Searching for evidence of resistance development of *Parascaris* spp. against fenbendazole in the Netherlands

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Abstract

Background information: Worldwide *Parascaris* spp. are common infections in foals that can lead to respiratory signs due to hepato-tracheal migration of larvae and impaction due to adult stages in the small intestines.
Relevance of the study: It is vital to have suitable treatment options for foals infected with *Parascaris* spp., since impactions can have fatal consequences. There are a number of studies that reported reduced efficacy of fenbendazole in other countries, however the current state of resistance development of *Parascaris* spp. against fenbendazole in the Netherlands is unknown.
Aim of the study: To investigate the current efficacy, and thereby to determine if there currently is reduced efficacy, of fenbendazole against *Parascaris* spp. in foals aged 3 to 12 months on farms in the Netherlands.
Materials and methods: In order to find foals that were infected with *Parascaris* spp., 297 fecal egg counts for individual foals have been performed at 11 farms in the Netherlands. All detected eggs were counted during the process, not only *Parascaris* spp. eggs. To investigate the current state of resistance development of *Parascaris* spp. against fenbendazole, fecal egg count reduction tests were performed with 24 foals which were treated with Panacur®. Additionally, there was a questionnaire send to the 11 farms about their anthelmintic policy and general management.
Results: Only 72 of 297 foals tested positive for *Parascaris* spp., of which 49 foals had a fecal egg count of 50 *Parascaris* spp. eggs per gram (EPG) or more. Of the 24 fecal egg count reduction tests performed with fenbendazole; one foal had a reduction of 66% while the other 23 foals had a reduction of 100% of *Parascaris* spp. eggs. Twenty foals had strongylid type eggs in their feces with an average count of 347 EPG. The reduction of fecal egg counts of strongylid type were on average 62,6%, with a standard deviation of 49,7%. Remarkable data from the questionnaire was the amount of anthelmintics that are used on the visited farms.
Discussion: Fourthy-nine out of 297 foals tested positive for *Parascaris* spp. which is lower than prevalences reported in earlier studies. A possible explanation is the extent of deworming that the foals are exposed to and / or building-up of immunity to *Parascaris* spp.. The individual foal with a reduction of 66% of *Parascaris* spp. eggs meant that there was still one egg detected in the feces, two weeks after treatment.
Conclusion: There is no clear evidence for existing fenbendazole resistance development, monitored with Fecal Egg Count Reductions Test of *Parascaris* spp. in 23 foals. However, as previously has been reported in other studies, there was reduced efficacy of fenbendazole against strongyles.

Keywords: Parascaris spp.*, fenbendazole, Panacur®, resistance, efficacy, the Netherlands, FECRT.*

Introduction

Parascaris spp. are common infections in foals and young horses and can be life threatening (Clayton and Duncan, 1979b; Kaplan, 2004; Nielsen et al., 2019). In order to make accurate treatment decisions it is important to have data on current local anthelmintic resistance development (Nielsen et al., 2019). Currently, it is unknown if there is resistance of *Parascaris* spp. to fenbendazole present in the Netherlands.

Parascaris spp.

In this research study *Parascaris univalens* and *Parascaris equorum* will both be referred to as *Parascaris* spp.. In most literature two different species of *Parascaris* spp. are reported, however they are not distinguishable morphologically and are only differentiable by karyotyping (Nielsen et al., 2014). Most publications are about *P. equorum.* However, research done by Nielsen et al. (2014) showed that *P. univalens* might be the main species of *Parascaris* spp. found in foal's feces. This uncertainty about terminology made Nielsen et al. (2014) advise to use the common term *Parascaris* spp. when specific species determination isn't performed. A recent DNA study showed that *P. univalens* and *P. equorum* are closely related and may even represent the same species (Gao et al., 2019).

Life cycle and transmission

Parascaris spp. have a direct life cycle that is pictured in Figure 1. Ingested eggs of *Parascaris* spp. nematodes will hatch in a foal as a third stage larvae and start a hepato-tracheal migration (Fabiani et al., 2016). When a foal gets experimentally infected with *Parascaris* spp. first post-mortem signs of infection were found in the liver within 48 hours at necropsy, starting with hemorrhagic spots (Clayton and Duncan, 1979b). Furthermore, Clayton and Duncan (1979b) discovered that larvae can be recovered from the liver seven days after infection. In one to two weeks after experimental infection the larvae migrate to the lungs where they will molt into fourth stage larvae and will be coughed-up and ingested (Nicholls et al., 1978; Clayton and Duncan, 1979b). After 23 days *Parascaris* spp. can be found in the small intestines where they will molt into an adult stage (Clayton and Duncan, 1979b; Clayton, 1986). Adult female *Parascaris* spp. lay immature eggs that pass the small intestines with the feces and so will contaminate the environment (Clayton, 1986). Reported prepatent periods range from 72 to 110 days (Clayton and Duncan, 1977; Clayton and Duncan, 1979b; Lyons et al., 1976). The eggs are not infective to foals until they have developed to a stage three larvae. This process takes around 10 days under optimal conditions of 25-35°C (Clayton, 1986). *Parascaris* spp. eggs are very resistant to environmental influences. In colder climates with temperatures below 10°Celsius eggs remain viable; however, development of the eggs stagnates (Clayton, 1986). When temperatures range between 35 and 55°C for a week, *Parascaris* spp. eggs will die (Gould et al.,2013). Besides temperature, humidity also plays a role in development rate of *Parascaris* spp. (Nielsen, 2016). Studies with ascarids of other species show a direct relation of higher relative humidity and the rate of development of the eggs (Gamboa, 2017; Seamster,1950). Furthermore, ascarid eggs remain longer viable when the soil has a higher humidity (Gaspard et al., 1997; Gaasenbeek and Borgsteede, 1998). A study by Ihler et al. (1995) showed that removing horses from a paddock for an 18 months period did not significantly decrease the number of infective eggs in the soil. Clayton (1986) suggest that a *Parascaris* spp. eggs can remain viable up to several years. Due to the climate in the Netherlands, the period of developing of

the eggs on the field, and the length of the prepatent period, foals will generally get infected by infected foals of the previous year that were on the same pasture or confinement.

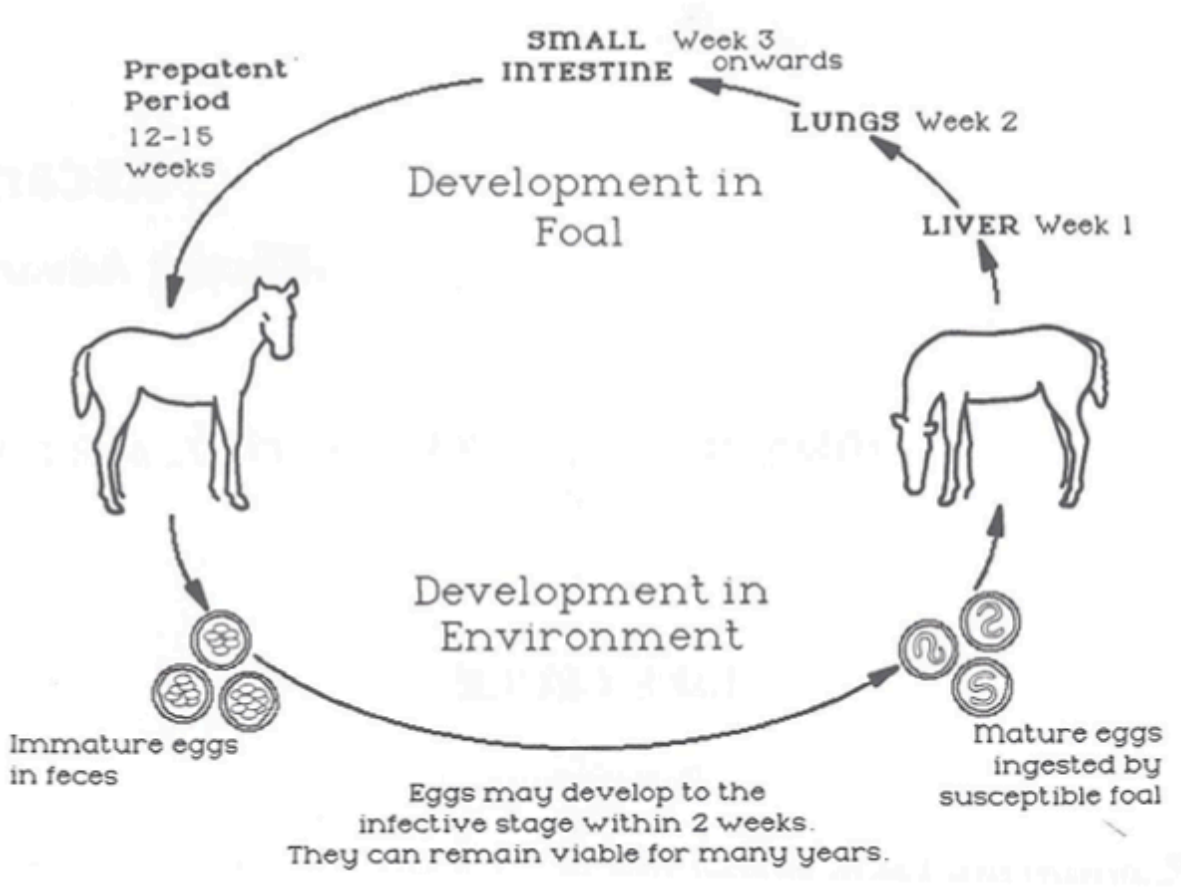

Figure 1: The direct life cycle of *Parascaris* spp. (Clayton, 1986 p. 314).

Clinical signs

Clinical signs of a *Parascaris* spp. infection may include respiratory symptoms of a bronchopneumonia like coughing and mucopurulent nasal discharge caused by the migrating larvae through the lungs (Nicholls et al., 1978; Clayton and Duncan, 1978). Foals experimentally infected at 2 to 4 weeks of age, developed previously named respiratory signs 13 to 25 days after infection (Nicholls et al., 1978; Clayton and Duncan, 1978). Moreover, a poor body condition score, decrease in growth, lethargy, anorexia and colic because of impaction can be seen (Clayton and Duncan, 1978; Cribb et al., 2006; Nielsen, 2016).

Immunity

When horses of 6 to 12 months old were exposed to *Parascaris* spp. they had a lower probability of having patent infections (Clayton and Duncan, 1979a). When these foals shed *Parascaris* spp. eggs after the prepatent period passed, the fecal egg counts (FEC) were lower compared to foals that were infected at 2 to 4 weeks of age (Clayton and Duncan, 1979a). Whether the foals were raised under worm-free conditions or were exposed to natural worm burdens on their pasture had no significant influence on the immunity build up after being experimentally infected (Clayton and Duncan, 1979a). Furthermore, Clayton and Duncan (1979a) found that in horses from 6 to 12 months old fewer larvae reached the small intestines. The majority of larvae were destroyed or otherwise died in the liver and lungs.

Therefore, it can be concluded that egg shedding reduces in foals, starting from 6 months of age, due to the development of immunity (Clayton and Duncan, 1979a). The faecal egg counts of *Parascaris* spp. peak at 4 months of age, while adult worm burdens in the intestines is highest at 5 months of age (Fabiani et al., 2016). Additionally, a secondary peak of immature *Parascaris* spp. is seen at 9 months of age, however this peak isn't accompanied by a peak of adult *Parascaris* spp. or a peak in FEC (Fabiani et al., 2016). Fabiani et al. (2016) also proved that there was no association with FECs or worm burdens of *Parascaris* spp. to seasonality. Therefore, it was concluded that the ageing of the foal changed worm burdens and FECs, because of the development of immunity. *Parascaris* spp. infections can appear in adult horses when immunity build up failed, however these infections often are not clinically relevant (Nielsen et al., 2019).

Reported reduced efficacies of anthelmintics around the world

The definition of anthelmintic resistance that is currently being used is defined by Sangster in 1999 (Nielsen et al., 2019). Sangster says: "Resistance is the ability of worms in a population to survive treatments that are generally effective against the same species and stage of infection." (1999). When any anthelmintic is excessively used over a period of time, selection of resistant-associated alleles is likely to occur (Kaplan, 2004). There are three major groups of anthelmintic therapy for horses: benzimidazoles (including fenbendazole), tetrahydropyrimidines (pyrantel) and macrocyclic lactones (moxidectin and ivermectin) (Coles et al., 2006). In Australia resistance against all the previously mentioned anthelmintic groups was found (Armstrong et al., 2014). In North America and Finland reduced efficacy against pyrantel and macrocyclic lactones was found (Craig et al., 2007; Lyons et al.,2008; Näreaho et al., 2011; Hautala et al., 2019). In Canada fenbendazole and pyrantel were found to be very effective, however resistance against ivermectin and moxidectin was found (Slocombe et al., 2007). In Sweden Martin et al. (2018) found resistance to pyrantel, while *Parascaris* spp. were still susceptible to fenbendazole at the majority of farms. Resistance against fenbendazole was found in Saudi Arabia, and although to a lesser extent, reduced efficacy of ivermectin was also present (Alanazi et al., 2017). In conclusion there are many studies in several countries that have reported resistance of *Parascaris* spp. against macrocyclic lactones and a number of studies that reported reduced efficacy of fenbendazole and pyrantel. In the Netherlands resistance of *Parascaris* spp. against macrocyclic lactones has been described previously (Boersema et al., 2002). There is no data on resistance development against either pyrantel or fenbendazole in the Netherlands.

Fenbendazole

Benzimidazoles were introduced to the veterinary market around the 1960's mainly for the treatment of nematodes and specifically in horses against *S. vulgaris* (Campbell, 1990). They were often used for dogs, cats, horses and farm animals because of their broad spectrum against helminths, including larval stages, and because there were no known side effects (Campbell, 1990). Benzimidazoles bind to ß-tubulin, thereby preventing polymerization and decreasing available energy for the cells of *Parascaris* spp. (Nielsen et al., 2019; Tydén et al., 2014; Malekpour et al., 2019). Resistance against benzimidazoles for other parasites than *Parascaris* spp. can be associated with single nucleotide polymorphism (SNP) in the ß-tubulin gene that lead to structural changes of the binding site of the drug (Kwa et al., 1993; Tydén et al., 2014). The molecular origin for resistance development of *Parascaris* spp. against benzimidazoles is still unclear. Tydén et al. (2014) found no SNP's of *Parascaris* spp. that were known for causing reduced efficacy of benzimidazole in other nematodes at that

time. Malekpour et al. (2019) recently studied the ß-tubulin isotype-1 gene of *Parascaris* spp., however found no SNP's or substitutions accountable for resistance against benzimidazoles. Since more widespread resistance is known against pyrantel and macrocyclic lactones in the US, the preferred treatment for *Parascaris* spp. is a benzimidazole, according to AAEP (Nielsen et al., 2019). Unfortunately, there is no guideline available based upon data originating from the Netherlands. Moreover, within a day after deworming of a foal with a heavy infection of *Parascaris* spp. with an anthelmintic that paralyses parasites can cause small intestine impaction (Nielsen, 2016; Cribb et al., 2006). This effect has not been observed with fenbendazole treatments (Nielsen, 2016). Thereby fenbendazole might be a safer alternative than macrocyclic lactones or pyrantel (Nielsen et al., 2019).

Currently there are two registered products with fenbendazole for horses in the Netherlands: Panacur equine guard® suspense 100 mg/g and Panacur® paste 187,5 mg/g (College ter Beoordeling van Geneesmiddelen, 2021). Panacur equine guard® suspense is mainly aimed at targeting larval stages of cyathostomes with a recommended daily dose of 7,5 mg fenbendazole per kilogram bodyweight during five consecutive days. Panacur® paste is developed for one-off treatment of gastro-intestinal parasites including *Parascaris* spp. The same dosage of 7,5 mg fenbendazole per kilogram bodyweight is advised. According to its prescription it is safe to use during gestation and lactation, no adverse effects are recorded, and it has a registered waiting time for slaughter of 20 days. Panacur® paste was used for this study.

Hypotheses

There were two hypotheses made for this study. H0 is there is no resistance of *Parascaris* spp. against fenbendazole in foals on farms in the Netherlands. And H1 is there is resistance of *Parascaris* spp. against fenbendazole in foals on farms in the Netherlands.

Aim of the study

The aim of this study is to investigate the current efficacy, and thereby to determine if there is currently is reduced efficacy, of fenbendazole against *Parascaris* spp. in foals aged 3 to 12 months on farms in the Netherlands.

Material and methods

Study design

For this study fresh fecal samples from foals aged, 3 to 12 months, were collected from 11 farms in the Netherlands. Collecting feces was done by picking fresh droppings from the ground. Samples were collected in plastic bags and analyzed directly at the farms or were put in a container with cooling elements for transport as recommended by the *American Association of Equine Practitioners* (AAEP) guidelines (Nielsen et al., 2019). To determine efficacy of fenbendazole treatment against *Parascaris* spp. the faecal egg count reduction test (FECRT) was used. Foals that were included in the study were treated with an anthelmintic, either fenbendazole or pyrantel (this last study was executed by researcher Groen). After 14-17 days another fresh fecal sample was collected of that individual foal to determine the effect of the treatment.

Farms

Farms were selected through the internet and contacted by phone. All spoken agreements were confirmed by email. After the first contact the following conversations were continued by phone or email. The owner of the farms had to be willing to participate and expect at least 20 foals by the end of September 2020. Furthermore, an inclusion criterion was that farms had to stop giving anthelmintics for at least 14 days prior to our first visit. The selected farms offered grouped housing for foals owned by others. These types of farms are common in the Netherlands. The foals are brought to the stable mostly from August to November after weening on 5 or 6 months of age. The foals stay until approximately 3 years of age on the farms. During that period the owners of the foals pay the farmer an agreed amount per month or year and in return the foals are looked after and housed in groups. These types of farms were selected because they are often larger than breeding farms in the Netherlands and therefore it was reasoned that large groups of foals could be tested. Before collecting the feces, a questionnaire was sent to the owners of the farms about their anthelmintic policy and general stable management. The questionnaire can be found in Dutch in ‘Attachment 1’. The questionnaire had to be filled in by owners of farms to be enlisted in the study. The goal of the questionnaire was to gain information about management choices and anthelmintics policies on these types of farms.

Animals

Foals were identified by a chipreader where possible. Additionally, color, markings, estimated age, gender and stable were noted in order to differentiate foals. For this study an inclusion criterion was that foals had to be between 3 and 12 months of age. Furthermore, foals were included if they were not treated with an anthelmintic for at least two weeks prior to the first visit.

Modified McMaster technique

The number of *Parascaris* spp. eggs were counted using the modified McMaster technique on 3 grams of feces with a sensitivity of 17 eggs per gram (EPG). The McMaster counting chamber is pictured in Figure 2. The sensitivity is calculated with the volume of one compartment being 0,15 ml and the fact that 3 grams of feces is suspended with 42 ml of sucrose solution. There are 3 chambers (6 compartments) counted and therefore 0,9 ml is

analyzed per fecal sample. Three grams of feces with 42 ml is around 45 ml, therefore 1 gram of feces equals 15 ml of suspension. Fifteen milliliters divided by 0,9 ml is 16.67. Therefore, every egg that is counted equals 17 eggs per gram feces (De Kool-van der Woude J.W., 2015). The protocol for the modified McMaster method can be found in 'Attachment 2'. It was important that the correct flotation solution with the right amount of osmotic forces was being used, in order to float the *Parascaris* spp. eggs and preserving the structural integrity. Norris et al. (2018) reports a specific gravity of 1.903 of *Parascaris* spp. eggs, thereby having a higher density than anoplocephalid eggs and strongylid type eggs. This is due to the thick proteinaceous capsule. According to O'Grady and Slocombe (1980) a solution with a density of between 1.22 and 1.38 specific gravity is suitable for *Parascaris* spp. eggs. For this study a sucrose solution was used with a density of 1.30 g/cm^3. The solution was made by dissolving 1280 grams of white crystal sugar in 1 liter of heated tap water (De Kool-van der Woude J.W., 2015). The specific gravity of the solution was confirmed using a scale; Ten ml of sucrose solution should weigh 13 grams.

If the EPG was below 50 the foal was still considered positive for *Parascaris* spp., however the foal was excluded for the FECRT. When the EPG of *Parascaris* spp. was above 50, the foal was included for the FECRT. This meant 3 or more eggs had to be found in 3 counting chambers. Additionally, strongylid-type and possible other eggs that were found were also counted.

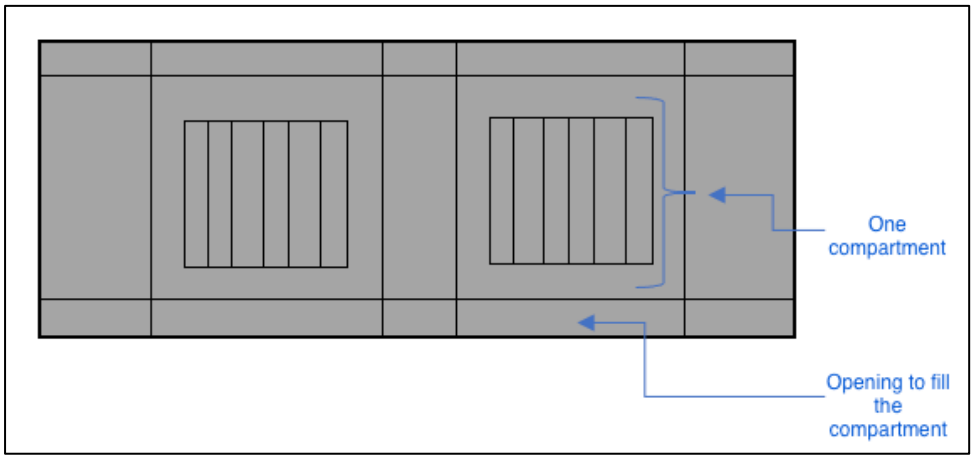

Figure 2: Schematic view of a McMaster counting chamber with two compartments.

Treatment

Due to a simultaneous study on resistance development against pyrantel, foals that had an EPG of 50 or above of *Parascaris* spp. were divided into two treatment groups on each farm. Assigning the foals into two treatment groups was done on each separate farm after the first FEC for each foal was determined. The foals included for the FECRT were paired by similar EPG counts for *Parascaris* spp.. Secondary estimated age and lastly gender were considered when matching the foals. Afterwards one foal of each pair was randomly assigned to a treatment group. This process resulted in two equal treatment groups. One group was treated with pyrantel (Nematel-P®) and the other group was treated with fenbendazole (Panacur®).

Treatment was the same day that the FEC were established or by exception on a second visit. Both treatments were performed according to the manufacturer's recommendation and dosage was based on the weight of the individual horse. The weight of the foals was estimated by the two researchers. Foals were given at least 10% more than their estimated body weight to prevent under dosing. When any anthelmintic was spilled or

otherwise dropped on the floor the foals were given the estimated lost anthelmintic on top of the original dosage.

Calculation method

14-17 days after treatment of for FECRT included foals, the second fresh fecal samples of the individual foals were collected. Collecting and processing was done the same way as the first visit. For calculating means, the arithmetic mean was used as advised by Dash et al. (1988) and Dobson et al. (2019). Furthermore, where possible geometric means were also calculated when all results were positive and greater than zero. A recent article showed that geometric means are more reliable for FECRTs because it is less effected by outliers (Moser et al., 2020). There are currently no validated guidelines for reduction percentages that indicate whether *Parascaris* spp. are still susceptible or resistant to fenbendazole. Therefore, the guidelines of the AAEP for strongyles were followed as described by Martin et al. (2018). According to the most recent AAEP guidelines expected efficacy of fenbendazole against strongyles is 99% (C.I. 95%) if there is no resistance (Nielsen et al., 2019). When the EPG has a reduction of 95% (C.I. 95%) or more after 14-17 days after treatment with fenbendazole then there is no evidence for resistance development. An outcome between 90-95% (C.I. 95%) would mean possible resistance development. Furthermore, a fecal egg count reduction of less than 90% (C.I. 95%) indicates resistance. The equation that was used to calculate the percentage reduction of FEC of an individual foal was the same as Nielsen et al. (2019) recommends.

$$\mathrm{FECRT} = \frac{\mathrm{FEC}_{\mathrm{pre-treatment}} - \mathrm{FEC}_{\mathrm{14-17\ days\ post-treatment}}}{\mathrm{FEC}_{\mathrm{pre-treatment}}} \cdot 100\%$$

Results

The feces of 297 foals, from 11 farms, in The Netherlands were collected. The visited farms were located in four different provinces. Four farms were located in Gelderland, three in Friesland, two in Utrecht and two in Overijssel (see Figure 3).

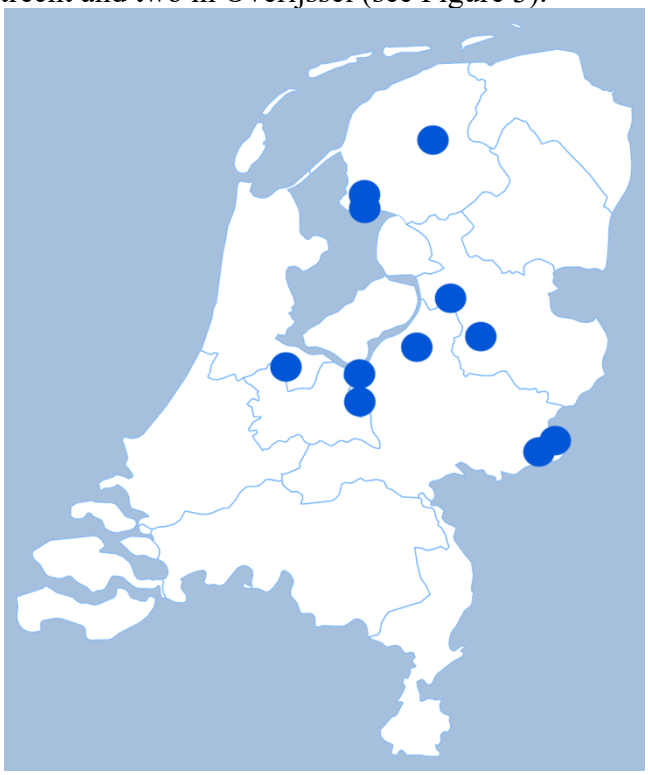

Figure 3: Distribution of the 11 visited horse farms across 4 out of 12 provinces of the Netherlands.

Fecal egg count reduction test

The 11 farms had an average (arithmetic mean) of 31 foals, with a standard deviation of 15,9 at the first visit (see Table 1). The fecal samples were examined within 32 hours after collecting. On three farms there were no foals present with a FEC of *Parascaris* spp. of 50 or above. Furthermore, one farm had a foal that could have been included however it was decided to treat this foal with pyrantel, in order to have two equal treatment groups. Seventy-two of the performed modified McMasters on the farms came out positive for *Parascaris* spp. Forty-nine of the 72 had an EPG of 50 or more and because of the inclusion criteria that were established on forehand these foals were included within the FECRTs. One of the 49 foals turned out the be dewormed the day before the first visit, and this foal was thereby excluded for the FECRT. Resulting in inclusion 48 foals within this study of efficacy of pyrantel and fenbendazole against *Parascaris* spp.. In total, for this study, there were 24 foals treated with Panacur® (fenbendazole) on 7 farms (Table 1). All horses underwent treatment without problems and no colic signs, or other adverse effects were noted by the farmers.

Table 1:
Overview of number of foals present at each farm, number of foals that were positive for Parascaris spp. *and number of foals that had an EPG of 50 or above. These foals were included within FECRT and treated with pyrantel or fenbendazole.*

Farm	Total foals on farm[1]	Number of fecal egg counts performed	Foals with *Parascaris spp.* eggs	Foals with EPG>49	Foals treated with pyrantel	Foals treated with fenbedazole
1	15	15	7	6	3	3
2	20	20	1	0	0	0
3	60	52	10	7	4	3
4	21	21	11	9	4	5
5	34	32	11	6	2	3
6	40	33	0	0	0	0
7	55	43	13	7	4	3
8	23	23	3	1	1	0
9	13	13	1	0	0	0
10	40	25	7	6	3	3
11	22	20	8	7	3	4
Total	343	297	72	49	24	24
Arithmetic mean	31	27	7	4	2	2
Geometric mean	28	25	-	-	-	-
Standard deviation	15,9	12,0	4,6	3,4	1,7	1,8

[1]*Total foals between 3-12 months present on the first visit to the farm.*

In Table 2 the age of foals is shown for the foals treated with pyrantel and fenbendazole. Age of the foals was estimated in consultation with the farmowner, since farmers often did not know precisely. Foals who were housed with their mares were said to be below 5 months of age, while weened foals were considered to be 5 months or older. Six out of 10 of the foals younger than 5 months had a FEC of *Parascaris* spp. above 50. While the incidence of *Parascaris* spp. eggs in the feces of the 287 older foals that were already weaned was 43. There were 21 foals of 5 months or older and 3 foals of less than 5 months of age in both treatment groups.

Table 2:
The age of foals with an EPG of 50 or above for Parascaris spp.*. These foals were included within FECRT and treated with either pyrantel or fenbendazole.*

	Treated with pyrantel	Treated wit fenbendazole	Total
< 5 months of age	3	3	6
5 months of age or older	21	21	42
Total	24	24	48

In Table 3 the distribution of the gender groups was shown for both treatment groups. Out of 48 treated foals, 30 foals were stallions, and 18 foals were mares. Both treatment groups consisted of 15 stallions and 9 mares.

Table 3:
The gender of foals with an EPG of 50 or above for Parascaris spp.*. These foals were included within FECRT and treated with either pyrantel or fenbendazole.*

	Treated with pyrantel	Treated wit fenbendazole	Total
Mares	9	9	18
Stallions	15	15	30
Total	24	24	48

In Table 4 the FECRT data on 24 foals that were treated with Panacur® are given. There was one farm where the foals were not treated on the day the FECs were determined. Two foals (nr 19 and 20) were treated 22 days after FEC was determined and one foal (nr 21) was treated with fenbendazole 3 days after FEC was determined. All other 21 foals were treated with fenbendazole on the same day FEC was determined. *Parascaris* spp. as well as strongylid type eggs were counted pre-treatment and after 14-17 days post-treatment. No eggs other than *Parascaris* spp. and strongylid types were found in the foals treated with Panacur®.

Strongylid type eggs were not present in the feces of every foal, since it was not part of the inclusion criteria of this study. 20 foals had strongylid type eggs in the feces with an average count of 347 EPG. The reduction of FEC of strongylid type was on average 62,6% with the highest being at 100% and lowest 0%.

The 24 foals treated with Panacur® had an average of 709 EPG *Parascaris* spp. eggs in the examined feces. The lowest EPG was 50, being the minimal EPG to be included in the study. And the highest was an EPG of 8818. The FECR of *Parascaris* spp. after treatment with Panacur® was overall 98,6%. One foal had a 50 EPG pre-treatment and it reduced to 17 EPG post-treatment. Therefore, this foal had a reduction of 66%. All 23 other foals had a reduction of 100% of *Parascaris* spp. eggs.

The full datasheet is archived at the department of Biomolecular Health Sciences of the University of Utrecht.

Table 2:*Overview of age and gender of the 24 foals on 7 Parascaris* spp. *egg count positive farms that were treated with Panacur® together with the results of FECRT for Parascaris* spp. *and the strongylid type eggs that were additionally counted.*

Foal	Age (months)	Gender	EPG strongylid type pre-treatment	EPG strongylid type post-treatment	FECRT strongylus (%)	EPG *Parascaris spp.* pre-treatment	EPG *Parascaris spp.* post-treatment	FECRT Parascaris (%)
1	> 5 months	Stallion	17	33	-94,1	567	0	100,0
2	> 5 months	Stallion	183	50	72,7	1233	0	100,0
3	> 5 months	Mare	33	0	100,0	283	0	100,0
4	> 5 months	Mare	0	0	-	117	0	100,0
5	> 5 months	Stallion	17	17	0,0	250	0	100,0
6	> 5 months	Mare	33	0	100,0	170	0	100,0
7	> 5 months	Mare	617	17	97,2	300	0	100,0
8	> 5 months	Stallion	767	100	87,0	50	0	100,0
9	> 5 months	Stallion	17	0	100,0	67	0	100,0
10	> 5 months	Mare	333	200	39,9	267	0	100,0
11	> 5 months	Stallion	17	17	0,0	250	0	100,0
12	> 5 months	Stallion	0	0	-	50	17	66,0
13	> 5 months	Stallion	0	0	-	8817	0	100,0
14	> 5 months	Mare	17	0	100,0	433	0	100,0
15	> 5 months	Stallion	450	0	100,0	250	0	100,0
16	< 5 months	Stallion	1633	133	91,9	83	0	100,0
17	< 5 months	Stallion	1133	400	64,7	850	0	100,0
18	< 5 months	Stallion	1783	650	63,5	183	0	100,0
19	> 5 months	Mare	167	0	100,0	150	0	100,0
20	> 5 months	Stallion	83	67	19,3	367	0	100,0
21	> 5 months	Mare	383	200	47,8	717	0	100,0
22	> 5 months	Stallion	267	50	81,3	1150	0	100,0
23	> 5 months	Stallion	0	0	-	267	0	100,0
24	> 5 months	Mare	367	67	81,7	150	0	100,0
Arithmetic mean	-	-	347	83	62,6	709	1	98,6
Geometric mean	-	-	-	-	-	283,7	-	98,3
Standard deviation	-	-	509	153	49,7	1757	3	6,9

Questionnaires

The data retrieved from the questionnaires show that every farm had their own anthelmintic policy, and most dewormed foals that were younger than 6 months every 2-3 months. However, farm 8 dewormed a second time after two weeks if a foal shed worms in the feces. The anthelmintics that are used throughout the year for foals on the farms are shown in Table 5.

Table 5:
Anthelmintics that the owners of the 11 visited farms administer to their foals outside of this research project. + = regularly used, (+) = has been occasionally used, - = never used.

Farm	Pyrantel	Fenbendazol	Ivermectine	Moxidectine
1	+	+	+	+
2	+	-	+	+
3	+	-	-	+
4	+	+	+	+
5	+	+	+	+
6	+	-	-	+
7	+	-	+	+
8	+	+	-	+
9	-	+	+	-
10	+	-	+	+
11	+	(+)	+	+

Table 6 gives an overview of most recent administered anthelmintics, before the first visit of this study which visits took place from the 1st to the 31st of October in 2020. Almost all farmers dewormed a foal when it arrived at the farm. Most farms used pyrantel at this time, however occasionally fenbendazole was used. Several farms (nr 1,2,5,6,8 and 10) had a deworming schedule that was based on month of the year. Two farms (nr 4 and 9) had a predetermined treatment plan based on the foal's age. In contrast one farm (nr 7) did not retain a fixed scheme and relied on fecal egg counts and advice given by the veterinarian. Another farm (nr 3) relied on FEC results for the choice of anthelmintic, however the timing of treatment was based upon month of the year. A different farm (nr 11) chose to deworm their foals every 9 to 10 weeks. These choices around anthelmintic policies are also shown in Table 6.

Table 6:
Last administered anthelmintic and anthelmintic policy on each farm.

Farm	Anthelmintic that was most recently used (before first visit)	Althemintic policy based upon
1	Pyrantel	Month of year
2	Pyrantel	Month of year
3	Pyrantel	Month of year and FEC
4	Pyrantel	Age of foal
5	Pyrantel	Month of year
6	Pyrantel	Month of year
7	Pyrantel	FECs
8	Fenbendazole	Month of year
9	Fenbendazole and ivermectin	Age of foal
10	Pyrantel	Month of year
11	Pyrantel	Weeks past since last treatment

There were three farmers (nr 3,7 and 10) who performed a FEC regularly for their foals, this is shown in Table 7. One of these farms (nr 3) collected individual samples twice a year to monitor worm burdens in foals. On the second farm (nr 7) they performed a FEC every 6-8 weeks using group samples. On this farm a veterinarian was consulted about the outcome of the FEC to determine if the foals should be dewormed at that moment, and if so what type of anthelmintic should be used. At the third farm (nr 10) group samples were collected for FEC a few times a year. Furthermore, three other farms (nr 1,2 and 8) performed FECs of the herd on indication or to verify if treatment was successful. On the remaining 5 farms (nr 4,5,6,9 and 11) no FECs were performed on foal's feces. Only on three farms (nr 3,7 and 10) there have been FECs positive for *Parascaris* spp. in the past. Sick foals due to *Parascaris* spp. were in recent years only identified on two farms (nr 3 and 8).

Table 7:
Answers of the farmers to the questions how often FECs are generally performed for foals on their farms, whether this is done with group or individually collected fecal samples and if they ever came across positive FECs for *Parascaris* spp. or clinically sick foals due to *Parascaris* spp. infections.

Farm	FEC performed on foals	Individual or group sampels	Positive FEC samples for *Parascaris* spp.	Sick foals due to *Parascaris* spp.
1	Only on indication	Group	No	No
2	Only on indication	Group	No	No
3	2 times a year	Individual	Yes	Yes
4	No	-	-	No
5	No	-	-	No
6	No	-	-	No
7	Every 6-8 weeks	Group	Yes	No
8	Only as check after deworming when Parascaris spp. was seen in feces	Group	No	Yes
9	No	-	-	No
10	Few times a year	Group	Yes	No
11	No	-	-	No

The general management of farmers is visualized in Table 8. In the winter the foals were housed in groups ranging from 2-20 foals per stable. On all 11 farms the foals had access to some type of sand paddock for a few hours per day or had full access day and night. Two stables (nr 3 and 5) had an outside area with a stone surface instead of a sand paddock. During summer all foals would get access to a pasture. Two farms (nr 3 and 7) gave the foals pasture access until September. The other farms (1,2 4-6, 8-11) chose not to let the foals on their fields until the year after they were brought. There was no farm where the foals would get pasture access where other animals grazed at the same time and all foals were housed solely with horses born in the same year. Feces were never removed from the fields; however, most fields were dragged once a year. How many hectares of pasture the foals had access to turned out a to be a question the farmers didn't have a reliable answer to. Furthermore, we asked the farmers if they changed the pasture the foals had access to from time to time. On two farms (nr 1 and 7) the foals changed pasture every week, on two other farms (nr 2 and 6) every other week, farms nr 5 and 9 let the group foals on another field when the grass was

vacant, and farm nr 4 declared not to change fields. Most paddocks were not mucked out (nr 2,4,6-10), however there were some farms who said to clean them regularly ranging from every 2-3 weeks to daily (nr 1,3,5 and 11). Stables were cleared out once per two weeks to once per two months. All stables were annually cleaned with high pressure either with warm or cold water. One farm added detergent and three farms added disinfectant when cleaning the stable.

Table 8:

Groupsize in relation with general management choices shown through the questionnaire data of the 11 participating farms.

Farm	Groepsize	Feces removed from field	Change of field	Feces removed from paddock	Stable emptied	Stable cleaned	Detergent or desinfection used with cleaning?
1	10	Never	Weekly	Daily	Monthly	Annually	No
2	18	Never	Every 2 weeks	Never	Monthly	Annually	Desinfection
3	15	Never	Every 2-3 weeks	Two times a week	Monthly	Annually	No
4	10	Never	Never	Never	Every 2 weeks	Annually	Detergent
5	6-8	Never	When the grass is vacant	Weekly	Monthly	Annually	No
6	20	Never	Every 2 weeks	Never	Every 2 weeks	Annually	No
7	20	Never	Weekly	Never	Weekly	Annually	No
8	6	Never	Monthly	Never	Every 2-3 weeks	Annually	No
9	14	Never	When the grass is vacant	Never	Once every 2 months	Annually	No
10	20	Never	Every 3 weeks	Never	Monthly	Annually	Desinfection
11	2	Never	Never	Once every 2-3 weeks	Every 2 weeks	Annually	Desinfection

Discussion

Results of FECRT

The FECRT with fenbendazole was 100% for 23 foals, however one foal had a fecal egg count reduction of 66%. Although there is no data available for expected efficacy of fenbendazole against *Parascaris* spp., with a FECRT of 100% for the 23 foals there are no indications for resistance. On the other hand, an efficacy of 66%, that was found for one foal, would mean there are indications for resistance when the cut off values within the guidelines of AAEP, for resistance of strongyles against fenbendazole, are followed (Nielsen et al., 2019). However, in this individual 66% meant that post-treatment there was only 1 egg found in three counting chambers, since the FEC pre-treatment was 50 EPG. This means the outcome could have been significantly different when the sensitivity of the modified McMaster would have been lower by counting more than three McMaster slides. Statistically, it does not hold to establish the existence of resistance based upon one egg in the feces of one foal. FEC is an indirect parameter, however currently the only method to determine the actual worm burden are post-mortem findings. Moreover, there is always a possibility of human error, for example anthelmintic could have been spit out without it being noticed. This individual might have resistant *Parascaris* spp. although this is precarious. A follow-up study could establish the efficacy of fenbendazole on this particular farm where this foal got infected, since resistance is a herd problem (Nielson et al., 2019).
For the individual foal, with a reduction of 66% of *Parascaris* spp. eggs after treatment, raises the question how to act now? The FEC merely gives insight in whether or not there are adult *Parascaris* spp. present. There is no direct correlation between EPG and the extent of the worm burden of *Parascaris* spp.. Furthermore, there is also no information on the amount of larval stages present, since there are currently no diagnostics to evaluate this (Nielsen, 2010). An EPG of 17 seems to be low, however this means this foal is infected with at least a few adult *Parascaris* spp. and might even be heavily infected.
Reinemeyer et al. (2009) stated that anthelmintic treatment for the removal of *Parascaris* spp. should ideally be based on positive diagnostics. Nielsen et al. (2016) researched the possibility of estimating the worm burden with transabdominal ultrasound, however implementing this in treatment decisions would be expensive and results were inconsistent. The only parameter that we currently have is the FEC, which was positive for this foal. The FEC of this foal did decrease. This might be due to the anthelmintic, however another possibility is that it is partially or completely due to immunity build-up. As described earlier *Parascaris* spp. infections can have fatal consequences. Waiting for complete removal of *Parascaris* spp. due to immunity development is therefore considered to be a high risk (Leathwick et al., 2017). The anthelmintic to consider for the consecutive treatment would preferably be pyrantel for this foal, since resistance against macrocyclic lactones has already been established in the Netherlands (Boersema et al., 2002). A mineral oil can be administered by a gastric tube which could make expelling the worms easier (Nielsen, 2016). When a heavy infection is expected, for example when a foal has a poor body condition, the foal should be monitored for a few days. When the foal shows signs of colic a veterinarian should be consulted. In order to determine if the foal is free of *Parascaris* spp. after the second treatment a FEC should ideally be repeated two weeks after administering the anthelmintic.

Two foals were treated 22 days after FEC was determined and one foal was treated 3 days after FEC was determined. Not being able to treat these three foals on this farm on the same day their FEC for *Parascaris* spp. was determined was due to practical issues. Another appointment had to be scheduled, since the farm owner had to arrange a suitable space to

administer the anthelmintics to the foals that were not used to human handling. An attempt was made the attain a second sample to confirm the FEC on a later visited, however there were no fresh feces for these individuals to collect. Especially for the two foals treated after 22 days, the decrease in FEC of *Parascaris* spp. might not have been solely caused by fenbendazole, since the immunity build-up could have interfered.

Number of FEC and FECRT performed

AAEP guidelines recommend including at least six horses in a FECRT on each farm (Nielsen et al., 2019). Reported prevalence of *Parascaris* spp. infections in foals are 58,3% in Australia (Armstrong et al., 2014), 50% in Finland (Hautala et al.,2019) and 53% in Saudi Arabia (Alanazi et al., 2017). Therefore, during this study, the aim was to collect feces from at least 12-15 foals at each farm for each treatment group (pyrantel and fenbendazole). Previous studies to determine the resistance of *Parascaris* spp. against anthelmintics around the world have collected samples ranging from 1 farm and 26 horses (Boersema et al., 2002) to 95 farms and a total of 376 horses (Hautala et al., 2019). For this study the goal was attaining samples from 10 farms. These targets were reached, since there were samples collected from 297 foals on 11 farms. If the prevalence of *Parascaris* spp. was indeed around 50% this would have resulted into admitting around 60 foals in both treatment groups. This is comparable to the number of horses used in other studies who did not have specific indications for resistance on forehand (Armstrong et al., 2014; Martin et al., 2018). However, there were less positive FECs for *Parascaris* spp. than was expected. That resulted in not being able to include 6 foals per treatment group at each farm.

Resistance is a herd problem, since foals on a farm are exposed to the same *Parascaris* spp. population. It is not likely that some foals are infected with resistant *Parascaris* spp. and some are not when a group of foals is raised together on the same location. However, the foals in this investigation did not get infected on the farm where the samples were collected, therefore these foals cannot be compared. This is the reason for investigating resistance at an individual level. Therefore, it was decided to still divide foals that could be included in two treatment groups on every farm, instead of choosing an anthelmintic per farm.

Nielsen et al. (2019) recommends including foals with a minimal EPG of 150, with a detection limit of at least 25 EPG, to be able to detect differences in FEC pre-and post-treatment. Since in this research there were less foals positive for *Parascaris* spp. and the sensitivity of the modified McMaster method was as low as 17 EPG, it was decided to include foals with an EPG of 50 and upwards.

A first possible explanation for the low number of positive FECs for *Parascaris* spp. is that immunity development was more advanced in the tested foals due to their age (Clayton and Duncan, 1979a; Fabiani et al., 2016).

A second explanation could be that the foals were dewormed prior to sample collection, and the inclusion criteria were not met in every case. Nielsen at al. (2019) recommends including horses in FECRTs when they have not been treated with anthelmintics for at least 8 weeks prior to the study. However, this was not possible in this study since the foals were often present for less than 8 weeks on the farms that were visited. Farm owners were asked to stop giving anthelmintic treatments when they were approached late August/ early September 2020. However, if owners were hesitant, they were asked to stop anthelmintic treatment at least two weeks prior to our first visit. This was one of the inclusion criteria for a farm to be able to participate. Many farms give anthelmintic treatments when an individual foal arrives at the stable, or the foal is dewormed just before it is brought to the farm. Farm owners often did not recall exactly when the individual foals had arrived, and the history of anthelmintic treatments were often absent for the individual foals. Moreover, one

farm turned out to have dewormed seven days prior to the first visit, whilst the farmer stated at arrival that inclusion criteria were met. This shows the custom of farmers giving frequent anthelmintic treatments to foals.

A third possible explanation for the few *Parascaris* spp. positive FECs could be that the prevalence is lower in the Netherlands than percentages found in other countries. Van Doorn et al. (2007) found 88 out of 332 (27%) examined foals to be positive for *Parascaris* spp. in the Netherlands. On 26 out of 43 participating farms *Parascaris* spp. were discovered (Van Doorn et al., 2007). Furthermore, Boersema et al. (2002) found 10 positive FECs for *Parascaris* spp. on a farm in the Netherlands where 25 fecal samples were collected (40%). In this present study 72 out of 297 foals were positive for *Parascaris* spp., this means 24% of the tested foals had at least an EPG of 17 for *Parascaris* spp.. This percentage is significantly lower than reported in most other studies, however it seems to be consisted with the prevalence reported by Van Doorn et al. (2007). Unfortunately, Van Doorn et al. (2007) does not specify the detection limit of their performed modified McMaster technique. If the detection limit is higher than the detection limit of 17 EPG used for this study, this prevalence might be incomparable with the prevalence found in this study.

There were 11 horse farms visited across 4 provinces of the Netherlands. Horse farms in other provinces were approached as well, however they often did not house enough foals to reach the inclusion criteria: 'expecting at least 20 foals by the end of September'. This is within expectations, because western provinces are highly populated and land prices are relatively high. Additionally, some farm owners were not willing to participate.

Efficacy of fenbendazole for encountered strongyles

Additionally, strongylid type eggs were also counted. The efficacy of Panacur® against strongylid type eggs ranged from 0% to 100%. The outcome of the FECRT in one case was a negative number of -94,1%, because more strongylid type eggs were found in the second feces sample. An anthelmintic cannot increase number of eggs in the feces, so a negative number should be explained as the number of eggs increased in the feces despite the anthelmintic that was administered. The geomatric mean of the FECRT for strongylid type eggs was 62,6%. These numbers indicate that there is resistance of equine parasites producing strongylid type eggs against fenbendazole (Nielsen et al., 2019). Most likely this concerns small strongyles (cyathostomes). This is not surprising because first reports of resistance against benzimidazoles started only a few years after benzimidazole became available on the veterinary market (Kaplan, 2004). First articles of reduced efficacy of benzimidazole on equine strongyles in the Netherlands were published around 1990 (Eysker et al., 1988; Boersema et al., 1991). In theory it is possible that when an anthelmintic is not used for a period of time resistance developed against that anthelmintic can reverse (Kaplan, 2004). This is due to genetic variation and the absence of selecting for that anthelmintic resistant alleles. Although fenbendazole was only (occasionally) used at 6 of the 11 visited farms, results of FECRTs of this study show that significant reduced efficacy of fenbendazole to strongyles is still present. Resistance of equine strongyles against benzimidazole is not solely found in the Netherlands. There are articles reporting resistance of cyathostomes against benzimidazole published all over the world (Varady et al., 2000; Piché et al., 1989; Wirtherle et al., 2004; Traversa et al., 2007).

Analyzing the questionnaires about management

As mentioned before, it is unlikely that these foals did get infected on these farms, since the prepatent period is 72 to 110 days (Clayton and Duncan, 1977; Clayton and Duncan,

1979b; Lyons et al., 1976). Therefore, management choices or anthelmintic policies on these particular farms can only be partially related to outcomes of FECs. Additionally, individual information of deworming, precise age, previous housing conditions and other history often were not available. Pre's and cons for the choice of these types of farms are further illustrated in the advice for future studies.

Analyzing the questionnaires two things stand out.

First, anthelmintics are frequently administered to foals on these farms. Almost all stables deworm a foal when it arrives. When inquired, there were farmers who told that they repeated the deworming, with interval times ranging from 2 weeks to 2 months. When asked what dosage was given most answered: 'half a syringe'. This equals a dose for 300 kilograms. However, there was one owner who admitted sometimes giving a whole syringe which equaled at a dosage for 600 kilograms. Fenbendazole is used on 6 of the 11 farms, while ivermectin is only used on 8 farms and pyrantel and moxidectin is used on 10 out of the 11 farms. This might indicate that fenbendazole is used less on these types of farms. Farmers said Panacur® is more liquid and therefore it is more difficult to prevent spilling. Contradictory, other farmers said to prefer Panacur® because *Parascaris* spp. worms do not harden and are therefore easier expelled, preventing an impaction. Fenbendazoles might also be used less frequent because of the known wide-spread resistance against strongyles.

Second, every farm has its own unique anthelmintic policy. Anthelmintic policies are based upon month of the year, age of the foal, weeks after last treatment or based upon FECs together with the advice of a veterinarian. As mentioned before administering anthelmintics should preferably be done based on positive outcomes of FECs (Reinemeyer et al., 2009). However, the majority of farms do not perform FEC regularly for their foals. Only three farms had FECs positive for *Parascaris* spp. in the past. Sick foals due to *Parascaris* spp. were only seen on two farms in recent years. This might be due to the fact that most foals are already 5 months of age when they arrive on the farms. However, it might also be that this is due to the heavy use of anthelmintics. In order to prevent, slow down, and control further development of resistance against anthelmintics of *Parascaris* spp. it is essential to make reasoned decisions when (not) to administer anthelmintics. Studying these questionnaires, it stands out that there is room for improvement by informing farmers about the threat of resistance development and the need to consider if giving anthelmintics is beneficial or if there are other ways to limit worm burdens other than solely medicinal (Reinemeyer, 2012). Their veterinarians are the suppliers of anthelmintics, so they should monitor when and what type of anthelmintic is administered and design an anthelmintic policy suitable for the specific farm together with the farmer.

When analyzing the general stable management, it becomes clear that group sizes vary greatly between farms. Furthermore, there were no farms that removed feces from the fields. This is interesting because if the feces would be removed, eggs of present helminths would be removed with the feces, thereby reducing the infection pressure (Fritzen, 2010). Instead, farms drag their fields, which will spread the eggs, thereby limiting foals in their selective grazing by which they naturally minimize intake of with parasites contaminated grass (Fritzen, 2010). However, it will also expose eggs of parasites more to environmental influences. Ultra-violet light in sunlight will affect the immature eggs in particular, and also to a lesser extend the embryonated eggs of ascarids (Shalimov, 1935; Spindler, 1940; Wright and McAlister, 1934). Moreover, the eggs will be more susceptible to dry weather conditions, which could shorten the time of viability (Gaspard et al., 1997; Gaasenbeek and Borgsteede, 1998). Since farmers couldn't tell how much hectare field they had for each group of horses, the question about how often they change fields does not hold much value. Feces are removed regularly from the paddock by some farmers and some never remove the feces out of the paddock. Stables on the different farms are cleared out every 2 weeks to every month.

Management of the whole environment of foals, not solely field management, can suppress the number of eggs foals are exposed to, however little data is available on this (Nielsen, 2016). All farmers said to clean stables annually with high pressure and some use detergent or even disinfectant, unfortunately it is not known what is needed to remove *Parascaris* spp. eggs successfully (Nielsen, 2016).

Advice for future studies

A benefit of the current research design is that foals originating from all over the country were included. Furthermore, it was reasoned that excessive deworming on these types of farms would lead to a higher chance of finding resistance. However, there was no clear evidence found in this study for resistance against *Parascaris* spp.. This might mean that *Parascaris* spp. in the Netherlands have not yet developed resistance against fenbendazole or that there is development of resistance, however it was not found in this study. Furthermore, breeding farms that have at least 12-15 foals every year are not common in the Netherlands. Whereas the type of farms used in this study are common on larger scale. Therefore, the goal of testing at least 12-15 foals at a farm was thought to be better achievable when choosing these types of farms.

There were 10 foals under 5 months of age that were not yet weaned included in this study. Sixty percent (6 out of 10) of these younger foals could be included in this study because of an EPG of 50 or higher for *Parascaris* spp., which is relatively high in comparison to 15% of the remaining older foals (42 out of 287). Although these percentages should not be mistaken for prevalence's, this might indicate that the incidence of *Parascaris* spp. infections are higher in foals aged 4 to 5 months old than in foals aged 6 months or older. Taken into account that immunity build-up starts at an age of 6 months this is also explainable (Clayton and Duncan, 1979a; Fabiani et al., 2016).

Advice for future studies into anthelmintic resistance in the Netherlands of *Parascaris* spp. would be including younger foals of 3 to 5 months of age for example at breeding farms. More positive FECs for *Parascaris* spp. are to be expected in that population, because immunity build-up has not started at that age and possibly there will be less deworming performed at these studs. When more foals test positive for *Parascaris* spp. foals should be included from EPGs of 150 and upwards, with a detection limit of 25 EPG or lower, so reduction can be measured more precisely as advised by the guidelines of American association for equine practitioners (Nielsen et al., 2019).

Foals included for FECRT should be treated the same day that their FEC is established or in de next few days after, to reduce the influence of immunity build-up on the outcome of the FECRT. When this is not possible a second fresh feces sample should be attained before treatment to make sure the FEC has not significantly changed.

When the original breeding farm is being visited, history of anthelmintic treatments is known and the requirement of not giving anthelmintics before the study can be monitored more closely and it might be possible to extent this period. Advised by the AAEP is withholding anthelmintic treatment 8 weeks prior to the start of the study (Nielsen et al., 2019). Eight weeks is a long period and therefore this might be a risk for the foals, however getting closer to those 8 weeks would be beneficial for the study. Another advantage is that the *Parascaris* spp. infections can be correlated to management factors of that specific stable, because foals are born and also infected at that stable. If possible, it would be interesting to include the stud where the foal with an FECRT of 66% was born. Including individual foals from private owners is also possible, although less favorable because since resistance is a herd problem it is easier identified on a farm where multiple foals are housed.

Conclusion

Fecal egg count reduction tests with fenbendazole performed on 24 foals with *Parascaris* spp. eggs at 7 farms in The Netherlands showed no clear evidence for resistance against fenbendazole. Only one foal had a reduction of 66% while the other 23 foals had a reduction of 100% of *Parascaris* spp. eggs in the feces after being treated with Panacur®. In accordance with previously published studies, there was reduced efficacy of fenbendazole against equine strongyles. Of the 297 foals included in the entire study, only 72 foals were positive for *Parascaris* spp.. This might be due to the extent of deworming that the foals are exposed to, build-up of immunity and / or a lower prevalence of *Parascaris* spp. in comparison to other countries. For future studies in the Netherlands to resistance of *Parascaris* spp. it would be recommended to visit large breeding farms in the summer months. This would lead to a younger population of foals where immunity to *Parascaris* spp. is less developed. When more foals test positive for *Parascaris* spp., foals should be included from EPGs of 150 and upwards, with a detection limit of 25 EPG or lower, so reduction can be measured more precisely. Furthermore, when foals are born on the same farm it is likely that foals are infected on that same farm, which makes it possible to correlate management factors to possibly discovered resistance. The requirement of not giving anthelmintics before the study can be monitored more closely when foals haven't been relocated and it might be possible to extent this period.

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Attachments

Attachment 1: Dutch questionnaire

Vragenlijst

U mag deze vragenlijst online invullen of uitprinten en een scan of foto sturen van de door u ingevulde lijst. U kunt uw antwoorden doorsturen naar: spoelworm.uu@gmail.com
Mocht dit voor u lastig zijn of voor als er onduidelijkheden zijn graag contact opnemen met: Elvie Karman: 06-37474773 (e.m.karman@students.uu.nl) of Amanda Groen: 06-15267098 (a.l.m.groen@students.uu.nl)
Onderzoeksbegeleider: Dr. D.C.K. (Deborah) van Doorn (Universiteit Utrecht)
*= Graag doorstrepen wat niet van toepassing is.

Algemene bedrijfsgegevens:
Bedrijfsnaam:
Plaats:
Contactpersoon:
E-mail:
Telefoonnummer:
Paardenweegschaal aanwezig op uw bedrijf: ja / nee
Behandelend dierenarts:
Telefoonnummer behandelend dierenarts:

Totaal aanwezige opfokveulens eind september/begin oktober 2020:
Aantal veulens geboren 2020:

Vragen betreffende ontwormingsbeleid:

1. Op dit bedrijf wordt ontwormt *op basis van mestonderzoek / is er een vast schema betreffende ontwormen/ wordt er mestonderzoek gedaan en is er een schema/ wordt er niet behandeld noch mestonderzoek uitgevoerd**.

2. Hoe vaak wordt er mestonderzoek gedaan op uw bedrijf?

3. Bij mestonderzoek, wordt een *groepsmonster / individueel monster per paard* * genomen.

4. Indien u gebruik maakt van een vast schema: hoe ziet dit schema eruit?

5. Met welk(e) middel(en) wordt er ontwormt op uw bedrijf?

6. Wanneer zijn de paarden voor het laatst ontwormd? Datum:

7. Welk middel is de laatste keer gebruikt?

8. Hoe vaak overlegt u met uw dierenarts omtrent het ontwormingsbeleid?

9. Zijn de laatste jaren op uw bedrijf mestmonsters verzameld die positief waren op spoelwormen? *Ja / nee / onbekend* *

10. Zijn er in de laatste jaren zieke paarden geweest die gerelateerd zijn aan een infectie met spoelwormen? *Ja / nee / onbekend* *

Vragen betreffende huisvesting:

11. Hoe worden de paarden gehuisvest? *Weide / stal / stal met uitloop (zand/gras) / wisselend per seizoen* *

12. De paarden worden *individueel / in groepen** gehuisvest.

    12a. Indien groepen gehuisvest; wat is de groepsgrootte?

    12b. Indien de paarden in groepen staat gehuisvest; *paarden staan met leeftijdsgenoten / paarden staan in groep met verschillende leeftijden**

13. Lopen de paarden *wel/ niet* * met andere diersoorten in de wei. Indien wel: welke dieren?

14. De mest wordt *dagelijks / wekelijks / maandelijks / nooit / anders namelijk:.....** opgeruimd op het weiland.

15. Hoeveel hectare hebben jullie met hoeveel paarden?
    Hectare: …… Paarden:.....

16. Worden de paarden *dagelijks / wekelijks / maandelijks / nooit / anders namelijk:.....** omgeweid.

17. De mest wordt *dagelijks / wekelijks / maandelijks / nooit / anders namelijk:.....** opgeruimd in de paddock.

18. De stallen worden *dagelijks / wekelijks / maandelijks / nooit / anders namelijk:.....** uitgemest.

19. Wordt de stal *dagelijks / wekelijks / maandelijks / nooit / anders namelijk:.....** ontsmet of schoongemaakt met schoonmaakmiddelen. En zo ja, welke middelen (merknaam):.....

Attachment 2: Protocol for modified McMaster technique

Following this protocol will lead to a modified McMaster with a detection limit of 17 eggs per gram (EPG). This protocol is derived from De Kool-van der Woude J.W. (2015).

Supplies needed:

- Fresh fecal sample
- Scale
- Two Falcon tubes
- Pipet
- Sucrose solution (S.G. 1.30)
- Sieve (for example a tea strainer)
- Three McMaster slides
- Microscope

1. Weigh 3 grams of freshly collected feces in a Falcon tube.
2. Measure 42 mL of sucrose solution in another Falcon tube.
3. Add the sucrose solution to the Falcon tube with 3g of feces and mix well.
4. Filter the solution with added feces through a sieve.
5. Mix the filtered solution well before filling the first McMaster slide.
6. For filling the McMaster slides, withdraw 1mL of sample out of the falcon tube, directly after mixing.
7. If any air bubbles are present in a compartment, the slide is emptied and refilled.
8. Mix again well before filling the second and third McMaster slide.
9. Give the eggs the possibility to float in the compartments by waiting one or two minutes.
10. Scroll systematically through the 3 McMaster chambers (6 compartments) using a microscope with a 10x objective lens and count every egg that is encountered.
11. Clean all equipment with tap water before starting with another sample.

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