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Variation in fecal egg counts in horses managed for conservation purposes: individual egg shedding consistency, age effects and seasonal variation

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SUMMARY

Cyathostomins are the most prevalent equine intestinal parasites and resistance has been reported in these nematodes against all 3 licensed anthelmintic classes. Strategies need to be developed that are less dependent upon drugs and more reliant on management-based control. To develop these we need to understand natural transmission patterns better. Here, we analysed longitudinal fecal egg count (FEC) data from 5 pony populations used for conservation purposes. We tested how egg excretion varied amongst populations and individuals, and how this was affected by age and climate. There was evidence for consistency in FECs over time at the individual level; this was generally weak and accounted for <10% of the total variance. Animals <5 years old had higher FECs and there was profound seasonal variation in FECs, with highest levels recorded in spring/summer. Effects of monthly temperature and rainfall explained most, but not all, of the observed seasonal variation and associations between climate measures and FECs were stronger in younger versus adult animals. One population was occasionally treated with anthelmintics and analysis of this population suggested that treatment substantially altered the seasonal dynamics. This paper highlights the variability in strongyle egg excretion amongst individuals and the factors involved in this variation.

Key words: horses, nematodes, cyathostomins, faecal egg counts, consistency, age, climate.

INTRODUCTION

The high prevalence and pathogenicity of cyathostomins, coupled with high levels of anthelmintic resistance, places these parasitic nematodes and the study of their epidemiology, at the forefront of research into equine welfare (Nielsen et al. 2010a). There are 50 recognized species of cyathostomins, 40 of which have been found in the caecum and colon of horses (Lichtenfels et al. 2008). The adult parasites live in the large intestine and shed their eggs in the feces. Within feces, eggs hatch to release larvae which undergo 2 molts to the infective third larval stage (L3); the latter migrate onto pasture and can develop from eggs in as short as 3 days (Corning, 2009). Once ingested, the larvae enter the mucosa of the caecum and colon, where they can encyst and can persist for many months (Matthews, 2008). Larval encystment is thought to primarily be a seasonal phenomenon and in some horses, encysted larvae can comprise over 90% of an individual’s cyathostomin burden in the autumn and winter (Dowdall et al. 2002). Large numbers (i.e. several million) of encysted larvae can build up before emerging; this leads to a clinical disease known as larval cyathostominosis which has a case fatality rate of up to 50% (Giles et al. 1985).

Anthelmintic resistance presents a major threat: almost every population of nematodes in managed horses in developed countries has been subjected to intense selection pressure via frequent anthelmintic administrations. This is exacerbated by regular under-dosing due to inaccuracies in weight assessment (Naeraho et al. 2011). Reversion to anthelmintic sensitivity does not seem to occur readily in nematodes; this has been observed in cyathostomins with benzimidazole anthelmintics, with reduced efficacy observed in resistant populations even after several years without exposure to the associated compounds (Hodgkinson et al. 2005). There are 3 classes of anthelmintic available for use in horses; benzimidazoles, tetrahydropyrimidines and macrocyclic lactones. A high prevalence of resistance to the benzimidazole and tetrahydropyrimidine classes has already been reported and there is evidence to support the emergence of resistance to the most frequently used class, the macrocyclic lactones (von Samson-Himmelstjerna et al. 2007). As no
new equine anthelmintics appear to be under development for licensing in the short to medium term (Kaplan and Nielsen, 2010), it is crucial that efficacy of the current products be maintained for as long as possible. Management practices that involve less intensive and more targeted treatment strategies are being advocated (Proudman and Matthews, 2000; Kaplan and Nielsen, 2010; Matthews, 2011). Development of such strategies depends on an understanding of the basic epidemiology of these nematodes and of natural transmission patterns in populations where treatment and, hence, selection pressure for resistance, is absent or reduced.

Over the last decade, researchers in the subject area have advised the application of anthelmintic applications to horses based on projected patterns of FECs using a limited sampling window (Eysker et al., 2006; Becher et al., 2010). In some instances, it has been implied that, in the case of mature horses with consistently low FECs, it is not necessary to repeat FEC examination each time a treatment decision is to be made. This is based, in part, on the fact that fecal egg output in horses is presumed to be hereditary (Eysker et al., 2006). There has been much discussion on the consistency of FECs in individuals over time, yet there is limited published evidence available from the analysis of long-term datasets. In one study, feces from 484 horses were sampled twice with an interval of 6 weeks without intervening treatments (Döpfer et al., 2004). Analysis showed that 55·2% horses had low FECs (i.e. <100 or ≥100 eggs per gramme, EPG) at both samplings, whilst 32·0% had higher FECs (i.e. EPG >100). Levels of FEC were linked to sex and anthelmintic treatment interval adopted. Analysis was not performed to examine season-to-season or year-on-year variation in an individual’s FECs. In a separate study, samples from 424 horses were analysed twice a year over 3 years (Nielsen et al., 2006). The results, presented as probabilities of ‘FEC outcome’ when 2 preceding FEC data were available, demonstrated that an individual with a FEC of 0 EPG in the 2 previous samples had an 82% probability of 0 EPG and a 91% probability of having a FEC of <200 EPG at the third. In addition, an animal that displayed 2 consecutive FECs <200 EPG, had an 84% probability of the next sample having a FEC of <200 EPG. In contrast, when FECs >200 EPG were obtained in 2 consecutive samples, the probability of the third having a FEC of >200 EPG was 59%. The authors concluded that most individuals (subjected to targeted treatments when their FECs exceeded 200 EPG) displayed consistent fecal egg shedding from one grazing season to the next.

Here, using FEC data derived from populations of ponies used for conservation purposes, we investigated longitudinal strongyle egg shedding patterns in animals not subjected to regular anthelmintic treatments. One might expect that, in these more ‘natural’ circumstances, where treatments are not applied with regularity, environmental effects on parasite transmission may come to the fore and that within-individual (rather than between-individual) variation is accentuated. In this study, we explored this hypothesis by studying FEC patterns over 8 years for 4 populations in which anthelmintic applications were virtually non-existent and for 1 population in which anthelmintics were applied. Specifically, we (1) estimated and compared the repeatability of FECs at the individual level in untreated and treated populations, (2) tested whether FECs were associated with environmental conditions, including year-to-year variation, seasonal variation and associations with local temperature and rainfall, (3) tested whether FECs varied with age and whether observed environmental associations with FECs were age dependent, and (4) tested the effects of age, season and climate and examined the magnitude and duration of the effect of anthelmintic treatment on FECs in the treated population.

**MATERIALS AND METHODS**

**Study populations, fecal sampling and fecal egg count analysis**

Five populations of ponies were considered here, each managed by the Norfolk Wildlife Trust. The populations are kept for conservation purposes at various locations around the Norfolk Broads (UK), with each being maintained to graze pastures. Details of the structure, habitat, numbers of individuals, total number of FECs performed and duration of sampling for each population are shown in Table 1. Norfolk Wildlife Trust staff performed the fecal sampling and strongyle FECs on veterinary advice. This was done to monitor pasture contamination and to ensure that the welfare of the animals was maintained. Freshly voided droppings were collected from identified individuals; the vast majority (94·7%) of samples were collected at between 1- and 4-month intervals. Sample collection from identified individuals was achieved by observing the ponies over time until they voided feces and the feces were immediately collected into labelled storage bags. Strongyle FECs were assessed using a modified McMaster technique (Ministry of Agriculture, Fisheries and Food, Britain. Manual of Veterinary Parasitological Laboratory Techniques. 1980, pp. 1–21.). This method has a sensitivity of 50 EPG. Only animals of known age were included in the analyses, and individuals were classified as foals (<2 years), youngsters (≥ 2 and <5 years), adults (≥ 5 and <18 year) or geriatric (≥ 18 years) depending on their age at the start of sampling. In addition, monthly average temperature (average of monthly recorded minimum and maximum temperatures) and rainfall data were obtained from the nearest UK Meteorological Office
weather station in Lowestoft (http://www.metoffice.gov.uk/climate/uk/stationdata/lowestoftdata.txt).

Four of the 5 populations (‘Konik’ and ‘Dartmoor’ breed populations) were not administered anthelmintics during the study period. This was because of possible ecotoxicological risks associated with anthelmintics when they are excreted in the feces of treated animals, in particular the macrocyclic lactones, ivermectin and moxidectin (McKellar, 1997). Two animals were removed from the Thompson Common dataset prior to the analysis because they had received occasional anthelmintic treatment due to ‘loss of condition’. Exclusion was based on the concern that treatment was likely to have considerably altered their egg shedding dynamics relative to the 16 other ponies in this population. It should be noted that exclusion of these 2 ponies in this population represented only a small fraction of the population, but may have removed the highest fecal egg shedders. Another pony was excluded from the dataset because its exact age was not known (excluded from Table 1). The population of Welsh ponies at the Hickling South site was regularly administered with a variety of anthelmintics; this was due to a history of clinical disease associated with cyathostomins at this site. These animals received a benzimidazole drug (fenbendazole; Panacur™, Intervet Schering Plough, ‘BZ’) 3 times; a tetrahydropyrimidine (pyrantel embonate (Stronglid-P™, Pfizer, or Pyratape-P™, MSD Animal Health, ‘TH’) 6 times; and a macrocyclic lactone (moxidectin, Equest™, Pfizer, ‘MOX’) 6 times (Table 2). At each treatment, the ponies were administered with anthelmintic per os at the manufacturer’s recommended dose rate. The treatments were administered at irregular intervals over the course of the 8-year sampling period (Table 2, Fig. 1).

Statistical analyses

All statistical models were run in R version 2.13 (R Core Development Team, 2005), using the packages ‘nlme’ (Pinheiro and Bates, 2000) and ‘mgcv’ (Wood, 2006). We used generalized additive mixed-effects models (GAMMs) to estimate the repeatability of parasite burdens within individuals, to test for differences in mean FEC among populations and age classes, and to examine seasonal patterns of FECs and the contribution of temperature and rainfall to those patterns. GAMMs combine the attributes of generalized linear mixed-effects models (GLMMs), which allow random effects to be fitted alongside fixed-effects terms to account for among-group heterogeneity and pseudo-replication (Pinheiro and Bates, 2000), with additive models which incorporate non-parametric smoothing functions into a linear model framework to model complex non-linear relationships without making a priori assumptions about the

<table>
<thead>
<tr>
<th>Location</th>
<th>Habitats</th>
<th>Breed</th>
<th>Number of individuals (fecal samples)</th>
<th>Population structure</th>
<th>Treated?</th>
<th>Monitoring duration</th>
<th>K (aggregation parameter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hickling Northside</td>
<td>Coastal wetland</td>
<td>Konik</td>
<td>5 (726)</td>
<td>3 stallions, 2 geldings</td>
<td>No</td>
<td>March 2000–Sept 2008</td>
<td>1.46</td>
</tr>
<tr>
<td>Roydon Common</td>
<td>Coastal heather, heath</td>
<td>Dartmoor</td>
<td>12 (387)</td>
<td>12 geldings, 10 mares</td>
<td>Yes</td>
<td>Nov 2005–Nov 2008</td>
<td>1.61</td>
</tr>
<tr>
<td>Thompson Common</td>
<td>Grassland, fen, scrub, woodland</td>
<td>Konik</td>
<td>16 (151)</td>
<td>11 geldings, 4 geldings</td>
<td>No</td>
<td>Aug 2006–Sept 2008</td>
<td>1.61</td>
</tr>
<tr>
<td>BA Hickling</td>
<td>Coastal wetland</td>
<td>Konik</td>
<td>15 (698)</td>
<td>1 stallion, 4 geldings, 10 females</td>
<td>No</td>
<td>Oct 2000–April 2011</td>
<td>0.83</td>
</tr>
</tbody>
</table>

1 This excludes 2 ponies that were excluded from analyses because they were treated with anthelmintics early in the study period due to perceived loss of condition due to worm infection.
Table 2. Details of treatments and fecal egg count reduction (FECR) in Welsh ponies at Hickling Southside

(Anthelmintic abbreviations are as follows: BZ, benzimidazole; TH, tetrahydropyrimidine; MOX, moxidectin. NS, – pre- and/or post-treatment sample(s) not obtained at this treatment. The percentage reduction in FEC between pre- and post-treatment sampling is given for each individual, where it was possible to calculate it. Individuals whose FEC increased or did not change following treatment were excluded. Cases where animals were not included due to apparent health issues or a failure to deworm were excluded. Average (FECR3) and individual average (iFECR3) FEC reductions are presented, with values ≥ 95% in bold (for method of calculation, see text.)

<table>
<thead>
<tr>
<th>Treatment date</th>
<th>Pre-treatment sampling date</th>
<th>Days post-treatment sample was taken</th>
<th>Drug class or type</th>
<th>Percentage reduction in FEC from pre- to post-treatment sample in each individual</th>
<th>Number of horses included in iFECR3 calculation</th>
<th>FECR3</th>
<th>iFECR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>15/03/2000</td>
<td>08/03/2000</td>
<td>10</td>
<td>BZ</td>
<td>94.12</td>
<td>3</td>
<td>65</td>
<td>70</td>
</tr>
<tr>
<td>26/05/2000</td>
<td>26/05/2000</td>
<td>14</td>
<td>TH</td>
<td>100.00</td>
<td>2</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>08/08/2000</td>
<td>27/06/2000</td>
<td>14</td>
<td>TH</td>
<td>NS, NS</td>
<td>7</td>
<td>85</td>
<td>97</td>
</tr>
<tr>
<td>22/01/2001</td>
<td>22/01/2001</td>
<td>18</td>
<td>BZ</td>
<td>NS, NS, 75.00, 93.75, NS</td>
<td>4</td>
<td>73</td>
<td>81</td>
</tr>
<tr>
<td>22/10/2001</td>
<td>22/10/2001</td>
<td>14</td>
<td>MOX</td>
<td>NS, NS, 100.00, 100.00, 100.00, 100.00, 100.00, 100.00, 100.00</td>
<td>4</td>
<td>73</td>
<td>81</td>
</tr>
<tr>
<td>13/01/2003</td>
<td>15/01/2003</td>
<td>14</td>
<td>MOX</td>
<td>NS, 100.00, 100.00, 100.00, 100.00, 100.00, 100.00, 100.00, 100.00</td>
<td>8</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>16/02/2004</td>
<td>10/02/2004</td>
<td>46</td>
<td>MOX</td>
<td>85.71, 100.00, 100.00, 100.00, 100.00, 100.00, 100.00, 100.00, 100.00</td>
<td>8</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>15/12/2004</td>
<td>26/11/2004</td>
<td>37</td>
<td>MOX</td>
<td>No change in FEC, 100.00, 100.00, 100.00, 100.00, 100.00, 100.00</td>
<td>7</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>06/05/2005</td>
<td>18/03/2005</td>
<td>7</td>
<td>TH</td>
<td>No change in FEC, 100.00, 100.00, 100.00, 100.00, 100.00, 100.00</td>
<td>3</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>15/12/2005</td>
<td>04/11/2005</td>
<td>36</td>
<td>MOX</td>
<td>100.00, 100.00, 100.00, 100.00, 100.00, 100.00, 100.00, 100.00, 100.00</td>
<td>9</td>
<td>89</td>
<td>97</td>
</tr>
<tr>
<td>28/06/2006</td>
<td>19/05/2006</td>
<td>16</td>
<td>TH</td>
<td>97.78, 85.71, 96.15, 95.24, Increase in FEC, 100.00, 100.00, 100.00, 100.00</td>
<td>5</td>
<td>91</td>
<td>95</td>
</tr>
<tr>
<td>11/12/2006</td>
<td>24/11/2006</td>
<td>53</td>
<td>MOX</td>
<td>100.00, 100.00, 100.00, Not treated, 100.00, 100.00, 100.00, 100.00, 100.00</td>
<td>8</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
form of such relationships (Wood, 2006; Zuur et al. 2009). We calculated the aggregation parameter, \( k \), for FECs in each of the populations, following Grenfell et al. (1995), as: \( k = \text{mean}^2 / (\text{variance} - \text{mean}) \) (see Table 1). Helminth burden data are typically over-dispersed and, although our FEC data were unusual in having a relatively low proportion of zero values (12% of all FECs) and low values (22% of all FECs were \( \leq 250 \) eggs/gramme), they still showed deviations from normality. Model residuals suggested some evidence of heteroscedasticity. FEC data are commonly log-transformed to deal with such issues, but transformation can make interpretation of results on the original scale (here, in EPG) difficult (Zuur et al. 2009). Analyses of log-transformed FEC data yielded similar results to those presented here, so for ease of interpretation of the patterns and relationships observed, we discuss models of untransformed FECs throughout. We began by fitting a generalized additive model of FEC incorporating fixed effects for population, age class and year and month of measurement. Population, age and year were fitted as factors. Preliminary analyses suggested that FEC varied from month to month across a year in a complex, non-linear fashion and so we modelled this relationship by including a non-parametric smoothing function for month, restricting the number of knots assigned to the smoother to 7 to avoid over-parameterization (Zuur et al. 2009). To this base GAMM, we added individual identity as a random effect, to account for pseudo-replication associated with the many repeated observations from the same individuals in our data set and to ensure that the significance of fixed terms in
the model was assessed against the appropriate degrees of freedom (Pinheiro and Bates, 2000). Additionally, we incorporated a continuous autoregressive function to account for temporal autocorrelation of residuals within individuals from month to month (Zuur et al. 2009). We then evaluated the significance of all fixed and random terms in this model using likelihood ratio tests (LRTs) comparing models with and without the relevant term, removing any terms that were not significant (Crawley, 2007; Zuur et al. 2009). Using estimates in the resulting model (‘Model 1’), we calculated a measure of consistency of FEC within individuals as the ratio of the variance among individuals to the sum of the variance among and within individuals estimated in the random effects structure of GAMMs. This is a standard approach and widely used approach to estimate ‘repeatability’ in quantitative genetics (Falconer and Mackay, 1996). It reflects the proportion of the variance in FEC that is not explained by the fixed effects (e.g. population, age, season) that can be attributable to consistent differences among individuals (i.e. differences in mean FEC across the study period). Since this ratio will vary depending on the fixed effects included in the model, we also calculated repeatability from a model in which no fixed terms were included (Wilson, 2008). We went on to include smoothing terms in our GAMMs for monthly average temperatures and rainfall, as well as the term already fitted for month, to determine the effects of weather on FECs and to what extent those effects explained evident seasonal variation captured by the ‘month’ term (‘Model 2’). We tested the extent to which each smoother improved model fit and, if it did, whether it explained more variation than a simple linear relationship would, by comparing models with relevant smoothing terms present, absent or replaced with linear functions using LRTs.

To test the prediction that seasonal or weather effects on FEC might differ amongst age class, we subsequently fitted models which included interactions between either month and age class, or interactions between age class and both rain and temperature (‘Model 3’). Where smooth functions had been retained for a term in the final model, these interactions were fitted as separate smoothers for each age class. Age composition varied between populations: BA Hickling was the only population in which ‘geriatric’ (i.e. >18 years old) ponies were measured, and there were no foals measured in Hickling South and Hickling North. To ensure that none of the observed age effects were being driven by a small proportion of very young and old individuals in certain populations, we re-ran models with a simplified age term with only 2 levels: young: 0–5 years and adult: >5 years. Finally, we analysed the treated population of Welsh ponies at Hickling South separately to establish to what degree treatment with different anthelmintics influenced FEC dynamics. All 9 individuals in the Hickling South population were geldings of the same age at first FEC measurement, making it impossible to separate the effects of year and age in this population. We therefore ran Model 1 for this population, as above, but including year of measurement as a fixed factor (to account for year-to-year variation, and associated changes between ages), excluding age and population. Random and fixed effects were included and tested as described above. We then added smoothers for temperature and rainfall to the preferred model to test for climate effects. To examine the effects of anthelmintic treatment of FECs in this population, we then took our preferred model and added a fixed factor coding whether or not FEC was measured within an anthelmintics’ remaining standard egg reappearance period (ERP) or not (i.e. for BZ and TH: up to 6 weeks, for MOX up to 12 weeks). Note that FECs taken within 2 days of treatment were considered as pre-treatment counts. We compared this to a model including a 4-level factor which specified the anthelmintic used (i.e. not within a drug’s ERP, within BZ ERP, within TH ERP, or within MOX ERP) to test if the particular drug type used mattered. Finally, to test whether FECs continued to increase after a drug’s standard ERP, we included a fixed linear covariate for the number of weeks post-treatment an FEC was taken.

We also tested the effect of anthelmintic treatments on strongyle FEC based on methods described by Cabaret and Berrag (2004). Using available pre- and post-treatment FEC data, we calculated the percentage FEC reduction (FECR) for each individual pony where this was possible, as well as the group ‘FECR’ (FECR3 in Cabaret and Berrag 2004), as:

\[ \text{FECR}_3 : 100 \times \left(1 - \frac{T_i}{T_{i1}}\right) \]

where \( T_1 \) and \( T_2 \) are the pre- and post-treatment arithmetic mean FEC across the group, respectively.

The individually-based FECR (iFECR3 in Cabaret and Berrag, 2004), as:

\[ \text{iFECR}_3 : \frac{1}{n} \Sigma (100 \times \left(1 - \frac{T_{i2}}{T_{i1}}\right)) \]

where \( T_{1i} \) and \( T_{2i} \) are individual \( i \)’s pre- and post-treatment FECs, respectively and \( n \) is the number of treated hosts.

It should be noted that, due to the nature of the study, the number of days between pre-treatment sampling, treatment and post-treatment sampling was variable. Here, to be able to calculate ‘FECRs’ for most applied treatments, we included post-treatment samples if they were within the drug’s standard ERP, rather than within the 14 to 17-day period after treatment. We excluded from these ‘FECR’ calculations, individual cases where researchers in the field had noted doubts of an individual pony receiving the anthelmintic or about an individual’s state of health, and where pre-treatment FECs were <150 EPG pre-treatment. We also excluded individual cases where
FECs increased from pre- to post-treatment (4 observations from 3 different individuals), as there were never more than 2 cases per treatment, and it was unclear whether this response reflected true lack of efficacy or a failure to administer any or sufficient anthelmintic at the time of treatment. Full details are presented in Table 2.

RESULTS

Individual repeatability and population, age and seasonal variation in FEC

Average FECs were significantly, but weakly, repeatable at the individual level, and showed a significant, albeit relatively low, level of temporal autocorrelation within individuals. Inclusion of a random effect for ‘individual’ substantially improved model fit, as did the subsequent incorporation of a temporal autocorrelation function within individuals (Table 3).

Fecal egg count variation in conservation ponies

<table>
<thead>
<tr>
<th>Term</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location (vs. BA Hickling)</td>
<td>53·4 ***</td>
<td>53·4 ***</td>
<td>53·4 ***</td>
</tr>
<tr>
<td>Hickling North</td>
<td>+363·1 (132·5)</td>
<td>+341·9 (137·5)</td>
<td>+354·6 (134·8)</td>
</tr>
<tr>
<td>Thompson Common</td>
<td>+732·8 (126·7)</td>
<td>+618·0 (131·3)</td>
<td>+630·1 (128·0)</td>
</tr>
<tr>
<td>Royden Common</td>
<td>+272·5 (102·1)</td>
<td>+291·8 (105·2)</td>
<td>+329·3 (103·0)</td>
</tr>
<tr>
<td>Hickling South</td>
<td>−344·3 (107·2)</td>
<td>−386·7 (111·3)</td>
<td>−379·2 (109·4)</td>
</tr>
<tr>
<td>Age class (vs. foal)</td>
<td>65·9 ***</td>
<td>65·9 ***</td>
<td>65·9 ***</td>
</tr>
<tr>
<td>Youngster</td>
<td>+413·3 (83·0)</td>
<td>+289·4 (84·7)</td>
<td>+479·9 (130·0)</td>
</tr>
<tr>
<td>Adult</td>
<td>−17·8 (98·4)</td>
<td>−120·0 (100·1)</td>
<td>+209·9 (136·5)</td>
</tr>
<tr>
<td>Geriatric</td>
<td>+227·1 (227·2)</td>
<td>+171·8 (228·8)</td>
<td>498·6 (326·3)</td>
</tr>
<tr>
<td>Year</td>
<td>Not presented</td>
<td>Not presented</td>
<td>Not presented</td>
</tr>
<tr>
<td>Month (smoother)</td>
<td>See Fig. 2C 244·4 ***</td>
<td>244·4 ***</td>
<td>244·4 ***</td>
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Table 3. Generalized additive mixed-effects models of FECs

(For each model of increasing complexity, random effects are reported as standard deviations and the estimated temporal autocorrelation among samples separated by 1 month. Fixed effects are reported as estimated mean differences and slope differences relative to a selected level for factors and interactions with factors, respectively. ‘LRT’ is the likelihood ratio test statistic comparing the model in question to one without the term in question and once significance is established in a previous model, these are not reported in subsequent models. Significance: ***, P<0·001, **, P<0·01.)
The estimated correlation within individuals of FEC observations 1 month apart is 0.26, which represents the continuous autoregressive structure applied in our models. This structure specifies that the correlation decreases exponentially as the temporal gap between observations increases (Pinheiro and Bates, 2000; Zuur et al., 2009).

In GAMMs accounting for repeated measures on individuals and temporal autocorrelation, there were significant differences in mean FECs among populations, age classes, years and also evidence for non-linear seasonal variation within years (Fig. 2, Table 3). FECs varied between populations, and this was not simply related to variation among breeds or between treated and untreated populations (Fig. 2A). The anthelmintic treated population of Welsh ponies at Hickling South had significantly lower FECs on average than all 4 other populations, as would be expected. The Konik ponies at BA Hickling had the next lowest average FECs, which were again significantly lower than the remaining 3 populations. The Koniks at Hickling North and the Dartmoor ponies at Royston Common had higher average FECs that were not significantly different from one another, whilst the Koniks at Thompson Common had significantly higher FECs than all other populations. FECs also varied between age classes. The raw data (summarized in Fig. 2B) suggested that FECs were higher in foals and youngsters compared to adults; however, model estimates, which would also account for differences among populations, years and months (presented in Table 3), suggested that youngsters had significantly higher FECs than both foals and adults, but not geriatrics. As well as significant year-to-year variation in average FECs, the non-parametric smoothing term fitted for month revealed a highly significant, non-linear pattern of variation in FECs within years (Table 3). Average FECs increased from their lowest point in February through spring until May, after which they remained high until September, before declining again through autumn and winter (Fig. 2C).

**Climatic effects on FEC**

When additional non-parametric smoothing functions were included for monthly temperature and rainfall, both significantly improved the explanatory power of the model (Table 3). Examining the relationship between FEC and month, rainfall and temperature predicted by smoothers in this model suggested that a complex, non-linear relationship with temperature was evident; however, independent of this temperature effect, both month and rainfall had positive linear relationships with FEC. This was confirmed by comparing the model with smoothers fitted for temperature, rainfall and month with one in which only temperature was fitted with a smoother.

![Fig. 2. Variation in FECs among populations, age classes and seasons.](image-url)
and rainfall and month were fitted as simple linear covariates (LRT comparing models: $\chi^2(2) = 0.00$, $P=1.00$). This model shows that FEC increases linearly with average monthly rainfall (Fig. 3A). It also increases with average monthly temperature from around 8 °C to around 14 °C (Fig. 3B). Below 8 °C, FECs are low and stable, whilst above 14 °C, slightly lower FECs are observed (Fig. 3B). The model also suggests that effects of temperature and rainfall account for the complex non-linear pattern of season variation predicted in Fig. 2C, because once these climate effects are controlled for in the model, the month effect collapses into a much simpler linear increase across the course of the year (Table 3).

Finally, we tested whether climatic effects on FEC differed among age classes, specifically that parasite burdens in younger animals would be more sensitive to climatic variation. Inclusion of a linear interaction between rainfall and age class, and a separate temperature smoother for each age class, significantly improved the explanatory power of the model (Table 3). The increase in FEC with increasing rainfall was strongest in foals and was still significant, although of around half the magnitude, in youngsters, while no significant relationship with rainfall was observed in adults and geriatrics (Fig. 4A). The increase in FEC across intermediate temperatures was most pronounced in foals and youngsters, with a weaker increase observed in adults and no evident relationship with temperature observed in the small number of geriatrics (Fig. 4B). Patterns were similar when age classes were collapsed into young (0–5 years) and adult (>5 years) classes (data not shown).

**Effects of anthelmintic treatment**

Administration of MOX and TH reduced post-treatment FECs by $\geq 95\%$ in all 6 treatments for MOX, and in all 4 treatments for which FECR could be calculated for TH (Table 2). FECs were available pre-treatment and within the standard ERP for 2 of the 3 BZ administrations and only 3–4 ponies were re-sampled after both treatments. In these cases, iFECR3 was 70% and 81%, indicating poor uptake of anthelmintic or reduced efficacy (Table 2): hence, use of this anthelmintic class was terminated at this site. GAMMs fitted to the Hickling South population alone suggested that anthelmintic treatment profoundly dampens the previously observed climate effects on FECs, but does not have much influence on estimates of repeatability and temporal autocorrelation. Random effects for individual and temporal autocorrelation were highly significant ($\chi^2(1) = 11.10$ and $7.18$, respectively, both $P<0.001$), with an estimated repeatability of 0.18 and a temporal autocorrelation coefficient of 0.25, which are similar in magnitudes to estimates from across all populations. The fixed effect of year was significant ($\chi^2(8) = 26.38$, $P<0.001$), although the smoother for month in FEC suggested a linear increase within years, which was confirmed by comparing the model with a smoother to one with just a linear term for month (comparison of models with smoother versus linear term for month: $\chi^2(1) = 0.00$, $P=0.99$; models with and without linear effect of month: $\chi^2(1) = 15.26$, $P<0.001$; estimated effect of month: $+40.84$ EPG/month $+/−10.26$ s.e.). In contrast to the analyses across all populations, the addition of smoothers for rain and temperature did not explain additional variation in
FEC in the treated population (adding temperature: \(\chi^2_{(2)} = 2.63, P = 0.27\); rainfall: \(\chi^2_{(2)} = 2.43, P = 0.30\)). Unsurprisingly, FECs performed within the anthelmintics’ ERPs were significantly lower than those FECs undertaken outside them (\(\chi^2_{(1)} = 31.34, P < 0.001\), estimated difference: 463.29 EPG +/− 81.34 S.E.; Fig. 5A). There was no evidence for any significant difference in the overall effect of the different anthelmintic types, as assessed by comparison of models including just whether or not a FEC was within an anthelmintic’s ERP and with the effect broken down by specific anthelmintic type: \(\chi^2_{(2)} = 0.01, P = 0.99\). FECs continued to increase significantly post-treatment beyond each anthelmintic’s ERP (addition of linear term for weeks since treatment to model including whether or not a FEC was within an anthelmintic’s ERP or not: \(\chi^2_{(1)} = 35.95, P < 0.001\), estimate effect: 21.78 EPG per week +/− 3.60 S.E.; Fig. 5B). Interestingly, inclusion of an ERP effect and weeks since treatment completely removed any effect of month in the model (estimated effect: −1.73 EPG per month +/− 11.08 S.E.), suggesting that much of the apparent increase in FECs within a year in this population was associated with maturation of surviving worms after treatment or the acquisition and development of new infections after treatment.

**DISCUSSION**

This work has provided a unique insight into the natural biology of cyathostomin infections. Based on this analysis it can be concluded that climate, and hence season, have a significant impact on FEC in the absence of anthelmintic treatment. It can also be
concluded that strongyle egg excretion is associated with age, with young animals, aged between 2 and 5 years, exhibiting higher FECs than adults. The interactions between age and climate imply that highest FECs would be expected in populations with younger age structures experiencing warmer and wetter climate conditions and emphasizes the importance of interplay between extrinsic environmental factors and development of immunity to the parasites in determining strongyle egg-shedding dynamics, especially in untreated populations.

Over the last decade, instead of routine, all-group treatments, the targeted application of anthelmintics to only those individuals excreting the highest number of strongyle eggs has been promoted in horses (Matthews, 2008; Kaplan and Nielsen, 2010). However, the long-term effects of such changes in management practices are unknown and few studies have been performed to assess these (von Samson-Himmelstjerna et al. 2009). Indeed, there is a paucity of publications that describe the basic epidemiology of cyathostomin infections, especially in horses that have been exposed to no, or limited, anthelmintic treatments. Here, we took advantage of the availability of long-term FEC data derived from 5 populations of ponies used for conservation purposes to examine egg-shedding patterns in different breeds and ages and in populations given no anthelmintics. Studies such as this, which use direct comparisons among treated and untreated populations, remain very rare. Our findings suggest that the clear differences in mean FECs observed over time were not only due to potential variation amongst breeds, but also to local climate conditions and their interactions with age. In the sequential pattern of strongyle egg excretion in individual ponies, significant, but relatively low, repeatability and temporal autocorrelation were observed. To some degree, this contrasts with studies on equine populations subjected to chemical control measures (Döpfer et al. 2004; Nielsen et al. 2006; Becher et al. 2010) in which observed consistency in individual FEC patterns form the basis of control strategies that target anthelmintics to only those animals that are classified as ‘high shedders’. In our study, complex within-individual dynamics were observed in longitudinal FEC patterns: these were associated with year, month, temperature, rainfall and age, with interactions observed between the latter 3. These effects were undetectable in the anthelmintic-treated population of ponies. This would be expected, as treatments in anthelmintic-sensitive nematode populations should profoundly alter and, indeed, dominate the infection dynamics (Klei and Chapman, 1999).

Differences in fecal egg excretion patterns observed here among the non-treated pony populations indicate that fixed local differences in environment matter. This could be associated with habitat quality: in small ruminants, at least, malnutrition has been shown to lead to immune function impairments that affect helminth burden (Coop and Holmes, 1996). Inter-population variation could also

Fig. 5. FECs in the anthelmintic treated population. (A) In the anthelmintic-treated pony population, FECs were considerably reduced if they were within the expected ERP of an anthelmintic, and there was little difference in this effect amongst anthelmintics, when taking the entire ERP into account. The plot shows distributions of FECs separated by whether or not the count was made within the ERP or not, and additionally separated by which anthelmintic was applied for treatments within an ERP (BZ, benzimidazole; TH, tetrahydropyrimidine; MOX, moxidectin). The ‘outside ERP’ group of FECs is represented on the graph by a box with mid-line at the median spanning the inter-quartile range and whiskers spanning the 5% to 95% quantiles, with the mean marked by a cross. The distributions of FECs that fell within the anthelmintic’s ERP are presented as jittered data points, with a dashed line at the mean. (B) FECs continue to increase after the expected ERP. The mean FEC measured different time-periods after anthelmintic treatment (with standard error bars) are plotted, with the first period (up to 12 weeks, equivalent to the longest ERP of the drugs applied) highlighted in red, with a linear regression line through the points.
be associated with stocking density differences or availability of attractive grazing sites. One implication would be that when animals are forced to graze closer to areas of fecal contamination, they are more likely to ingest nematode larvae. A major determinant of feeding area selection is availability of areas of different parasite ‘risk’ (measured as distance from contaminated fecal pats) and sward height. Controlling for availability, it has been demonstrated that horses graze tall grass (>16 cm), which is of poorer nutritional quality, less than expected, whether grass is contaminated or not, and select short patches >1 m from feces, where risk is low (Fleurance et al. 2007).

The effects of year, month and local rainfall and temperature suggest that these are important drivers of strongyle egg excretion: although there will be a time lag between transmission and downstream egg shedding, the climatic parameters will affect levels of infection to which the ponies were exposed in the preceding period and one would predict that this would have most impact where there is likely to be less larval encystment in the host, i.e. in spring and summer. Year-to-year variation in FEC, as evident from significant year effects in our models, seemed likely to reflect annual variation in climate. While local temperature and rain in the month of measurement explain most of the monthly variation in FECs, there is still a residual increase in FEC through the year (Models 2 and 3 in Table 3). This is likely to echo gradual build up of pasture contamination through the grazing season: this will comprise L3 derived from eggs released from adult worms that have matured from overwintered intestinal wall encysted larvae or from new infections acquired in the same season. The observed relationship between temperature and fecal egg counts agrees with previous studies (Baudena et al. 2000; Ramsey et al. 2004; Couto et al. 2008; Quinelato et al. 2008). At higher temperatures pre-parasitic strongyle larvae develop faster and temperature is linked to survival of free-living stages (Baudena et al. 2000; Ramsey et al. 2004; Couto et al. 2008; Quinelato et al. 2008). Above a certain threshold, however, free-living larval survival time is reduced, leading to lower environmental infection levels (Baudena et al. 2000). Likewise, cooler temperatures have a negative impact on free-living larval development (Baudena et al. 2000). Moreover, in autumn, it is likely that a proportion of L3 consumed would encyst in the large intestinal wall (Ogbourne, 1975; Eysker et al. 1990) resulting in a downstream reduction in FECs, especially once resident adult worms reach the end of their lifespan. We also observed a positive relationship between FECs and monthly mean rainfall; this can be attributed, in part, to the effect that water has on the free-living stages facilitating dispersal of feces and translation of larvae onto grass from where they are more likely to be ingested (Ramsey et al. 2004; Couto et al. 2008; Santos et al. 2011). Throughout the present study we considered immediate effects of climate at the time of sampling and have not explored longer-term, lagged effects of past climate on egg shedding patterns. Work on Soay sheep suggests that interpreting data taking into account temporal lags may be of relevance: for example, summer strongyle FECs were observed to be of greater magnitude following warm, wet winters (Hayward et al. 2009). These complex effects of previous climatic conditions could be important for understanding nematode egg-shedding dynamics in horses and will be considered in future studies.

The observation of age effect on FEC fits with the prediction that younger horses are more likely to have higher burdens than older animals due to a lack of acquired immunity and is in agreement with previous research (Love and Duncan, 1992; Klei and Chapman, 1999). The group that we categorized as ‘youngsters’ are known to be the age category at most risk of developing larval cyathostominosis, which is associated with emergence of large numbers of encysted larvae from the intestinal wall (Reid et al. 1995; Dowdall et al. 2002). Foals are likely to have lower strongyle FECs than ‘youngsters’ because they are less likely to ingest contaminated pasture whilst relying on the mare’s milk for nutrition. Because of the low sample size, we cannot extrapolate too much regarding egg shedding in geriatrics; however, there was a trend for higher strongyle egg output in this category and this could be associated with immunological senescence (Horofov et al. 2010). Our analyses suggest that the effects of acquired immunity (as indicated by the lower FECs in the ‘adult’ class) act to reduce the impact of climatic and seasonal variation on strongyle egg excretion.

In the anthelmintic-treated population, the climatic effects so clearly evident among the other populations were not detectable. To avoid clinical disease, anthelmintics were administered to this population each time high FECs were measured. Where pre- and post-treatment FECs were available, calculated FECRs suggested that MOX and TH were efficacious, with BZ less so. Our analyses suggest that the effects of anthelmintic administration dampened climatic and seasonal variation. This dataset serves to demonstrate the profound effect that anthelmintics have on the epidemiology of equine strongyle infections, when efficacy is high, and, in particular, when ERP is extended, as observed with a persistent active ingredient such as MOX.

Horses vary in parasite burden, with a relative lifelong susceptibility observed in some (Ogbourne, 1976). It has been estimated that, in treated populations, approximately 20% of animals will excrete around 80% of the total burden of the population at any one point (Kaplan and Nielsen, 2010). Here, we did not observe such strong consistency/repeatability
in FECs at the individual level, nor did we identify especially high correlations among an individual’s FECs when they were taken a month or two apart. To produce estimates of ‘consistency’, previous analyses have split horses into a range of categories (zero, low, high shedders); however, these have not been methodologically consistent or directly comparable as they have applied different shedding categories, repeat sampling periods, treatment repareable as they have applied different shedding categories, repeat sampling periods, treatment regimes and overall time-periods of study (Döpfer et al. 2004; Nielsen et al. 2006; Becher et al. 2010). Whilst this approach is intuitive and straightforward to implement, it is important to follow FEC patterns over time, rather than predict a pattern based on a limited sampling window for any given individual. Perhaps most saliently, a simple probability of an individual remaining in a particular FEC category does not account for the different factors that might influence longitudinal patterns of shedding, such as yard management, animal age and sex, changes in climate and time since last treatment. Furthermore, in most cases, these probabilities are only assessed in animals initially measured in zero or low shedding categories, because animals in high FEC categories will be treated and will change category as a result. It should also be taken into account that there is poor correlation between FECs and total cyathostomin burden, especially when proportions of encysted larvae present are high (Dowdall et al. 2002; Nielsen et al. 2010). For this reason, assessment of FEC only gives an indication of that individual’s capacity to contaminate the environment at a given point and not its overall cyathostomin burden and, hence, future capacity to contaminate the environment with eggs.

In the present study, we used a generalized additive mixed-effects model (GAMM) approach to produce an estimate of consistency of FEC at the individual level. We derived this estimate from a decomposition of variance in FEC between 2 levels—the between-individual (differences in individual mean FEC) and within-individual (residual variance). Individual repeatability was expressed as the ratio of between-individual variation to the sum of both between and within-individual variation. The current analysis has a sound statistical basis, based on standard quantitative genetic approaches (Falconer and Mackay, 1996), and should produce an estimate that is comparable across studies. However, it should be noted that when deriving repeatabilities from the random effects structure of mixed-effects linear models as we have done, the repeatability estimate will vary depending on the fixed effects included in the model, because the estimates of variance associated with random terms in the model are conditioned upon the fixed effects fitted (Wilson, 2008). Alternative and equally well-established methods also exist for calculating comparable repeatabilities using classical analysis of variance (Lessells and Boag, 1987; Wollack et al. 2012), and preliminary investigations with this approach suggested that they would yield similar estimates of repeatability (data not shown). The rather low level of individual consistency estimated using our approach, relative to previous studies, probably reflects the longer time series and larger data set, as well as the fact that our models account for factors such as population, climate and age, as well as the absence of anthelmintic treatments in 4 of the populations. It is certainly possible that individuals are more consistent than we have estimated in their egg shedding within particular seasons or age groups. However, the temporal autocorrelation estimates indicate that a FEC obtained in 1 month rather poorly predicts FEC the following month. We also note that estimates of repeatability and temporal autocorrelation were low in our treated population. We suggest that, in future, attention be paid to how ‘repeatability’ or ‘consistency’ is measured at the individual horse level, and to how high such metrics need to be to represent a faecal egg excretion pattern that would support individually-targeted treatment strategies.

In the last 20 years, anthelmintic resistance has been identified in cyathostomins on many occasions (Matthews, 2008). The ubiquitous nature and potential consequences of disease with these nematodes make them the primary target of parasite control programmes worldwide. Traditional control methods depend on the repeated application of anthelmintics. Several factors can influence the rate at which resistance develops; high frequency of treatment being one of the most important, so control strategies need to attempt to reduce the number of these applications (Matthews, 2011). A number of pasture and on-farm management practices have been found to be negatively associated with anthelmintic resistance; in regions where targeted therapies are mandatory and anthelmintics cannot be obtained without prescription, it is indicated that the spread of resistance can be slowed (Larsen et al. 2011). Nevertheless, the impact of these changes in management programmes needs to be followed over time and based on robust epidemiological knowledge of the parasites under scrutiny. The study of the epidemiology of strongyle infections in horses represents a relatively small proportion (~20%) of research findings published on these pathogens (Kornas et al. 2010) and, given that advice now relates to control measures where chemicals should be used less, it is important that the base-line patterns be investigated.

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