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Short communication

Parascaris equorum in foals and in their environment on a Swedish stud farm, with notes on treatment failure of ivermectin

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Abstract

Environmental contamination and the egg excretion pattern of the ascarid *Parascaris equorum* (Nematoda) was investigated in relation to anthelmintic treatment on a Swedish stud farm. Faecal samples from 15 foals, dewormed every 8th-week with a paste formulation of ivermectin at the standard dose rate of 0.2 mg/kg bodyweight, were collected at five sampling occasions between August and November 2006. In addition, soil samples were obtained from four paddocks used by these foals in November 2006. The number of eggs per gram (epg) was counted in both faeces and soil. Egg excretion started when the foals were 3–4 months, and reached the highest levels when they were approximately 5-month-old, and was then followed by a decline. Egg excretion seemed to be unaffected by ivermectin despite these foals were dewormed at regular intervals. In four out of five foals examined 10 days after treatment, epg actually increased. In contrast, when either fenbendazol or pyrantel embonate were used instead of ivermectin, treatments were effective. The number of eggs in soil was significantly higher in the permanent paddock compared to in the temporarily used soil paddock and in the summer paddocks.

Keywords: Horse-nematoda; Foals; Anthelmintic resistance; Epidemiology; Soil

1. Introduction

The large roundworm of horses, *Parascaris equorum*, is an important cosmopolitan nematode parasite of foals. The prepatency period of *P. equorum* is about 10–15 weeks (Clayton, 1986). Attempts to demonstrate lactogenic or prenatal transmission have been unsuccessful (Andersson, 1992). Thus, ingestion of infective eggs in the environment is the major route of transmission (Boyle and Houston, 2006). Moderate to high infection levels may cause respiratory symptoms and bad appetite associated with weakness, decreased growth, enteritis and occasionally obstruction and peritonitis (Boyle and Houston, 2006).

Problems with control of strongyles that are resistant to anthelmintics including macrocyclic lactones (ML) have increasingly been documented wherever horses are grazed intensively (Kaplan, 2004). Lately, reduced

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efficacy of ML to *P. equorum* has also been reported from a stud farm in the Netherlands in foals treated with either moxidectin or normal and double doses of ivermectin (Boersema et al., 2002). Also, Hearn and Peregrine (2003) observed a high number of ascarid eggs as soon as 12–13 days after treatment. Furthermore, according to Slocombe et al. (2007) the overall efficacy of ivermectin was only 34% in foals treated with ivermectin in Canada. Recently similar observations have been made both in Denmark (Schougaard, 2005) and Germany (von Samson-Himmelstjerna et al., 2007).

In temperate regions, eggs of *P. equorum* become infective from spring to late autumn, and in conjunction with when a high number of foals spend most of their time outdoors in paddocks and/or on pasture. *P. equorum* infected horses may shed several thousands of eggs per gram faeces. Thus in a single day a foal can contaminate the environment with millions of eggs (Clayton and Duncan, 1979). Still, few environmental studies have been conducted concerning where roundworm eggs occur and for how long they remain infective. According to Bello (1982), eggs of *P. equorum* survive for many years in the soil. DiPietro et al. (1988) reported that soil samples from a paddock, which had been used by foals for the previous 25 years, contained on average 30 epg soil but only 52% of these were embryonated. In a Norwegian study (Ihler, 1995), 15 paddocks without grass cover at 12 different stud farms were investigated. Soil profiles were taken to a depth of 15 cm and it was found that paddocks with gravel had a lower total content of eggs compared to paddocks with clay or moraine soil.

The aim of this study was to provide baseline data on: (1) the egg expulsion pattern in foals on a Swedish stud farm, and (2) the egg content in soils from different paddocks used by these foals. The efficacies of oral paste formulations of commonly used anthelmintic compounds were also examined.

2. Materials and methods

Between August and November 2006, foals were examined for roundworm eggs on a commercial stud farm in south central Sweden. On this farm approximately 30 mares were housed in single-box stalls, whereas the younger horses were kept in loose housing. Between mid May and October the dams and their foals were on pasture for 24 h a day.

At the stud farm, the standard recommendations, starting with a treatment of the dams with a paste formulation mainly of ivermectin at foaling, followed by deworming of the foals every 2nd month, had been adopted for many years. The study was initiated because a massive burden of *P. equorum* was discovered at necropsy, in one foal that died in November 2005, despite it had been dewormed.

The occurrence of ascarid eggs was investigated by monitoring the faecal egg excretion pattern for 6 months in all 15 foals born in 2006, and by analyses of ground surface soil samples from four paddocks (see below). The foals that were born between early March and late June, were all subjected to faecal egg counts on five occasions at monthly intervals. Accordingly, all foals were examined on the same days but they were between 1.5 and 5 months old at the first sampling occasion. Information about the management of the paddocks and of foals and dams, as well as the crop rotation, was gathered by interviewing the stud farm manager.

Throughout the study period, all foals were dewormed every 8th-week with a paste formulation of ivermectin (Ivomec®, Merial) according to the recommended dose rate of 0.2 mg/kg bodyweight (bw). The doses were determined based on the heaviest individual using a girth measuring tape. The five foals with the largest epg counts at the ivermectin treatment the 1st-week in August 2006 were, in addition to the regular monthly samplings, re-examined 10 days after treatment in August. As the ivermectin treatments were ineffective, all 15 foals received paste formulations containing either pyrantel embonate (Banminth®, Pfizer) at a dose rate of 19 mg pyrantelpamoat per kg bw, or 7.5 mg fenbendazole per kg bw (Axilur®, Intervet), in late October 2006.

The soil sampling was conducted to investigate the level of contamination by ascarid eggs in paddocks that had been used by the foals. In general all foals spent some time in all four paddocks beginning with paddock A, with the exception of the three youngest foals (number 1, 2 and 3) that never had access to paddock B. All paddocks (A, B, C and D), were investigated in November 2006. Paddock A was a 0.2 ha paddock with clay soil and no grass cover, while paddocks B, C and D were larger (2.5, 7 and 7 ha, respectively) and mainly covered by grass. The time spent in paddock A could be up to 2–3 weeks for those foals that were born early in spring (March–April), but usually only 1–3 days for those born later in the spring (May). Paddock B was only temporarily used until mid May, before the foals were turned out to their summer pastures (paddocks C and D). Paddock C was a natural pastureland with a mixture of grass areas, rocky parts, bushes and trees and all foals grazed it more or less simultaneously. Also paddock D had a grass cover

(ley), and it was an extension of paddock C allowed to be grazed from June. When the study was conducted, the ley (which was incorporated in crop rotation) was 7 year old.

From each paddock up to two sampling areas were selected. Soil samples were then collected from these and analysed according to the method of Roepstorff and Nansen (1998). Briefly, soil samples were collected with a device taking equal samples from the upper ground layer (~3 cm depth) every seventh step and by walking along a W route. Care was taken to avoid fresh dung. The sampling was then repeated by walking a W route covering the same area up side down. Approximately 30 samples were obtained from each W route and then carefully mixed into a pooled sample with a total amount of 300–500 g. In the laboratory, a subsample of 10 g from each pooled sample was prepared and soaked overnight in 0.5 M NaOH. Nematode eggs were enumerated with a detection limit of 1 egg per 10 g soil, and identified to genus. If ascarid, their stage of development (with or without larva) was determined. The faecal samples were analysed using a modified McMaster technique based on 4.5 g faeces, with a detection level of 33 epg faeces (Monrad et al., 1999).

Data were summarised using Microsoft Excel® and analysed using the statistical analysis system (SAS) version 9.1 (2004). Factors that influenced the epg pattern were investigated with a repeated measures analysis of variance using the Mixed Model procedure, which uses a restricted maximum likelihood estimation technique and allows for missing values and an unbalanced design (Wolfinger and Chang, 1998). The model tested the fixed effects of age group (i.e. foals older or younger than 3 months at the start of the study), sampling date, interaction between age group and sampling occasion, and with the animal as a random effect. Three covariance structures were tested: compound symmetry (CS), Huynh–Feldt (HF) and unstructured (UN).

Statistical analysis of the results from the environmental sampling was made using generalized linear models (Olsson, 2002). The number of eggs was modelled by a Poisson distribution with a log link. The procedure Genmod of SAS was used for the numerical evaluations. The significance level was set to $p < 0.05$. The paddocks were divided into three types prior to the statistical analysis: type 1, small soil paddock temporarily used (paddock A); type 2, ley pasture used only during summer (paddocks B and D); and type 3, permanent natural pasture used all-year around (paddock C).

3. Results

Changes in faecal egg counts (FEC) are presented in Fig. 1. As can be seen only one foal, number 7, excreted eggs before the age of 3 months. Twelve foals out of the fifteen shed more than 1750 epg and 40% had a peak value of more than 4000 epg. Three foals, numbers 1, 10 and 13, had consistently low values throughout the study. According to the statistical analysis of the epg values there was a significant effect of the sampling date ($p = 0.006$), as well as the interaction between age group and time of sampling ($p = 0.014$), irrespective of the covariance structure. This shows that there was a clear seasonal pattern in the egg excretion, although this was slightly different between early and late born foals.

As shown in Table 1, the treatment of the five foals, which were sampled after being dewormed with ivermectin in August 2006, had no effect on the *P. equorum* egg output. In fact, four out of five foals had higher epg values 10 days after the ivermectin treatment.

The number of *P. equorum* eggs in the soil varied from no eggs in any of the four samples from paddock B, to four positive samples from paddock C with a maximum of 22 embryonated eggs, containing a larva, per 10 g soil (Table 2). The samples from the type 3

Table 1
Faecal egg count reduction test (FECRT), 10 days after administration with an oral paste formulation of ivermectin

Individual	08 August 2006 epg before treatment	18 August 2006 epg 10 days after treatment	Change (%)
Foal 9, age 4 months	1100	1900	173
Foal 11, age 4 months	3200	5200	163
Foal 12, age 4 months	3000	4300	143
Foal 14, age 5 months	2700	5200	193
Foal 15, age 5 months	6350	4200	66
Mean	3270	4160	147

For most individuals the number of eggs from *Parascaris equorum* was higher after treatment than before. Note that only unembryonated eggs were counted.

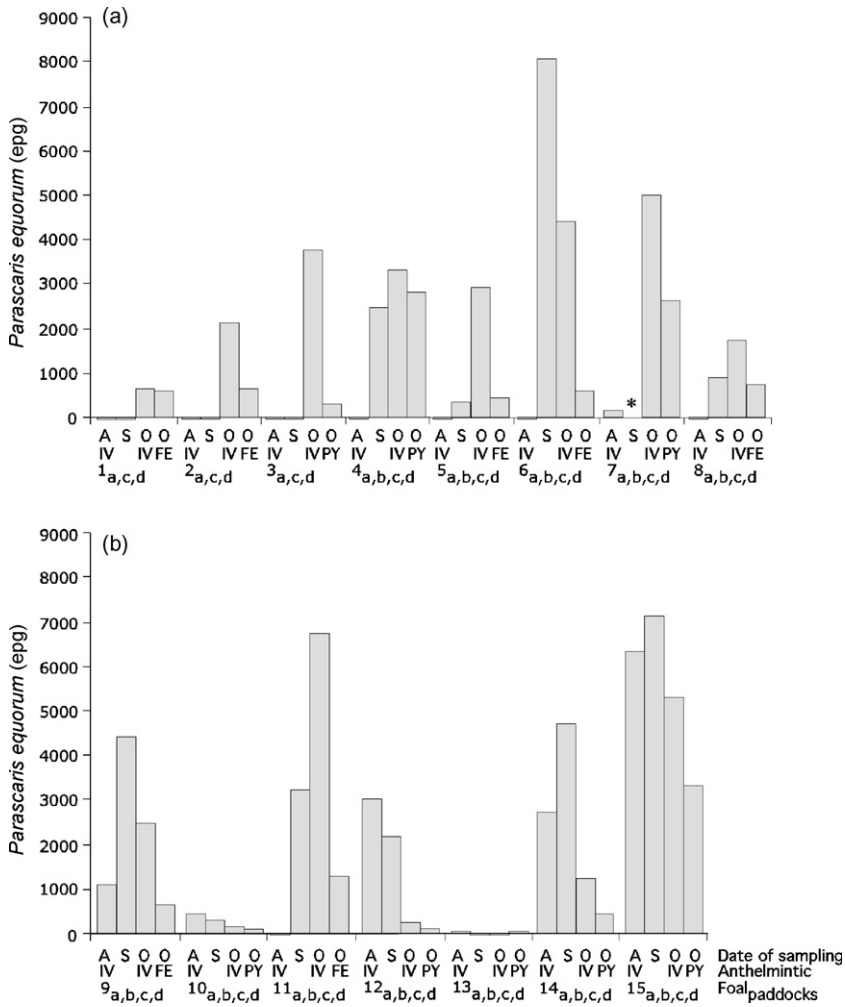

Fig. 1. Faecal egg counts (FECs) of *P. equorum* from foals on a Swedish stud farm. Data are shown for each individual but samples were collected simultaneously on 08 August (A), 06 September (S), 05 October (O), and 25 October (O). Throughout the study all foals had used the same paddocks apart from foal 1–3 that never had access to paddock B. They were also dewormed on the same day with an oral paste formulation of ivermectin (IV) at approximatly 8 week intervals, whereas on 25 October they were treated either with fenbendazole (FE) or pyrantel (PY). The results from the last sampling occasion in 06 November, have been omitted as all horses, apart from foal 3, had negative FECs. The star indicates that the September sample from foal 7 was missing. (a) Foals $\leq$3 months and (b) foals $\geq$3.5 months at the first sampling occasion.

paddock contained a larger number of *P. equorum* eggs compared to the types 1 and 2 paddocks (Table 2), ($p < 0.001$ in both comparisons).

4. Discussion

This study shows when and where foals became infected with *P. equorum* on a Swedish stud farm. Most foals did not excrete large number of eggs until August or September when they were approximately 4-month-old. Egg excretion peaked approximately 1–2 months later, and was followed by a decline. The soil samples from the paddock that was used all-year around were the most contaminated with ascarid eggs.

Interestingly, there was no reduction of eggs from *P. equorum*, despite all foals were treated throughout the study every 8th-week with ivermectin. In four out of five animals examined 10 days after treatment, the faecal egg counts were actually increased. This is in accordance with recent reports on the poor or reduced efficacy of macrocyclic lactones against ascarid infection in horses, both in the Netherlands (Boersema et al., 2002), Canada (Hearn and Peregrine, 2003; Slocombe et al., 2007), Denmark (Schougaard, 2005) and Germany (von Samson-Himmelstjerna et al., 2007). Hence, the results of the present study, indicated that *P. equorum* was not adequately controlled with ivermectin paste. This is not surprising as it also corresponds to

Table 2
The number of eggs in the pooled soil samples and the percentages of these eggs that contained a larva

Paddock	Pooled samples	Eggs per 10 g soil	Embryonated eggs (%)	Dry soil g per 10 g	Eggs per g dry soil
A	1A	0	–	7.2	0
	1B	2	100	6.9	0.29
B	1A	0	–	6.4	0
	1B	0	–	6.4	0
	2A	0	–	6.4	0
	2B	0	–	6.6	0
C	1A	19	100	6.3	3.02
	1B	14	86	6.0	2.33
	2A	3	100	5.7	0.53
	2B	22	100	5.8	3.80
D	1A	1	100	6.6	0.15
	1B	0	–	7.2	0

Samples were collected altogether from four paddocks (A–D) in November 2006.

previous reports from Swedish stud farms on the failure of macrocyclic lactones in the treatment of this parasite. Reduced efficacy of ivermectin in *P. equorum* infected horses was actually first recognised at the largest stud farm in Sweden more than 10 years ago. Recently, the same problem has also been documented on several other stud farms in Sweden (Osterman-Lind, unpublished data).

In contrast, a high efficacy of ivermectin paste against the parasitic stages of *P. equorum* has been reported earlier. For example, Austin et al. (1991) claimed that egg excretion was suppressed for 10–12 days post-treatment with ivermectin paste. The paste formulation has also been declared to be 100% effective against 11-day-old migrating larvae of *P. equorum* (French et al., 1989). In an American trial with 20 ponies (aged 2–11 years), ivermectin treatment reduced the burden of adult *P. equorum* from 2.9 to 0 (Klei et al., 2001). When DiPietro et al. (1997) treated 150 standardbred horses (aged 5 months to 23 years) with paste formulations of ivermectin or moxidectin, both substances were reported as being effective. However, the egg reduction pattern was not clearly presented. The same authors have also suggested incomplete efficacy of ivermectin paste against migrating stages of *P. equorum* (DiPietro et al., 1987).

On the other hand, treatment with either fenbendazole or pyrantel embonate proved to be effective in the present study. Only one foal that was treated with pyrantel embonate excreted >100 epg 3 weeks post-treatment. However, it needs to be emphasized that the number of animals in each treatment group was low with only 7 and 8 foals, respectively. Also the deworming with fenbendazol or pyrantel embonate was made in late October, when the foals were between 4 and 7.5 months old. Thus it cannot be excluded that the reduction at least in some cases was partly due to immune expulsion. Nevertheless, our results are in accordance with Slocombe et al. (2007), who noticed high overall efficacy of fenbendazole (100%) and pyrantel pamoate (>97%) against *P. equorum*.

The largest average number of eggs, 15 egg per 10 g soil, was found in the soil samples from the permanent paddock C. This paddock had been used all summer by dams and foals, both for creep-feeding and grazing and as a transport area to the leys used for grazing. Furthermore, paddock C had previously been used the whole year around for many years, since its rocky nature made it less vulnerable to tramping. From the other paddocks only a few eggs were recovered and in particular from paddock B, where no eggs were found. All eggs recovered from the soil samples were embryonated and contained a larva, showing that the conditions had been favourable for their development and survival. This shows that fresh dung was not the source of the eggs, despite dung distribution was large in particular in paddocks A and C. The contamination observed in paddock C was about 20 times lower than reported in the results from DiPietro et al. (1988), who found 30 eggs per of gram soil and of which only 52% were embryonated.

Calculating with a prepatency period of 10 weeks from the first sampling occasion, the foals that excreted *P. equorum* infective eggs in the beginning of August must initially have been exposed to *P. equorum* already in May, or sometimes even earlier than that. It has been established that lactogenic transmission is not a source of infection of foals (Andersson, 1992), and that older horses in general do not shed *P. equorum* eggs. Thus, the most likely

explanation is that these foals picked up a residual contamination that arrived from other foals that used the same paddocks in previous years. Hence, our results are in accordance with DiPietro et al. (1988), who claimed that foals are mainly infected by eggs derived from the previous generation of foals.

The proportion of eggs that overwinter and remain infective until the next generation of foals enter the paddocks is a question of major practical concern to understanding the infection dynamics of *P. equorum*. Unfortunately, the survival and distribution of the eggs in the outside environment has only been scarcely investigated (Bello, 1982; Ihler, 1995; DiPietro et al., 1988). For example, results from natural pastures have according to our knowledge never been presented. On this stud farm the natural pastureland in paddock C, which was used more or less the year around, was evidently the environment where these foals experienced the highest risk of being exposed to infective eggs.

Although the general opinion is that ascarid eggs are infective for many years, it remains speculative how many years a paddock must be spelled to become parasitologically safe. In experimental studies with *Ascaris suum*, only a few deposited eggs were recovered after 4–12 months (Larsen and Roepstorff, 1999). Furthermore, Kraglund (1999) found that <10% of the eggs were infective to tracer pigs after 8–18 months. These findings point out the great demand for similar studies as regards *P. equorum*. This is important, in order to understand when and where foals are infected. However, it is also of more general interest as it has been claimed that anthelmintic resistance is delayed when there is a large proportion of parasites in refugia (van Wyk, 2001).

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References

Andersson, R.C., 1992. Nematode Parasites of Vertebrates. CAB International, University Press Cambridge, 578 pp.

Austin, S.M., DiPietro, J.A., Foreman, J.H., Baker, G.J., Todd Jr., K.S., 1991. Comparison of the efficacy of ivermectin, oxibendazole, and pyrantel pamoate against 28-day *Parascaris equorum* larvae in the intestine of pony foals. J. Am. Vet. Med. Assoc. 198, 1946–1949.

Bello, T.R., 1982. Endoparasitism. In: Mansmann, R., McAllister, E., Pratt, P. (Eds.), Equine Medicine and Surgery, 3rd ed., vol. 1. American Veterinary Publications, Santa Barbara, CA.

Boersema, J.H., Eysker, M., Nas, J.W.M., 2002. Apparent resistance of *Parascaris equorum* to macrocyclic lactones. The Brit. Vet. Assoc. 150, 279–281.

Boyle, A.G., Houston, R., 2006. Parasitic pneumonitis and treatment in horses. Clin. Tech. Equine Pract. 5, 225–232.

Clayton, H.M., 1986. Ascarids. Recent advances. Vet. Clin. N. Am. 2, 313–327.

Clayton, H.M., Duncan, J.L., 1979. The migration and development of *Parascaris equorum* in the horse. J. Parasit. 9, 285–292.

DiPietro, J.A., Lock, T.F., Todd Jr., K.S., Reuter, V.E., 1987. Evaluation of ivermectin paste in the treatment of ponies for *Parascaris equorum* infections. J. Am. Vet. Med. Assoc. 190, 1181–1183.

DiPietro, J.A., Todd, M.S., Todd, K.S., 1988. Chemotherapeutic treatment of larvae and migratory stages of *Parascaris equorum*. In: Proceedings of the 34th Annual Convention of the American Association of Equine Practitioners, San Diego, CA.

DiPietro, J.A., Hutchens, D.E., Lock, T.F., Walker, K., Paul, A.J., Shipley, C., Rulli, D., 1997. Clinical trial of moxidectin oral gel in horses. Vet. Parasitol. 72, 167–177.

French, D.D., Klei, T.R., Taylor, H.W., Chapman, M.R., 1989. Efficacy of ivermectin in oral drench and paste formulation against migrating larvae of experimentally inoculated *Parascaris equorum*. Am. J. Vet. Res. 50, 1071–1073.

Hearn, F.P.D., Peregrine, A.S., 2003. Identification of foals infected with *Parascaris equorum* apparently resistant to ivermectin. J. Am. Vet. Med. Assoc. 223, 482–485.

Ihler, C.F., 1995. The distribution of *Parascaris equorum* eggs in the soil profile of bare paddocks in some Norwegian stud farms. Vet. Res. Commun. 19 (6), 495–501.

Kaplan, R., 2004. Drug resistance in nematodes of veterinary importance: a status report. Trends Parasitol. 10, 477–481.

Klei, T.R., Rehbein, S., Visser, M., Langholff, W.K., Chapman, M.R., French, D.D., Hanson, P., 2001. Re-evaluation of ivermectin efficacy against equine gastrointestinal parasites. Vet. Parasitol. 98, 315–320.

Kraglund, H.O., 1999. Survival, development and dispersal of the free living stages of *Ascaris suum, Oesophagostomum dentatum* and *Trichuris suis* at the pasture. Ph D Thesis, Royal Veterinary and Agricultural University, Copenhagen, Denmark, 124 pp.

Larsen, M.N., Roepstorff, A., 1999. Seasonal variation in development and survival of *Ascaris suum* and *Trichuris suis* eggs on pastures. Parasitology 119, 209–220.

Monrad, J., Bjørn, H., Craven, J., Pearman, M., Eiersted, L., 1999. Parasitologisk diagnostik i stordyrspraksis. Kvantitativ gødningsundersøgelse med henblik på involdsorm hos heste. Dansk Veterinärtidsskrift 82, 113–117.

Olsson, U., 2002. Generalized Linear Models—An Applied Approach. Studentlitteratur, Lund, Sweden.

Roepstorff, A., Nansen, P., 1998. Epidemiology, diagnosis and control of helminth parasites of swine. FAO Animal Health Manual No 3, Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark, 161 pp.

SAS Institute Inc., 2004. *SAS/Stat*® User's Guide: Version 9.1. SAS Institute Inc., Cary, NC.

Schougaard, H., 2005. Resistensudvikling hos hestens spolorm overfor ivermectin—det første beskrevne tilfælde i DK. Dansk Veterinærtidskrift 14, 22–23.

Slocombe, J.O., de Gannes, R.V., Lake, M.C., 2007. Macrocyclic lactone-resistant *Parascaris equorum* on stud farms in Canada and effectiveness of fenbendazole and pyrantel pamoate. Vet. Parasitol. 145, 371–376.

van Wyk, J.A., 2001. Refugia—overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. Onderstepoort J. Vet. Res. 68, 55–67.

von Samson-Himmelstjerna, G., Fritzen, B., Demeler, J., Schürmann, S., Rohn, K., Schnieder, T., Epe, C., 2007. Cases of reduced cyathostomin egg-reappearance period and failure of *Parascaris equorum* egg count reduction following ivermectin treatment as well as survey on pyrantel efficacy on German horse farms. Vet. Parasitol. 144, 74–80.

Wolfinger, R.D., Chang, M., 1998. Comparing the SAS GLM and MIXED Procedures for Repeated Measures. SAS Institute Inc., Cary, NC.