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Determination of ivermectin efficacy against cyathostomins and *Parascaris equorum* on horse farms using selective therapy

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ABSTRACT

Ivermectin resistance has recently been described in *Parascaris equorum* and there have been reports from several countries of a shortened egg reappearance period (ERP) following ivermectin treatment for cyathostomins. This study was aimed at determining the efficacy of ivermectin in treating cyathostomins and *P. equorum* in Danish horses. A total of 196 animals were selected from 52 farms, all of which were using a selective anthelmintic treatment strategy. ERP was investigated with weekly samples from 96 horses from nine farms. Horses were treated with ivermectin oral paste by their owners at an estimated dose rate of 0.2 mg/kg. Overall, faecal egg counts were reduced 10–14 days after treatment by 96.9% and 100% for *P. equorum* and cyathostomins, respectively. Mean faecal egg count reductions at 4 and 6 weeks post treatment were 99.5% and 96.9%, respectively. No signs of developing ivermectin resistance were found in either cyathostomins or *P. equorum* in the studied horses.

Introduction

Cyathostomins are ubiquitous parasites of grazing horses, and are considered the most important parasites in horses (Love et al., 1999) although large strongyles are still a potential threat when present. In foals and young horses, the roundworm *Parascaris equorum* also constitutes a major threat to equine health (Clayton, 1978). Over recent decades, increasing resistance of cyathostomins to several groups of anthelmintics has been reported (Kaplan, 2002, 2004). Furthermore, recent reports suggest that in some instances the cyathostomin egg reappearance period (ERP) following treatment with ivermectin has been reduced to 4–5 weeks (Lyons et al., 2008; Molento et al., 2008; von Samson-Himmelstjerna et al., 2007) from the 9 weeks initially reported (Borgsteede et al., 1993). Lyons et al. (2009) showed that this reduction was due to apparent ivermectin resistance in the luminal L4 stage. Reports from several countries of *P. equorum* isolates showing apparent ivermectin resistance have also been published (Boersema et al., 2002; Hearn and Peregrine, 2003; Schougaard and Nielsen, 2007; Veronesi et al., 2009). These developments constitute a major concern for the equine industry as ivermectin is the most widely used equine anthelmintic (Nielsen et al., 2006), and it is unclear when, or even if, a new anthelmintic class will be launched for equine usage.

To delay further development of anthelmintic resistance, recommendations have been made to reduce treatment intensity, and to base treatments on parasite surveillance (Kaplan, 2002; Nielsen et al., 2007). One widely recommended strategy is selective therapy, where faecal samples are taken from all horses and treatment is given only to individuals exceeding a predetermined cut-off egg count (Duncan and Love, 1991; Krecek et al., 1994; Little et al., 2003; Matthee and McGeoch, 2004). The use of selective therapy has been supported by changes in legislation and several European countries, including Denmark, Sweden, The Netherlands, Finland and Italy, now disallow prophylactic treatment and require a diagnosis before anthelmintics can be administered. In Denmark, where such legislation has been in place since 1999, the majority of horse establishments base their parasite control programs on selective therapy with faecal samples taken from all horses twice yearly (Nielsen et al., 2006). However, it remains unclear whether these restrictions have affected the development of anthelmintic resistance.

The aims of this study were to assess the current efficacy of ivermectin against *P. equorum* and cyathostomins on Danish farms practicing selective therapy, and to determine cyathostomin ERP after ivermectin treatment in these horses.

Materials and methods

The study was carried out between September 1st and November 1st 2008.

Horses

All investigated horses were from farms which had been using selective therapy for at least 2 years prior to the study, and which had been using ivermectin as the only anthelmintic for that period. On all farms faecal samples were taken from all

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horses twice a year, and individuals exceeding a faecal egg count (FEC) of 200 strongyle eggs per gram of faeces (EPG) or having a positive *P. equorum* egg count were treated. Farms were selected through a referral laboratory analysing faecal samples submitted from various regions in Denmark. A total of 196 horses from 52 farms were used in the study.

Egg reappearance period

The ERP following ivermectin treatment was determined on nine farms where at least 10 horses were treated. A total of 96 horses were included in the study. On the nine farms, 69 untreated horses with FECs <200 EPG were kept as a control group. There were ⩾5 untreated horses per farm.

Faecal egg counts

All FECs were determined by the McMaster method with a detection limit of 50 EPG (Roepstorff and Nansen, 1998). Larval cultures were performed on two individual horses sampled at random from each study farm to determine if any large strongyle species were present.

Treatment

In Denmark, owners are allowed to give medical treatments to their horses only after passing an authorised course in medicine handling. The veterinarian prescribing the drug specifies the dosage, and the dosing is then carried out by the owner. Thus the liveweight of each horse was estimated by its owner who then gave their horse an oral dose of 0.2 mg/kg ivermectin paste (Noromectin vet, Biovet ApS). After ivermectin treatment, the first author then estimated each horse's weight using a girth tape.

Ivermectin efficacy

Faecal samples were collected for faecal egg count reduction tests (FECRT) on the day of ivermectin treatment and 10–14 days post treatment. FECR was determined for each individual horse as:

$$FECR = (FEC_{pretreatment} - FEC_{posttreatment})/FEC_{pretreatment} * 100\%$$

Arithmetic means for each group and each farm were determined from individual FECR. Mean FECRT cut-off values selected for establishing the presence of resistance were 90% for *P. equorum* and 95% for strongyles (von Samson-Himmelstjerna et al., 2007).

Strongyle egg reappearance

Weekly FECs were performed from weeks 2 to 6 post treatment and FECR% were calculated. Cut-off values selected for declaring a shortened ERP was a farm mean FECR of <90% 6 weeks or fewer post treatment (von Samson-Himmelstjerna et al., 2007).

Statistical analyses

Data were analysed using SAS 9.1.3 software (SAS Institute). The distribution of data was examined by the univariate procedure, which showed that FEC post treatment and FECR were not normally distributed ($P = 0.0016$ and $P < 0.0001$, respectively). Hence, data were analysed using a variance component model (mixed linear models, mixed procedure). FEC post treatment was chosen as the response variable and efficacies were determined post modelling. FEC were analysed as a quantitative discrete variable. Explanatory variables were farm, horse, gender, date, dosage, age, FEC prior to treatment, mean FEC on the farm and proportion of treated horses on the farm. Horse and farm were chosen as random variables. Results were interpreted at the 0.05 level, and variables that were not significant were excluded to refine the model.

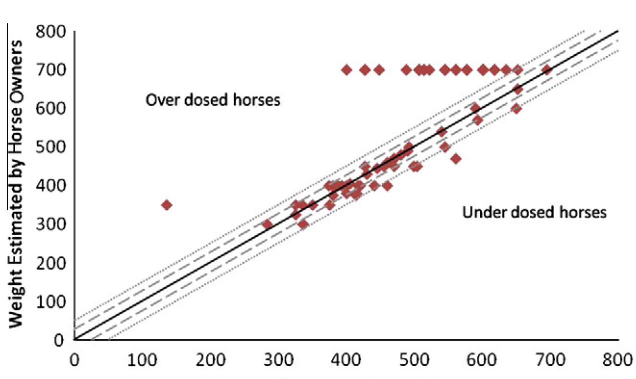

Fig. 1. Results of girth tape measurements as performed by one of the investigators compared to the bodyweight estimations of the horse's owner. Numbers on axes are in kg. The dotted lines on each side of the trend line represent deviations of ±25 kg (- - -) and 50 kg (···), respectively.

Results

Only cyathostomins were identified in the larval cultures. The large majority of the horses were dosed correctly, or marginally overdosed, according to the weight estimated by weight girth tape measurements. Eight horses were marginally under dosed. The weight estimations are presented in Fig 1.

Ivermectin efficacy

One hundred and seventeen horses (59%) were excreting strongyle eggs only. These horses were from 10 different farms and had a mean age of 8.7 years (95% confidence interval (CI): 7.6–9.4) and mean FEC of 491 EPG (95% CI: 421–562). Seventy-nine (40%) of the treated horses were excreting *P. equorum* eggs prior to treatment. These horses were from 42 farms and had a mean age of 2.1 years (95% CI: 1.6–2.5). A majority of these horses (84%) were co-infected with strongyles; mean pre-treatment strongyle FEC was 323 EPG (95% CI: 262–385).

The overall mean FECR 10–14 days following ivermectin treatment were 96.9% and 100% for *P. equorum* and strongyles, respectively.

Strongyle egg reappearance

Horses in this part of the study had a mean age of 9.0 (95% CI: 7.9–10.1) years. The results are presented in Table 1. One horse on one farm was shedding 200 EPG by week 4. By week 5, three farms and by week 6, five farms had horses shedding eggs. However, FECR remained high throughout the study period; the mean farm FECR at 6 weeks post treatment was 96.9%. Of the five farms where horses were shedding eggs, only one had a <90% reduction in FEC, and two other farms had an FEC that was higher but not significantly different from 90%. In the untreated group the mean FEC increased from 88 to 333 during the study.

Table 1
Arithmetic mean reductions in faecal egg counts (FECR) after treatment with ivermectin (0.2 mg/kg). Numbers in parentheses are 95% confidence limits for mean FECR.

FECR% post treatment					
Farm number	Week 2 (%)	Week 3 (%)	Week 4 (%)	Week 5 (%)	Week 6 (%)
1	100	100	100	100	100
2	100	100	100	94.9 (84.6–105.0)	89.6 (72.0–107.1)
3	100	100	100	100	100
4	100	100	95.8 (87.7–104.0)	96.6 (91.4–101.7)	92.6 (83.9–101.2)
5	100	100	100	100	100
6	100	100	100	100	100
7	100	100	100	98.5 (95.8–101.3)	99.2 (97.5–100.8)
8	100	100	100	100	93.8 (81.5–106.0)
9	100	100	100	100	96.9 (90.8–103.0)
Mean	100	100	99.5 (99.3–99.8)	98.9 (98.5–99.3)	96.9 (96.1–97.7)