015; Fabiani et al., 2016).

Selective treatment regimens have been recommended for the control of strongyles (Duncan and Love, 1991) and decisions may be based on cut-off values ranging from 20–500 eggs per gram (epg) (Becher et al., 2010; Gomez and Georgi, 1991; Krecek et al., 1994; Little et al., 2003; Matthee and McGeoch, 2004; Nielsen et al., 2006b). Although strongyle egg counts are not linearly correlated with worm burdens (Dowdall et al., 2002; Nielsen et al., 2010a), significantly higher worm counts above cut-offs ranging from 100 to 500 epg have been reported (Nielsen et al., 2010a). Selective treatment regimens in adult horses have been shown to reduce the number of anthelmintic doses administered by 36–86% (Becher et al., 2010; Gomez and Georgi, 1991; Krecek et al., 1994; Little et al., 2003; Matthee and McGeoch, 2004) and reduce pasture contamination (Becher et al., 2010). In contrast, no guidelines for an egg count treatment threshold are available for *Parascaris* (Nielsen et al., 2013). In one study, no correlation between *Parascaris* egg counts and worm burden was found and the diagnostic value of egg counts was considered low (Nielsen et al., 2010a). However, in other studies, some association between the magnitude of ascarid egg counts and worm burdens was found (Donoghue et al., 2015; Nielsen et al., 2016). Further investigation of the association with *Parascaris* worm burden is necessary before the quantitative merit of FECs can be determined. Although unproven, leaving young foals untreated is considered a potential health risk and interval administration of anthelmintics is frequently undertaken (Nielsen, 2016; Nielsen et al., 2014a; Robert et al., 2015). In addition, marked differences in relative levels of acquired immunity between juveniles and adults may increase the risk of parasitic associated disease in younger horses (Reinemeyer and Nielsen, 2017). Selective treatment regimens are currently not recommended for use in foals and weanlings (Erskine et al., 2016); however, FECs may still be useful on a population level to determine those animals that may need additional treatments other than those designated in a strategic drenching programme. Little is known about the epidemiology of *Parascaris* and determination of foals at greatest risk of *Parascaris*-associated disease would benefit the design of evidence-based control programmes. In addition, FEC surveillance for strongyles in foals will allow characterisation of worm burdens (Erskine et al., 2016) and the use of selective treatment regimens should be considered in young horses as the impact of low-moderate strongyle burdens is likely low (Reinemeyer and Nielsen, 2017). The use of FECs in a FECRT to monitor the efficacy of anthelmintics for both *Parascaris* and strongyle burdens is important to identify and delay the development of AR and methods to improve the accuracy of FEC results are required.

Increased selection pressure for AR is influenced by a variety of factors including parasite genetics and biology, host-parasite relationships (Wolstenholme et al., 2004) and management factors including intensive regimens of anthelmintic administration (Kaplan and Nielsen, 2010). Anthelmintic resistance in *Parascaris* may increase the risk of accumulation of worm burdens and disease in foals (Donoghue et al., 2015) and AR in *Parascaris* and cyathostomins is an important threat to effective control in foals and adults: e.g. macrocyclic lactone resistance in *Parascaris* (Armstrong et al., 2014; Lindgren et al., 2008) and benzimidazole resistance in cyathostomins (Kaplan, 2004) is widespread. Development of appropriate guidelines for endoparasite control in foals, including assessment of anthelmintic efficacy, prudent use of anthelmintics and safe implementation of parasite refugia (Leathwick et al., 2017; Nielsen, 2016; Peregrine et al., 2014) is necessary to reduce selection pressure for AR. Any strategy for selective anthelmintic use is reliant on the detection of animals requiring treatment, most commonly through FEC analysis.

The aims of this study were to determine (1) the potential impact of egg distribution in faeces and subsequent FEC variability on anthelmintic treatment decisions for foals in a selective treatment regimen scenario and (2) the reliability of a single FEC result of zero. Recently,

we demonstrated that the density of *Parascaris* eggs in faeces is inhomogeneous and a negative binomial distribution can be used to model egg count data (Wilkes et al., 2016). However, the impact of inhomogeneous egg density on decision-making for anthelmintic administration has not been assessed previously. We hypothesized that the use of negative binomial confidence intervals would provide a more appropriate estimate of the egg distribution mean than point estimates, including single counts and sample means, which do not account for sample variance.

## 2. Materials and methods

### 2.1. Faecal sample collection and FEC

For all components of the study, faecal samples obtained from foals were stored at 4 °C in airtight containers until egg counts were performed within two days of collection. The FEC method used was the modified McMaster technique, a quantitative flotation test (Mines, 1977), with an egg detection limit of 10 epg. Raw data were used for all analyses as a negative binomial distribution is defined on the integers 0, 1, 2, 3 etc., and not on the multiplied numbers (e.g. 0, 10, 20, 30 etc., using a multiplier of 10).

### 2.2. Treatment decision making using multiple faecal samples

Faecal samples were collected from foals aged between 4 and 18 months that presented to the Veterinary Clinical Centre, Charles Sturt University (CSU) for veterinary treatment and from foals recruited from participating studs. A sample from a fresh faecal pile passed in stables/yards was collected from 53 foals once daily for 3 days. For each sample, multiple faecal balls were collected and egg counts were performed on three 3 g portions, resulting in a total of nine egg counts for each foal. All egg counts were performed by one operator (E.J.A.W.).

For each foal, the two-sided negative binomial 95% confidence interval (CI) of nine counts ( $CI_{1-9}$ ) was taken as the gold standard for the best estimate of the egg distribution mean ( $\mu$ ) (Wilkes et al., 2016). For hypothesis testing for selection of foals for treatment, the one-sided negative binomial upper 5% confidence limit (UCL) for  $\mu$  from 9 counts was used ( $UCL_{1-9}$ ), as the null hypothesis is that treatment is not required (i.e. the parasite is present at a sufficiently low level, which occurs when the egg density is below a threshold value) (Wilkes et al., 2016). In addition, the mean of the first three counts ( $\bar{X}_{1-3}$ ), mean of nine counts ( $\bar{X}_{1-9}$ ) and the UCL for  $\mu$  from three egg counts (Day 1:  $UCL_{1-3}$ ) were calculated.

The number of foals selected for treatment was calculated based on results from the first egg count ( $C_1$ ),  $\bar{X}_{1-3}$  and  $\bar{X}_{1-9}$  UCL<sub>1-3</sub> and UCL<sub>1-9</sub> for treatment thresholds of 100, 200, 300, 400 and 500 epg (calculated from raw egg count data) for both *Parascaris* and strongyles. Using UCL<sub>1-9</sub> as the reference method, the percentage of foals with  $\bar{X}_{1-9} > 0$  (i.e. evidence of patent infection) for which the decision to treat would be different was calculated for  $C_1$ ,  $\bar{X}_{1-3}$ , UCL<sub>1-3</sub> and  $\bar{X}_{1-9}$  for each treatment threshold. Results were also calculated for foals with  $\bar{X}_{1-9} > 5$ . This egg count value was selected to remove foals with low egg counts (and unlikely to require treatment) from the analysis and not as a proposed treatment threshold.

### 2.3. Consistency of an egg count of zero

The percentage of foals with a  $C_1$  of zero was calculated and compared with the percentages of foals for which the three counts on Day 1 were zero and all nine counts were zero, for both *Parascaris* and strongyles. The sensitivity and negative predictive value (NPV) of  $C_1$  were calculated, using the results from nine counts as the gold standard. As the detection of one or more eggs was considered to represent infection, the specificity and positive predictive value were not calculated.

### 2.4. Statistical analysis

As the density of nematode eggs in faeces is inhomogeneous (Wilkes et al., 2016), CIs based on the negative binomial distribution were used for  $\mu$ , the distribution mean. A gamma approximation of the CI was calculated in Excel® using the GAMMA.INV function:

$$(\text{Gamma.Inv}(\alpha/2, n \hat{\theta}, \hat{\theta}n/\hat{\mu}), \text{Gamma.Inv}(1 - \alpha/2, n \hat{\theta}, \hat{\theta}n/\hat{\mu})(1),$$

The estimators of  $\mu$  and  $\theta$  are the method of moments estimators  $\hat{\mu} = \bar{X}$  and  $\hat{\theta} = \bar{X}^2/(S^2 - \bar{X})$ , where  $\bar{X}$  and  $S^2$  are the mean and variance of the sample, respectively. Negative binomial CIs cannot be calculated for a single count or if  $\hat{\theta}$  is negative ( $\bar{X} > S^2$ ).

An Excel® spreadsheet for calculation of the gamma approximation of the two-sided 95% CI and one-sided UCL is included in the Supplementary Material.

The study was approved by the Animal Care and Ethics Committee, CSU (ACEC 13/082).

## 3. Results

### 3.1. Treatment decision-making using multiple faecal samples

For *Parascaris*, 42/53 foals had patent infection (median  $\bar{X}_{1-9}$ : 3.4, range 0.1–238.9). For strongyles, 44/53 foals had patent infection (median  $\bar{X}_{1-9}$ : 4.7, range 0.1–140.3). Infections were highly overdispersed: 80% of the eggs counted were in 15.1% and 18.9% of foals for *Parascaris* and strongyles, respectively.

The  $CI_{1-9}$  could be calculated for 30/42 (71.4%) foals with patent ( $\bar{X}_{1-9} > 0$ ) *Parascaris* infections, of which  $C_1$  was outside the CI in 22/30 (73.3%). The  $CI_{1-9}$  could be calculated for 34/44 (77.3%) of foals with patent strongyle infections:  $C_1$  was outside the CI in 24/34 (70.6%).

Table 1 (*Parascaris*) and Table 2 (strongyles) present the percentages of foals with  $\bar{X}_{1-9} > 0$  and  $\bar{X}_{1-9} > 5$  for which the decision to treat would be different when  $C_1$ ,  $\bar{X}_{1-3}$ ,  $UCL_{1-3}$  and  $\bar{X}_{1-9}$  were used, compared with  $UCL_{1-9}$  for all selected treatment thresholds. For *Parascaris*, the percentage of foals with  $\bar{X}_{1-9} > 0$  for which the decision to treat was different when compared with  $UCL_{1-9}$  ranged from 0 to 17%, 0–13%, 0–10% and 0–22% for  $C_1$ ,  $\bar{X}_{1-3}$ ,  $\bar{X}_{1-9}$  and  $UCL_{1-3}$ , respectively. For strongyles, the decision to treat differed for 0–15%, 0–18%, 0–9% and 0–13% for  $C_1$ ,  $\bar{X}_{1-3}$ ,  $\bar{X}_{1-9}$  and  $UCL_{1-3}$ , respectively. For *Parascaris*, the percentage of foals with  $\bar{X}_{1-9} > 5$  for which the decision to treat was different when compared with  $UCL_{1-9}$  ranged from 0 to 25%, 0–20%, 0–15% and 0–31% for  $C_1$ ,  $\bar{X}_{1-3}$ ,  $\bar{X}_{1-9}$  and  $UCL_{1-3}$ , respectively. For strongyles, the decision to treat differed for 0–25% 0–30%, 0–15% and 0–19% for  $C_1$ ,  $\bar{X}_{1-3}$ ,  $\bar{X}_{1-9}$  and  $UCL_{1-3}$ , respectively.

**Table 1**

Number (%) of foals with patent *Parascaris* infection for which the decision to treat was different in comparison to  $UCL_{1-9}$  at selected treatment thresholds.

Raw egg count parameter comparison	Treatment threshold				
<i>Foals with <math>\bar{X}_{1-9} &gt; 0</math></i>					
$UCL_{1-9}/C_1$	FEC > 100 3/30 (10%)	FEC > 200 2/30 (6.7%)	FEC > 300 5/30 (16.7%)	FEC > 400 2/30 (6.7%)	FEC > 500 0/30(0%)
$UCL_{1-9}/\bar{X}_{1-3}$	2/30 (6.7%)	2/30 (6.7%)	4/30 (13.3%)	2/30 (6.7%)	0/30(0%)
$UCL_{1-9}/UCL_{1-3}$	1/18 (5.6%)	1/18 (5.6%)	4/18 (22.2%)	2/18 (11.1%)	0/18 (0%)
$UCL_{1-9}/\bar{X}_{1-9}$	0/30 (0%)	3/30 (10%)	1/30 (3.3%)	1/30 (3.3%)	0/30 (0%)
<i>Foals with <math>\bar{X}_{1-9} &gt; 5</math></i>					
$UCL_{1-9}/C_1$	FEC > 100 3/20 (15%)	FEC > 200 2/20 (10%)	FEC > 300 5/20 (25%)	FEC > 400 2/20 (10%)	FEC > 500 0/20 (0%)
$UCL_{1-9}/\bar{X}_{1-3}$	2/20 (10%)	2/20 (10%)	4/20 (20%)	2/20 (10%)	0/20 (0%)
$UCL_{1-9}/UCL_{1-3}$	1/13 (7.7%)	1/13 (7.7%)	4/13 (30.8%)	2/13 (15.4%)	0/13 (0%)
$UCL_{1-9}/\bar{X}_{1-9}$	0/20 (0%)	3/20 (15%)	1/20 (5%)	1/20 (5%)	0/20 (0%)

$C_1$ : first egg count;  $UCL_{1-3}$ : one-sided negative binomial upper 5% confidence limit for the egg distribution mean from 3 counts;  $UCL_{1-9}$ : one-sided negative binomial upper 5% confidence limit for the egg distribution mean from 9 counts;  $\bar{X}_{1-3}$ : mean of 3 counts;  $\bar{X}_{1-9}$ : mean of 9 counts.

### 3.2. Consistency of an egg count of zero epg

#### 3.2.1. *Parascaris* spp

Twenty four foals (45%) had  $C_1$  of zero: for 11 foals, all nine FECs were zero, while for 13 foals, at least one subsequent FEC was  $> 0$  (range: 1–3). The sensitivity and NPV of  $C_1$  were 69% and 46%, respectively.

#### 3.2.2. Strongyles

Seventeen foals (32%) had  $C_1$  of zero: for 9 foals, all nine FECs were zero and for 8 foals, at least one subsequent FEC was  $> 0$  (range: 1–9). The sensitivity and NPV of  $C_1$  were 82% and 53%, respectively.

## 4. Discussion

This study introduces the concept of integrating surveillance based methods into the anthelmintic treatment regimens of foals. The impact of inhomogeneous egg density in the same or multiple samples from the same foal has not been assessed previously and the results of this study demonstrate that variability in *Parascaris* and strongyle egg counts from individual foals may influence clinical decision-making.

Our results demonstrate that a single egg count is often not a good estimate of  $\mu$ . The first egg count ( $C_1$ ) was outside the 95% CI for  $\mu$  from nine counts for  $> 70\%$  of foals for which a CI could be calculated, for both *Parascaris* and strongyles (Supplementary Material). These findings are likely due to the inhomogeneous density of eggs in faeces (Wilkes et al., 2016), which cannot be accounted for by a point estimate.

Overall, there was poor agreement in treatment decision-making for individual foals when using  $C_1$  compared with  $UCL_{1-9}$ . The level of agreement varied for different egg count thresholds (Tables 1–2). Using  $C_1$  and a threshold of  $> 500$  epg, there were no differences in treatment-decision making for both *Parascaris* and strongyles. This likely reflects the distribution of eggs within the foal population used. In a population with higher egg distribution means, there may be expected to be a greater disagreement at higher egg count thresholds. When only foals with  $\bar{X}_{1-9} > 5$  (i.e. 50 epg) were analysed (representing foals more likely to be considered for selective treatment), the levels of agreement in decision-making for  $C_1$  and  $\bar{X}_{1-3}$  were lower, emphasizing the poor performance of point estimates to direct selective treatment. If FECs are used to determine infection patency, there would be little influence of the inhomogeneity of egg density on decision making. However, when using a treatment threshold, this inhomogeneity may influence treatment decisions. Similarly, the estimated efficacy of an anthelmintic could be inaccurate if based only on single FECs pre- and post-treatment, with implications for the detection of AR. Our results, and those of Lester et al. (2012), confirm that

**Table 2**Number (%) of foals with patent strongyle infection for which the decision to treat was different in comparison to UCL<sub>1-9</sub> at selected treatment thresholds.

Raw egg count parameter comparison	Treatment threshold				
<i>Foals with <math>\bar{X}_{1-9} &gt; 0</math></i>					
	FEC > 100	FEC > 200	FEC > 300	FEC > 400	FEC > 500
UCL <sub>1-9</sub> /C1	5/34 (14.7%)	5/34 (14.7%)	3/34 (8.8%)	1/34 (2.9%)	0/34 (0%)
UCL <sub>1-9</sub> / $\bar{X}_{1-3}$	6/34 (17.7%)	4/34 (11.8%)	2/34 (5.9%)	1/34 (2.9%)	0/34 (0%)
UCL <sub>1-9</sub> /UCL <sub>1-3</sub>	2/23 (8.7%)	2/23 (8.7%)	3/23 (13.0%)	1/23 (4.4%)	0/23 (0%)
UCL <sub>1-9</sub> / $\bar{X}_{1-9}$	1/34 (2.9%)	3/34 (8.8%)	1/34 (2.9%)	1/34 (2.4%)	0/34 (0%)
<i>Foals with <math>\bar{X}_{1-9} &gt; 5</math></i>					
	FEC > 100	FEC > 200	FEC > 300	FEC > 400	FEC > 500
UCL <sub>1-9</sub> /C1	5/20 (25%)	5/20 (25%)	3/20 (15%)	1/20 (5%)	0/20 (0%)
UCL <sub>1-9</sub> / $\bar{X}_{1-3}$	6/20 (30%)	4/20 (20%)	2/20 (10%)	1/20 (5%)	0/20 (0%)
UCL <sub>1-9</sub> /UCL <sub>1-3</sub>	1/16 (12.5%)	2/16 (12.5%)	3/16 (18.8%)	1/16 (6.3%)	0/16 (0%)
UCL <sub>1-9</sub> / $\bar{X}_{1-9}$	1/20 (5%)	3/20 (15%)	1/20 (5%)	1/20 (5%)	0/20 (0%)

C1: first egg count; UCL<sub>1-3</sub>: one-sided negative binomial upper 5% confidence limit for the egg distribution mean from 3 counts; UCL<sub>1-9</sub>: one-sided negative binomial upper 5% confidence limit for the egg distribution mean from 9 counts;  $\bar{X}_{1-3}$ : mean of 3 counts;  $\bar{X}_{1-9}$ : mean of 9 counts.

aggregation of nematode eggs occurs and the spatial distribution of eggs in faeces should be considered in the assessment of anthelmintic efficacy. In this study, faecal samples were collected soon after defaecation and stored at 4 °C for less than two days to avoid variation in egg counts due to prolonged storage. Our methods were in line with recommendations for optimal storage to minimise development or degradation of strongyle eggs (Mfitilodze and Hutchinson, 1987; Nielsen et al., 2010b) and the variability in egg counts was unlikely to be due to effects of storage or sub-optimal collection techniques.

The decision to treat a foal can be based on whether the one-sided 5% UCL of multiple egg counts is greater than a threshold value. In comparison to a point estimate (e.g. single FEC), the use of statistical inference through application of a UCL for decision-making is likely superior, as it is a more reliable estimate of  $\mu$  (Wilkes et al., 2016). The variation of the estimated egg count for a sample decreases as the number of counts is increased (Torgerson et al., 2012) and for interpretation of strongyle FECs, the average of two or more counts from the same faecal sample has been recommended (Carstensen et al., 2013; Vidyashankar et al., 2012). However, a mean FEC is a poor estimate of  $\mu$  and of limited use for statistical inference for hypothesis testing and repeat counts from a single sample are likely to be reasonably similar, compared with spatially separate portions of faeces (Wilkes et al., 2016). In up to 30% of foals with  $\bar{X}_{1-9} > 5$  (strongyles), the decision to treat was different when results of  $\bar{X}_{1-3}$  were compared with UCL<sub>1-9</sub>. Given the inhomogeneous density of eggs in faeces, multiple counts from non-adjacent portions of a faecal pile (or preferably, multiple faecal piles) will provide a more accurate estimate of  $\mu$ .

The egg count threshold values were selected to demonstrate the potential impact of FEC variability on treatment decisions and were based on previously described values for strongyle egg count data (Gomez and Georgi, 1991; Krecek et al., 1994; Little et al., 2003; Matthee and McGeoch, 2004; Nielsen et al., 2006b, a). The aim of this study was not to recommend the use of a specific threshold value and other cutoff values may be used for different animal populations and requirements. As such, the outcomes from the application of the methods described in this study will likely vary for different animal and parasite populations and different threshold values.

Although a linear correlation between egg count and worm burden does not exist for strongyles (Nielsen et al., 2010a), selective treatment regimens are recommended for sustainable strongyle control and to decrease pasture contamination (Becher et al., 2010; Duncan and Love, 1991; Kaplan and Nielsen, 2010; Krecek et al., 1994; Matthee and McGeoch, 2004; Nielsen et al., 2006a). Significantly higher strongyle worm counts above FEC cutoff values of 100–500 egg were found in one study (Nielsen et al., 2010a), suggesting selective treatment may be worthwhile. A tendency for similar patterns of strongyle egg shedding by individual horses over time has been reported (Becher et al., 2010;

Nielsen et al., 2006a; Scheuerle et al., 2016), supporting selective treatment of horses to reduce pasture contamination and maintain a parasite refugium. Regular (interval) administration of anthelmintics is expected to eliminate the majority of *Parascaris* egg excretion by infected foals, given the long pre-patent period of the parasite and decline in worm survival with increasing foal age (Leathwick et al., 2016). Sustained reduction in egg excretion by susceptible genotype worms provides an advantage for resistant genotypes to proportionally increase in subsequent *Parascaris* generations with development of AR (Leathwick et al., 2016), of which there is evidence of widespread occurrence (Armstrong et al., 2014; Hearn and Peregrine, 2003; Lindgren et al., 2008; Lyons et al., 2008; Nielsen, 2016). Changes in the management of *Parascaris* infection are necessary to avoid reliance on interval treatment regimens and delay development of resistance to anthelmintics that are currently effective.

While evidence-based approaches to sustainable control of nematodes in juvenile horses have yet to be established, the application of a model of *Parascaris* dynamics and genetics for AR to investigate effects of treatment regimens on development of AR has been described recently (Leathwick et al., 2017). In that study, monthly treatment rapidly resulted in AR, while a single treatment or two treatments given at 60 and 150 days of age slowed the development of AR, suggesting limited environmental contribution to parasite refugia and the importance of the contribution of susceptible genotype worms to subsequent generations (Leathwick et al., 2017).

Overdispersion of equine nematode infections within the population of foals in our study was present, similar to other studies of strongyles (Lester et al., 2013; Relf et al., 2013). This is the first time this has been demonstrated for *Parascaris* and may have implications for selective treatment regimens as part of integrated parasite control programmes designed to decrease the risk of AR. *Parascaris* are considered ubiquitous parasites of foals less than 6–8 months of age, prior to the development of age-dependent immunity (Leathwick et al., 2016; Nielsen, 2016; Reinemeyer, 2009). The principle concern with *Parascaris* infection is intestinal impaction by a large burden of luminal stages in foals older than 3 months resulting in abdominal pain and possibly fatal intestinal rupture (Bellaw et al., 2016; Nielsen, 2016; Reinemeyer, 2009), and it has been proposed that this syndrome is predisposed by inadequate anthelmintic treatment (Nielsen, 2016). Our findings suggestive of overdispersion of *Parascaris* in foals may reflect, in part, pre-patent infections; however, it remains possible that true differences in worm numbers exist. In a small study of ascarid burdens in 13 untreated foals aged 5–10 months, nine had less than 10 luminal worms (Donoghue et al., 2015), suggestive of overdispersion. The identification of high strongyle egg shedders is considered important for targeted treatment to decrease pasture contamination (Becher et al., 2010; Nielsen et al., 2006a), and while unproven, similar principles may be

applicable to programmes for *Parascaris* control.

The method of calculation of the negative binomial CI described in this study is simple; however, CIs or UCLs cannot be calculated if  $\bar{X} > S^2$ . If the CI/UCL is calculated from a small number of counts, the reliability of this estimate of  $\mu$  will be decreased. Further, because the egg density of separate samples/faecal piles/days can change considerably in an individual foal (Wilkes et al., 2016), the CI for  $\mu$  may increase, not decrease, when a larger number of counts are used. This explains why the CI was larger for 9 counts than for 3 counts in some foals as a consequence of the inhomogeneous egg density and increased variability with increasing  $n$  and the difference in the sample mean between days. However, because of the aggregation of eggs in faeces, the use of more egg counts will result in a better estimation of  $\mu$ . Despite these limitations, the method for calculation of negative binomial CIs in this study is applicable for use in veterinary practice.

Our results demonstrated that an observed single egg count of zero (*Parascaris* and strongyles) can come from an animal with a patent nematode infection. Although the decision to treat was not different and egg counts remained low, falsely classifying an animal as not having a patent infection may impact the assessment of the prevalence of patent infection, environmental contamination and anthelmintic efficacy. In our study, the sensitivity and NPV of  $C_1$  were low for *Parascaris*. Similarly, the high risk of false negative results using a single FEC of 0 has been described previously (Nielsen et al., 2010a; Torgerson et al., 2014, 2012). The sensitivity of  $C_1$  was higher for strongyles, however the NPV was low. Anthelmintic resistance is continuing to develop in nematodes of importance in horses and monitoring of anthelmintic efficacy is recommended (Kaplan and Nielsen, 2010).

## 5. Conclusions

The inhomogeneity of egg density in faeces can influence the interpretation of FECs and the decision of whether an animal requires treatment, particularly when based on a single count which is a poor estimate of  $\mu$ . More than one FEC should be performed from non-adjacent sections of a faecal pile to account for inhomogeneous egg density. Notwithstanding the limitations of the method, calculation of negative binomial CIs/UCLs will provide a better estimate of  $\mu$  in individual animals and represents a readily achievable method for statistical inference that may improve diagnosis and interpretation of FEC data. The concept of using surveillance based approaches for the use of anthelmintics in foals was introduced in this study. While there are concerns regarding the risks of leaving foals in a population untreated, little epidemiological data on *Parascaris* infestations and associated disease in foals are available for evidence-based control programmes. The overdispersion of infestations identified in this study supports the use of FECs to identify those foals with higher counts that may require treatments outside a specific interval-based or strategic deworming schedule. It is imperative that more sustainable approaches towards parasite control are considered in both adult and foal populations.

## Conflict of interest

No authors have any potential conflict of interest associated with this work.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.vetpar.2019.04.010>.

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