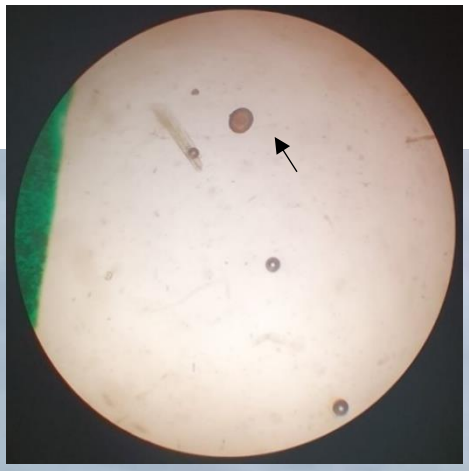
ASSESSING POSSIBLE RESISTANCE DEVELOPMENT OF *PARASCARIS* SPP. TO FENBENDAZOLE IN THE NETHERLANDS.

MASTER THESIS – MSC VETERINARY SCIENCE



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Abstract

Background and relevance. *Parascaris* spp. are a host specific gastro-intestinal equine nematode from the family Ascarididae and is known to be the most pathogenic parasite of juvenile equids. Age immunity develops around 6 months of age, though older horses could be a source of re-infection due to environmental contamination. Larvae migrating through the respiratory tract could cause respiratory signs. Small intestinal impaction caused by crowded adult *Parascaris* spp. results in ill-thrift and can even be the cause of death among young foals. Thee different types of high efficacy, broad spectrum anthelmintic drugs are available to treat Parascariasis. Though, frequent effective anthelmintic treatment has resulted in overwhelming selection pressure and emerging reports on anthelmintic resistance (AR) of *Parascaris* spp. populations to ivermectine worldwide. Several studies demonstrated that *Parascaris* spp. populations have also developed resistance to pyrantel. Recently, a study in Saudi Arabia (Alanzi et al., 2017) and a study in Australia (Armstrong et al, 2014) reported resistance of this nematode to the anthelmintic drug fenbendazole, though thus far resistance of ascarid species to fenbendazole in The Netherlands has not yet been demonstrated.
Aim of the study. The aim of the study is to assess the efficacy of fenbendazole against *Parascaris* spp. populations in foals from ten weeks to five months on Dutch farms.
Materials and methods. This study was simultaneously executed with a similar study on the efficacy of pyrantel. The Fecal Egg Count Reduction Test is the practical and golden standard for *in vivo* establishing efficacy of anthelmintic drugs expressed in percentages. Fecal Egg Counts (FEC's) were performed on the day of treatment (D0) and compared to fecal egg counts on day fourteen to twenty-one days post treatment (D14 – D21) for establishing FECRT's for fenbendazole. In this study egg counts were performed with the modified McMaster technique utilizing two McMaster slides, performed with a sensitivity of 25 eggs per gram (EPG). Foals ranging from ten weeks to three months with an EPG≥100 that didn't receive any anthelmintic treatment ≥4 weeks prior to our farm visit, were included in our investigation.
Results. 46 FECRT's were performed and in 42 foals fenbendazole achieved the maximum efficacy of 100%. Four foals showed reduced efficacy of fenbendazole of 0%, 0%, 79 and 84% respectively. Of 292 fecal samples, 114 were found to be positive. The seemingly high prevalence of 39 % within this study could be explained either by the repeated FEC's for 23 foals or by the young age of included foals. Interestingly, results from the questionnaire showed that most farms frequently used two different types of anthelmintic drugs to control infections with *Parascaris* spp..
Discussion Nowadays, no *in vivo* tests for direct detection of Anthelmintic Resistance (AR) in ascarid species have yet been validated, so the Fecal Egg Count Reduction Test by counting *Parascaris* spp. eggs shed in the feces (D0 and D14-D21) is the practical and golden standard *in vivo* for measuring anthelmintic efficacy. However, FEC's variability could affect results, including young age of foals, variability in egg shedding, sensitivity of the egg counting technique and missed spilled treatment. Additionally, farms are over reliant on the use of frequent anthelmintic treatment to control infections with *Parascaris* spp. and should implement more management measures that could contribute to effective parasite control.
Conclusions. This is the first time that reduced efficacy of fenbendazole to *Parascaris* spp. has been reported in The Netherlands. Therefor indications for AR of *Parascaris* spp. populations to fenbendazole on Dutch farms have been demonstrated. Though a follow-up study is recommended on these farms, by re-establishing FECRT's for fenbendazole with minimum limit of detection of 10 EPG, hence increasing the probability of egg counts being truly zero after

treatment and generating more accurate FECRT results. Increasing the interval between treatments (>60 days) and daily cleaning of stables is recommended on Dutch farms.

Introduction

Among nematodes from the family Ascarididae, *Parascaris* spp. is known to be the largest equine roundworm. Adult worms have a mean length of ten to twenty centimeters, whereas the eggs are around 100 µm in diameter (Reinemeyer & Nielsen 2018). *Parascaris* spp. are considered to be the most pathogenic parasites of juvenile horses. Colic after infection with this host specific gastro-intestinal helminth is very common in foals worldwide. Furthermore, small intestinal impaction or perforation could be the cause of death among young infected foals. Treatment is limited to three registered groups of broad spectrum anthelmintics and global reports of resistance to these anthelmintics are increasing. However, nowadays no evidence has been found for resistance of *Parascaris* spp. to fenbendazole in The Netherlands.

Terminology

Infection can occur with two different species of the genus Parascaris, which are morphologically identical and can only be identified by karyotyping. *Parascaris univalens* has only one chromosome pair, whereas *Parascaris equorum* contains two pairs (Goday and Pimpinelli 1986). Although *Parascaris equorum* is more commonly described, recent studies suggest that *Parascaris univalens* is actually accountable for most infections in equids (Nielsen et al., 2014a; Tyden et al., 2013; Gao et al., 2018). Gao et al (2018) considers that they may even represent the same species. In this study no specific species have been established, so both species will be referred to as '*Parascaris* spp.'.

Life cycle and epidemiology

To comprehend the clinical relevance and to asses possible factors contributing to the development of resistance to fenbendazole, it is necessary to have knowledge of the life cycle and epidemiology of these roundworms. *Parascaris* spp. are nematodes with a direct life cycle and is distinguished by a parasitical stage in equids and an environmental stage in the external habitat (figure 1). Infection of foals and young horses until 6 months of age with *Parascaris* spp. is very common worldwide. Prevalence among foals younger than one year has been globally reported to be up to 83% (Armstrong et al., 2014; Nielsen, 2015), concluding most foals endure an infection with this nematode.

Infection occurs when larvated infectious eggs are ingested from the pasture, stalls or paddocks. Thus far no evidence has been found for transplacental or lactogenic transmission (Andersson, 1992). Approximately 72 to 110 days (Clayton and Duncan, 1977) post infection *Parascaris* spp. have reached patency and female nematodes produce eggs which are excreted in the feces. Egg shedding of female adult worms could lead to high Fecal Egg Counts (FEC's). Clayton and Duncan (1977) demonstrated a foal shedding over 50 million of eggs per day. Another study by Sinniah (1982) examined egg shedding of the human ascaris species, *Ascaris* lumbricoides, and demonstrated females can produce over 200.000 eggs a day. Embryonated eggs excreted in the feces of infected foals can develop into infective larvae within nine to fourteen days. The optimal temperature for this development is 25-35˚C (Scala et al., 2021). Larvated eggs can survive for up to ten years in moist and cooler pastures (Bello, 1982), since they are very resilient due to their thick spherical outer shell (Burk et al., 2014), though the majority of eggs are expected to survive one year (Nielsen, 2016). In colder climates with temperatures below 10˚C eggs will not become infective, though they remain viable (Clayton, 1986). *Parascaris* spp. eggs will be eradicated when exposed to temperatures of below minus 20˚C (Schurer et al.,2014) or temperatures of 35 – 55 ˚C (Gould et al., 2013). However, more

research is required to determine the exact resilience of *Parascaris* spp. on a pasture, considering soil type, season and climate. (Ihler, 1995; Reinemeyer, 2009; Nielsen, 2016).

The Netherlands has a moderate maritime climate, characterized by moderate temperatures and frequent rains. In this climate, eggs shed by infected foals on the pasture, stalls or paddocks become infective during the next spring to late autumn, and can infect foals which will be born next year. (Lindgren et al., 2008)

The ingested embryonated *Parascaris* spp. eggs hatch once they arrive in the stomach. The larvae subsequently penetrate through the intestinal mucosa and veins and reach the liver through the portal vein. A post-mortem study by Clayton and Duncan (1977) of eight worm-free foals that received a single infection of 8000 embryonated *Parascaris* spp. eggs, showed that two days post-infection the larvae have reached the liver. After penetrating the liver tissue and veins, the larvae spread to the lung parenchyma through the vena cava and right heart, approximately 7 to 14 days after infection. Via the tracheoesophageal route 99% of the larvae consecutively have returned to the small intestine on day 23. Reappearing to the small intestine, the roundworms have accomplished an extreme growth. Infected horses can harbour worm burdens ranging from a single worm to a couple of thousand (Nielsen et al., 2010).

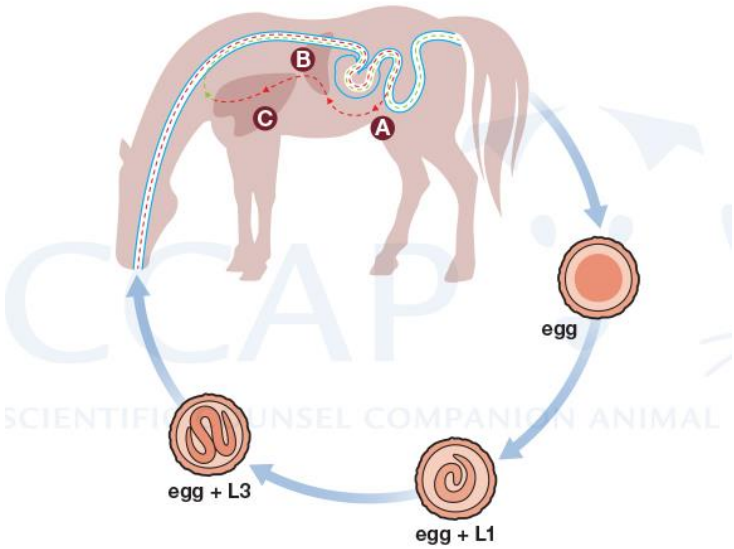

Figure 1: The schematic representation of the direct life cycle of Parascaris spp. (website ESCCAP, 2021).
A: Hatching of third stage larvae L3 in the stomach and small intestine, penetration of intestinal veins. B: Larvae reach liver via portal vein, migration through liver tissue and penetration of liver veins. C: Larvae reach lung via vena cava and right heart, penetration into lung alveoles and migration via trachea and pharynx to small intestine (moulting to L4 and St5 prior to development into adults).

Clinical signs

Clayton and Duncan (1977) demonstrated that respiratory signs can occur during the migrational phase, at approximately 2-3 weeks post infection. Most common signs of infection in foals until 6 months of age are coughing and mucoid or purulent nasal discharge. This suggests that migrating larvae damage respiratory tissue. Macroscopically lung parenchyma showed focal eosinophilia and hemorrhage 7-14 days post infection. The inflammatory response has been

histologically described as a pulmonary eosinophilia with active alveolitis, bronchiolitis and bronchitis. (Nicholls et al. 1978).

Clinical signs such as anorexia, poor body condition score and ill thrift could also develop. Studies suggest this is caused by adult worms competing for space and nutrients in the small intestine. (Nielsen, 2016)

Histologically the hepatic migration of *Parascaris* spp. induces multiple small, white fibrotic lesions. Nevertheless macroscopically few lesions have been reported, supported by blood analysis showing no elevated clinical liver values. (Brown and Clayton, 1979)

Probably the most severe risk of infection with this equid nematode is small intestinal impaction, which can result in intestinal rupture and death. This manifestation is associated with heavy worm burden followed by recent high efficacy anthelmintic treatment, though this has not been demonstrated with fenbendazole. (Nielsen, 2015) Furthermore two studies reported that less than 0,5% of all colic surgeries are compromised by Ascarid impactions (Cribb et al., 2006; Southwood er al., 1996). This demonstrates that morbidity is high, though mortality is low regarding infection with *Parascaris* spp..

Weanlings (6 to 12 months of age) and yearlings can be infected, though they generally have acquired immunity. Bello (1985) demonstrates increasing anti-body titres in plasma from aging foals, inclining that older foals develop an adequate immune response to this equid nematode. This is supported by another study (Lindgren, 2008), that reported egg shedding diminishes after 6 months of age. Though immunity isn't absolute and reinfection with generally small worm burdens occurs in few weanlings and yearlings. This is supported by a recent study that reported biphasic appearance of FEC, respectively peaking at about 4-5 months and a smaller peak at 8-10 months (Fabiani et al., 2016). When yearlings are infected, they can develop more severe respiratory illness. Radiographs at day sixteen post infection show visible signs of bronchopneumonia (Clayton and Duncan, 1977). Nielsen et al. (2010) suggests that an explanation could be the more efficient immune system and an adequate inflammatory response, caused by larvae trapped in the lungs. Older horses can occasionally get infected with the equid nematode and could be a possible source of re-infection due to environmental contamination (Kornas et al., 2006).

Diagnosis

Diagnosis is crucial before starting treatment and can be difficult regarding the life cycle of this Ascaris species. So far only patent infections (when adult worms are already present in the small intestine) can be diagnosed. The standard method is detecting eggs shed in the feces nine to sixteen weeks post infection, by performing a FEC utilizing a microscope. (Lyons et al. 1976) Note that a positive FEC demonstrates an infected foal, but a negative FEC doesn't exclude an infection with *Parascaris* spp.. Furthermore the number of eggs doesn't correlate with worm burden. (Nielsen et al.,2016) Nielsen (2016) suggests that adult worms in the small intestine could also be diagnosed by transabdominal ultrasound. Though, this isn't be suitable for routine diagnosis since equipment and specialized practitioners are required.

Treatment

Three different groups of high efficacy, broad spectrum anthelmintics were introduced to the health market for the control of the four groups of gastro-intestinal parasites; large strongyles, cyathostomins, ascarids and pinworms (Kaplan and Nielsen, 2010). Thus far these are macrocyclic lactones, tetrahydropyrimidines and benzimidazoles (Nielsen et al., 2016). The introduction of these anthelmintic drugs resulted in worldwide routine treatment of foals,

because of the economic advantage and the safety of the drugs. Furthermore, exposure to these anthelmintic drugs inhibits susceptible eggs on the pasture to become infective. (Campbell, 1990)

Preliminary observations on the mechanism of fenbendazole showed an inhibition of DNA synthesis, by selectively binding and damaging B-tubulin and preventing polymerization into microtubules (L.C. Davidse, 1986). Fenbendazole is a non-paralytic anthelmintic drug that inhibits cell metabolism, inherently starving the worms to death. Several studies suggest that fenbendazole also has high larvicidal and ovicidal activity in susceptible parasites, when treatment is administered for five consecutive days. Unfortunately, *Parascaris* spp. larvae aren't susceptible and treatment mainly targets adult worms crowding in the small intestine. (Reinemeyer et al., 2010). Usually no adverse effects occur, though, gastrointestinal side effects such as vomiting and diarrhea can occasionally develop. Toxicity subsequent to overdosing is very unlikely, because fenbendazole specifically targets susceptible parasites. (Campell, 1990; Plumbs.nl, 2021).

Fenbendazole was introduced as a single dose antiparasitic agent for the control of gastro-intestinal nematodes and several different formulations were developed for both farm animals and companion animals (Campell, 1990). In the Netherlands Panacur® paste and Panacur Safe Guard Paste® (100mg/g) are approved by the FDA (Food and Drug Administration) for oral administration for equids and bovines (Plumbs.nl, 2021).

The mechanism of action for ivermectin and pyrantel is inducing paralysis of adult worms, by inhibiting neuromuscular transmission. Macrocyclic lactones (ivermectin) interfere with the glutamate-gated chloride channels of Parascaris spp., though precise interaction is unknown (Craig et al., 2017). Tetrahydropyrimidines (pyrantel) are known to be potent agonists for Acetylcholine receptors in somatic cells of Ascaris spp. (Harrow et al., 1985).

Since only limited anthelmintic drugs are available to treat Parascariasis and development of new anthelmintic drugs is unlikely, monitoring efficacy and resistance is essential (Sangster, 1999).

Efficacy and establishing resistance

For several decades horses have been treated with these high efficacy anthelmintics frequently and repeatedly. Anthelmintic treatment failure was first described fifty years ago against Cyatostominae. Though, small strongyles don't generally cause any health issues on farms. However, in 2002 a study in the Netherlands first reported resistance of *Parascaris* spp. populations to macrocyclic lactones (Boersema et al., 2002). Subsequently various other studies have reported treatment failure of macrocyclic lactones globally (Hearn and Peregrine et al., 2003; Craig et al., 2007; Lindgren et al.,2008). Previously resistance in *Parascaris* spp. populations was considered unlikely, since efficacy in general had been reported as high as 100% (Drudge et al., 1975). However, overwhelming selection pressure and low proportion of worms in refugia allow resistant parasites to survive and increase in frequency overtime (Kaplan and Nielsen, 2010). The definition of anthelmintic resistance (AR) as described by Sangster (1999) is the ability of worms in a population to survive treatments that are generally effective against the same species and stage of infection.

Ivermectin was used most frequently to treat this widespread disease, hence most studies reported resistance to this anthelmintic drug (Reinemeyer, 2009). Furthermore, pyrantel and fenbendazole are non-persistent time-dependent drugs, whereas ivermectin is a persistent drug. Subsequently anthelmintic exposure of *Parascaris* spp. worms is more extentsive on farms using ivermectin. Note that worms that haven't been exposed to anthelmintic drugs slow the development of AR populations (Molento et al., 2008). In 2008 Molento et al reported reduced efficacy of ivermectin and pyrantel to *Parascaris* spp. in Brazilian horses. Nevertheless,

fenbendazole achieved maximum efficacy. Lyons et al (2011) also reported reduced efficacy of pyrantel against ascarids, but fenbendazole achieves maximum efficacy for treating ascarid infections.

Recently, reports on reduced efficacy of fenbendazole against *Parascaris* spp. are also emerging. In 2014 Armstrong describes AR of *Parascaris* spp. on farms in Australia to all three anthelmintic drugs. In 2017 AR of *Parascaris* spp. to fenbendazole has also been reported in Saudi Arabia (Alanazi et al., 2017). Some other factors could be contributing to these anthelmintic resistant *Parascaris* spp. populations (Reinemeyer, 2011). Fenbendazole is a broad spectrum anti-parasitic agent and achieves high efficacy (>90%) in treating foals infected with all equid nematodes. The dosage of 5 mg/kg PO is sufficient to eliminate large strongyles, cyathostomins and pinworms. However, *Parascaris* spp. is the dose limiting parasite for fenbendazole (and ivermectin) and for treating ascarid infections the double dosage is recommended (10mg/kg PO). (Plumbs.nl, 2021) In the Netherlands an dosage of 7,5 mg/kg is advised by the manufacturer of Panacur®, which contains the anthelmintic drug fenbendazole. The dose limiting parasite has a lower threshold for the development of AR, by increasing number of refugia and allowing more susceptible worm populations to survive and so decreasing the selection pressure. (Reinemeyer, 2009). However, the importance of refugia in the development of AR in ascarid species is unknown, since infected equids can show excessive egg shedding and embryonated eggs are very resilient in the external environment (Lindgren & Höglund 2010). Though, frequent treatment with high efficacy anthelmintic drugs causing few refugia to survive, has led to resistance development worldwide, so it's likely to be significant.

Unfortunately the mechanisms causing AR to the different anthelmintic drugs have not yet been discovered for ascarid species. In conclusion, reduced efficacy of fenbendazole to *Parascaris* spp. in The Netherlands could be expected, though precise mode of action causing AR in *Parascaris* spp. populations is still unknown.

Hypothesis

For this study a null hypothesis and an alternative hypothesis were drafted. H0 is deworming with fenbendazole shows expected efficacy of below 90% against *Parascaris* spp. in approximately 40 foals from ten weeks to five months in the Netherlands as established with the Fecal Egg Count Reduction Test. H1 is deworming with fenbendazole shows expected efficacy of above 90% against *Parascaris* spp. in approximately 40 foals from ten weeks to five months in the Netherlands, as established with the Fecal Egg Counting Reduction Test.
150 veulens en prevalentie ongeveer 25%

Research goal

The aim of the study is to assess the efficacy of fenbendazole against *Parascaris spp.* in as many foals as possible aged ten weeks to five months on Dutch farms included in our research.

Material and methods

Study design

This study reviewed the efficacy of the anthelmintic drug fenbendazole in foals infected with *Parascaris* spp. in the Netherlands. During our field research we collected fresh fecal samples from foals aged ten weeks to five months that had not received anthelmintic treatment for at least four weeks prior to inclusion within our study. After identifying the foals, the fresh fecal samples were collected in a disposable glove and immediately analyzed either on the farm predominately or at the KLIF laboratory. Concluding our field research, the final fecal samples were send to the Veterinary Microbiologic Diagnostic Centre (VMDC) and were preserved in the refrigerator, because *Parascaris* spp. eggs are very resilient. The fecal samples were analyzed in the KLIF laboratory at the Department of Biomolecular Health Sciences-I&I after fourteen to twenty-one days. FEC's were established using the modified McMaster technique and foals with an EPG ≥100 were included in our investigation.

This study was performed alongside a different study on efficacy of pyrantel against *Parascaris* spp. with a similar study design. For practical reasons most farms were visited together (see section result for specific farms). Foals with an EPG≥100 were treated with an anthelmintic, either fenbendazole or pyrantel. On farms participating on both studies, the foals with an EPG≥100 were divided into two equally distributed groups based on gender, age and EPG. One group was treated with fenbendazole and the other group was treated with pyrantel. At fourteen to twenty-one days post treatment new fresh fecal samples were collected, hence the corresponding Fecal Egg Counting Reduction Test could be established.

Farms

Leading up to our field research we've contacted several Dutch farms harbouring foals, by searching the internet. Farms were included if they were located in Gelderland, Brabant of Utrecht and a minimum of four weeks no anthelmintic treatment prior to our farm visit could be achieved. Because of the long prepatent period of Parascaris spp., eight weeks or over without deworming would have been preferable *(*Armstrong et al., 2014). However, most farms in The Netherlands were reluctant to extend this period for more than four weeks, so this was also approved by our supervisor as an acceptable inclusion criteria.

It is recommended by the American Association of Equine Practitioners (AAEP) guidelines to include at least six horses in a FECRT on each farm. Note that only foals with a positive egg count were included in the study. Assuming a prevalence of 27% on Dutch farms (Van Doorn et al., 2007) and targeting 6 horses with a positive EPG (e.g., ≥ 100 EPG), we would have preferably included approximately 22 foals on each farm. Including a large amount of foals ensures a more accurate representation of AR on a farm, since the results of one foal doesn't affect the group result to such a high extent. Unfortunately the farms included in our research didn't own that many foals (see section result for number of foals included on each farm). Different types of farms participated, but mostly stud farms housing a large number of foals were included in our field research. Other types of farms included in our research were farms that also housed foals, such as horse dairy farms, farrowing houses, riding schools and private housed horses.

In regard to previous studies, this and the similar study with pyrantel of Michelle Schellekens included roughly 300 foals together, respectively 150 foals within each study (Vidyashankar et al., 2012).

Foals

Foals from the participating farms range from ten weeks to five months of age. For identifying horses a chip reader was present. Unfortunately no foals were chipped just yet. Alternatively physical characteristics were noted to differentiate foals, including gender, age, color, markings and stable number. The fresh samples were collected when foals dropped feces on the ground. On some farms feces was collected from stall bedding and based on the size, shape, and consistency defecated by a foal (Lyons et al., 2011). The foals on each farm were numbered in the logbook and the corresponding number was marked on the plastic glove containing the feces. The fecal samples to VMDC were transported in a closed tube wrapped in an absorbant. The tube(s) were sealed in a 'sealbag' and were transported in a bubble envelope by PostNL, retrieved from a PostNL service point. Each tube and envelope were marked with a sticker, including farm name, horse name(s) or number(s), VMDC, post office box number of VMDC, the name of our supervisor (Deborah van Doorn) and the title of this master Thesis.

Treatment

After including foals, their weight was estimated by three people by observing the height, length, visibility of the ribs and tuber coxea and the abdominal distention of each foal. To prevent under-dosing we added 10% to the estimated weight. Anthelmintics were administrated orally. When spilling was observed, the spilled amount was accounted for. Unfortunately, the two specific foals that spilled treatment weren't noted in the logbook and therefore weren't included in the results.

On farms only contributing to research on fenbendazole foals with an EPG≥100 were treated with Panacur® paste 187,5 mg/g fenbendazole. The dosage is 7,5mg/kg fenbendazole and is indicated by graduation marks on the tubes (Diergeneesmiddeleninformatiebank.nl, 2021). On farms participating in both simultaneously executed studies the foals with an EPG≥100 were divided into two equally distributed groups. This equal division was based on age, gender and EPG and more feasible on farms harbouring several infected foals. One group was treated with fenbendazole and the other group was treated with pyrantel.

The modified McMaster technique

The modified McMaster technique was utilized for assessing the number of nematode eggs per gram of feces. This quantitative technique is a flotation technique that requires solutions with a different specific gravity to separate the worm eggs from debris. In this study we wielded a limit for detection of 25 EPG (eggs per gram), which is recommended by the AAEP guidelines and Kaplan and Nielsen (2010). (Vadlejch et al 2011) The protocol for the modified McMaster technique is included in the appendix (1).

Norris reported that P*arascaris* spp. eggs are denser than strongyle type eggs and anoplocephalid eggs and found a specific gravity of 1.0903. This is supported by another study which estimated SG for *Parascaris* equorum to be 1.0969 (David and Lindquist, 1982). The flotation medium we used, was a sugar solution with a density of 1.30 g/cm^3. The flotation medium was made by suspending 1280 grams of sucrose in 1000 ml of warm tap water. The specific gravity of the flotation medium was confirmed by a densimeter. Immediately after collecting fresh fecal samples we weighed 3 grams of feces on a scale and added the flotation medium until the dispenser bottle contained 45 ml of fecal solution. After thoroughly mixing, the fecal solution was filtered with a sieve separating solution and the fecal matter. We used a pipette to withdraw 1 ml of the well mixed sample and fill one of the two McMaster chambers of the McMaster slide, according to figure 2. Before analyzing the samples we waited one minute to allow *Parascaris* spp. eggs float to the surface.

The McMaster chamber consists of a square made with eight green lines, indicating which areas should be examined. We utilized a microscope with a 10x objective to scroll through the compartments. Each counting chamber enabled us to analyze 0,15 ml of fecal suspension. Since 1 gram of feces is suspended in 15 ml of suspension, counting one egg inside one counting chamber translates to 100 eggs per gram feces (EPG). For each analysis we evaluated not one but two McMaster slides, each containing 2 counting chambers. We measured 4 x 0,15 ml (0,6 ml), which resembled 1/25th of the total suspension. Consequently counting one egg inside a counting chamber during this study resembled 25 eggs per gram feces. This lowered the limit for detection from 100 EPG to 25 EPG.

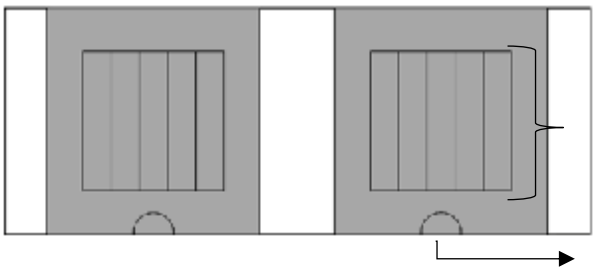

Figure 2: A schematic view of the modified McMaster technique slide, containing two counting chambers. (Kochanowski et al., 2013)

Testing efficiency

Fecal Egg Counting Reduction Test (FECRT) is the *in vivo* standard method currently available for detecting diminished efficacy in nematodes of horses. It's a method for assessing anthelmintic efficacy, expressed as percentages. The formula is as followed: 100(1 –FEC post-treatment)/FECpre-treatment) (figure 3). (Armstrong, 2014) Unfortunately no FECRT thresholds have been validated for *Parascaris* spp.. Therefor we used the thresholds acquired for strongyloides, another equid parasite with similar treatment. The amount of egg count reduction required to qualify as an acceptable efficacy is generally accepted as >90% or >95% FECRT, as stated in the AAEP guidelines (Reinemeyer, 2009). Resistance of *Parascaris* spp. to fenbendazole might be suspected when the reduction in egg count is between 90% and 95%. When a FECRT <90% is established, evidence of resistance to fenbendazole has been demonstrated (Bauer et al., 1986).

In this study FECRT was only established when the minimum quantitative standard of 100 EPG was measured. Hence, a significant reduction in EPG after treatment could be demonstrated, with an egg counting sensitivity of 25 EPG (Kaplan and Nielsen, 2010). Data analysis will be set out in tables and processed by Excel and SPSS.

$$\frac{\text{EPG (pre-treatment)} - \text{EPG (14 day post-treatment)}}{\text{EPG (pre-treatment)}} \times 100 = \text{FECRT}$$

Figure 3: FECRT calculation formula (AAEP, 2021)

Questionnaire

Prior to our field study we established a questionnaire based on the questionnaire by Karman and Groen regarding anthelmintic policy and housing management. Date of last deworming and age of the foals were confirmed in advance on the phone, hence these factors were necessary for including farms in our study. Questionnaires were either conducted on the farms or send by email in advance of the first visit.

General farm information such as address, contact information, veterinarian and number of horses and foals was collected. Furthermore current anthelmintic policy for foals, pregnant mares and horses was established. Fecal diagnostics can provide information on the accurate moment of deworming, so this was also included in the questionnaire.

Furthermore housing conditions were noted, including if foals were housed in stalls, paddocks or meadows. Additionally group or individual housing was inquired after. Questions on pasture management included dragging pasture, alternately using meadow, and grazing other species. Frequency of cleaning stables and disinfection routine were also included in the questionnaire. Assessing these possible risk factors is crucial for controlling and reducing infection pressure of *Parascaris* spp. among foals worldwide. This will be further illustrated in the section discussion. The complete questionnaire in Dutch is included in the appendix section (2).

Results

Farms

During our field study we visited 36 farms, located in the middle and partially the south of the Netherlands. Most farms included in our field research are stud farms. Specific details on farms identity is not included in this thesis, because of privacy policy. We will refer to farms using numbers 1 to 36. The majority of farms contributed to both studies. Farms 2, 24, 25, 33 and 35 participated exclusively within study on fenbendazole. Farms 3, 10, 13 and 15 participated exclusively in the similar study on pyrantel. Results on foals treated with fenbendazole (32 farms) will be mainly discussed, but results of all farms will be partially included.

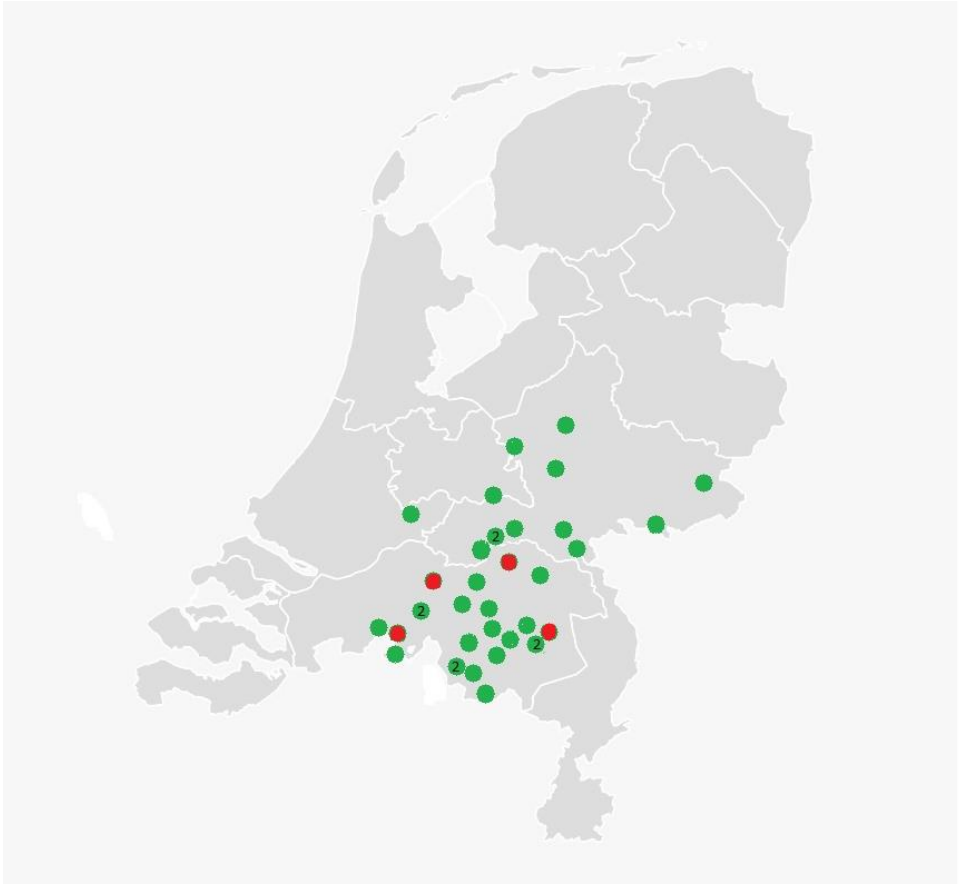

Figure 4: Distribution of the 36 visited farms in the Netherlands within the provinces Brabant, Gelderland and Utrecht. In four green dots the number '2' is added, to indicate two different farms were visited in that area. Four darts are marked red instead of green to indicate these farms participated exclusively in the research on Pyrantel.

On 11 of the 36 farms no *Parascaris* spp. infected foals (EPG≥25) were housed. The mean number of FEC's performed on each farm is 8 with a standard deviation of 4. The mean number of foals treated with fenbendazole, and included for FECRT, on each farm is 1,4 with a standard deviation of 1,7. All farms participating in this research are shown in table 1. Number of FEC's performed and foals treated with fenbendazole or pyrantel are included. Prevalence on each farm was calculated by dividing the sum of foals with an EPG≥25 on each farm by the sum of FEC's performed on that farm. Farm 1 in table 1 harbours the highest number of infected foals, associated with a prevalence of 100%. Farm 2 in table 1 has a similar prevalence. The mean EPG on each farm harbouring infected foals was calculated by Excel. The mean EPG pre-treatment of

all foals is 488 EPG and the highest mean EPG pre-treatment is 1898, corresponding with farm number 4 in the table.

Table 1: All 36 farms are included in this table including number of Fecal Egg Counts performed, infected foals with EggsPerGram≥25, foals treated with fenbendazole or pyrantel and mean EPG pre-treatment on each farm. Farms are sorted from high to low prevalence. The four cursively noted farms are participating exclusively in the research on pyrantel.

Farm	FEC's performed on each farm	Foals with *Parascaris* spp. eggs (EPG ≥25)	Foals treated with fenbendazole	Foals treated with pyrantel	Prevalence on each farm in percentages	Mean EPG pre-treatment
1	12	12	6	6	100%	1615
2	7	7	2	5	100%	1654
3	*7*	*5*	*0*	*5*	*71%*	*386*
4	13	9	5	4	69%	1898
5	11	7	1	6	64%	1375
6	16	10	5	3	63%	973
7	5	3	1	1	60%	200
8	7	4	2	2	57%	1043
9	9	5	5	0	56%	1253
10	*11*	*6*	*0*	*5*	*55%*	*234*
11	8	4	4	0	50%	1381
12	4	2	1	1	50%	100
13	16	7	2	5	44%	289
14	5	2	2	0	40%	360
15	5	2	1	1	40%	1120
16	15	6	2	3	40%	395
17	5	2	1	1	40%	115
18	11	4	4	0	36%	470
19	*3*	*2*	*0*	*1*	*33%*	*33*
20	*9*	*3*	*0*	*2*	*33%*	*344*
21	13	4	2	2	31%	765
22	10	3	2	1	30%	795
23	8	2	1	0	25%	34
24	14	3	2	1	21%	230
25	5	1	1	0	20%	495
26	3	0	0	0	0%	0
27	2	0	0	0	0%	0
28	5	0	0	0	0%	0
29	2	0	0	0	0%	0
30	7	0	0	0	0%	0
31	3	0	0	0	0%	0
32	4	0	0	0	0%	0
33	10	0	0	0	0%	0
34	4	0	0	0	0%	0
35	9	0	0	0	0%	0
36	13	0	0	0	0%	0
Mean	8,1	3,2	1,4	1,5	34%	488
Standard deviation	4,1	3,2	1,7	2	29%	574

Foals

Altogether we collected fresh fecal samples of 292 foals of which 271 were examined immediately after collecting. Some samples (21) were send to VMDC due to time restrictions. They were reserved in a refrigerator and analyzed in the laboratory at the Department of Biomolecular Health Sciences-I&I after fourteen to twenty-one days. As established through FEC's, using the McMaster technique, 114 foals were found to be infected with *Parascaris* spp. and 178 foals had a negative FEC. The inclusion criteria stated a required EPG count of 100>EPG, therefore 107 foals were suitable to participate in our investigation. These foals were treated with an anthelmintic, 52 were treated with fenbendazole and 55 were treated with pyrantel. After treatment with Panacur® no gastro-intestinal side effects were observed. Farm 36 wasn't able to participate in a post treatment FEC, so these foals were excluded for FECRT. In total 95 FECRT's were established. This study reviews the 46 foals treated with fenbendazole and examined with a FECRT, within the parallel study 49 foals treated with pyrantel and were examined through FECRT's.

The prevalence in this study is 38,9% with a 95% confidence interval of 33,3% - 44,5%. Thus it is 95% certain that the true prevalence of *Parascaris* spp. in our study lies between 33,3% and 44,5%. The mean age of the 292 foals is 102 days with a standard deviation of 24 days, see table 2 for specific numbers. The 46 foals treated with fenbendazole were constituted of 24 stallions and 22 mares. The mean age of these foals is 117 days and they have a mean EPG pre-treatment of 1669, with a standard deviation of 1900. The mean EPG post-treatment is 60 with a standard deviation of 301. The highest EPG pre-treatment is 7500, the highest EPG post-treatment is 2000, belonging to different foals on different farms.

Table 2: The mean age and the gender of foals included for Fecal Egg Counts that were either treated with fenbendazole or pyrantel.

Treatment	Mare	Stallion	Mean age
Infected foals	63 mares	51 stallions	113 days
Not infected	86 mares	92 stallions	95 days
Fenbendazole	22 mares	24 stallions	117 days
Pyrantel	30 mares	19 stallions	-
Total or mean age of all foals	149 mares	143 stallions	102 days

Fecal Egg Count Reduction (FECR) for fenbendazole

On day fourteen to twenty-one after treatment with Panacur®, 46 fresh fecal samples were collected. Egg counts were established to be able to establish efficacy through FECRT's. Forty two foals had an egg count of zero, corresponding with an FECRT of 100%. However, four foals had a positive FEC after treatment with fenbendazole and have a FECRT <90%, which is shown in table 3. Foals number 1 and 2 in table 3 have a FECRT of 0%. Foal number 3 has a FECRT of 79% and foal number 4 has a FECRT of 84%. The foals with a reduced efficacy percentage are housed on different farms. The arithmetic mean efficacy among these 46 foals as calculated with Excel is 94,8%, with a standard deviation of 21%.

Table 3: The four foals with a FECRT <90% on 14 – 21 days post treatment with fenbendazole.

Foal	Gender	Age in days, on day one of treatment	EPG pre-treatment	EPG post-treatment	FECRT expressed in percentages
1	Stallion	76	125	2000	0%
2	Stallion	73	225	300	0%
3	Mare	162	2775	75	79%
4	Stallion	113	2450	400	84%

Table 4 shows the twenty farms participating in FECRT. Sixteen farms have an average FECRT of 100% and fenbendazole achieved maximum efficacy. On four farms FECRT was below 100%, see table 4 for specific average FECRT'S on each farm. The foal with the highest EPG pre-treatment of 9325 was housed on farm number 16 in the table and didn't correlate with a lower farm FECRT.

Table 4: All farms that participated in a FecalEggCountReductionTest are shown in this table. The number of FecalEggCounts and FECRT's performed on each farm. The mean EggsPerGram pre-treatment, post-treatment and the highest counted EPG pre-treatment are also included. The last column shows the corresponding mean FECRT on each farm.

Farm	Number of foals/FEC's performed	Number of FECRT's performed	Mean EPG pre-treatment	Mean EPG post-treatment	Highest EPG pre-treatment	Mean FECRT fenbendazole
1	7	2	1654	0	2500	100%
2	13	5	1898	0	7500	100%
3	11	1	1375	0	5100	100%
4	16	5	973	60	5625	80%
5	5	1	200	0	600	100%
6	7	2	1043	0	5175	100%
7	9	5	1253	0	6400	100%
8	8	4	1381	100	4750	96%
9	4	1	100	0	200	100%
10	16	2	289	0	1625	100%
11	5	2	360	0	1675	100%
12	5	1	1120	0	5400	100%
13	15	2	395	0	2725	100%
14	5	1	115	0	400	100%
15	11	4	470	19	2775	95%
16	13	2	765	0	9325	100%
17	10	2	795	0	4425	100%
18	8	1	34	0	225	100%
19	14	2	230	1000	2700	50%
20	5	1	495	0	2475	100%
Arithmic mean	*9,4*	*2,3*	*747*	*59*	*3580*	*96%*
Standard deviation	*4,1*	*1,5*	*562*	*223*	*2559*	*12%*

Questionnaire

Altogether we've contacted 180 farms spread among Brabant, Gelderland and Utrecht in the Netherlands, of which 36 participated in our research. Some details are absent on the frequency of dragging meadow or providing a new meadow. Though, all questions included in the questionnaire are answered and discussed in the following chapter.

Anthelmintic policy

Most farms (30 farms is 83% of the farms) had a fixed deworming schedule which was advised by their veterinarian. Six farms didn't have a fixed anthelmintic policy. Frequency of treatment also varied a lot on farms, differing from no anthelmintic treatment to 5 or even 6 times within six months. All participating farms with corresponding frequency of anthelmintic treatment are shown in the table below (table 5). The proportion of farms for each subgroup of anthelmintic treatment frequency is also shown in table 5 and was calculated by the sum of the farms included in the specific deworming group divided by the sum of all 36 farms. Only 2 farms (5,6% of all farms) didn't treat foals with anthelmintic drugs. Most farms (52,8%) dewormed their foals on average three or four times within the first six months.

Fecal examination was performed on 14 farms (39%) of which 8 (22%) farms only performed fecal examination on indication when foals or horses showed clinical signs, like rough coat and diminished body condition score and distended abdomen. Six farms (17%) implemented Strongyle FEC's as parasite control. Farm 15 performed FEC's individually three times a year, farm 21 once a year individually and farm 22 once a year individually. Farm 33 performed FEC's twice a year with a pooled sample. Farm 34 performed fecal examination most often, specifically once a year individually, when foals reached the age of 4 months and on indication when horses showed clinical signs.

Table 5: Frequency of anthelmintic treatment of foals on each farm, the proportion of farms expressed in percentages is also included.

Frequency deworming	Farms	Number of farms	Proportion of all farms expressed in percentages
No anthelmintic treatment	Farm 7, 14	2	5,6%
1-2 times	Farms 3,4,16,27,29,21,31,32,33,35	10	27,8%
3-4 times	Farms 1, 6, 9, 10, 13, 14, 15, 17, 18, 20, 21, 22, 23,24,25,28,30,34,36	19	52,8%
5≥ times	Farms 2, 5, 8, 11, 26	5	13,9%

An overview of the different anthelmintic drugs that are used on farms is shown in table 6. Pyrantel is used most frequently on farms participating in this study. On 20 farms (56% of farms) pyrantel is used by default. Fenbendazole is used on 8 farms (22% of the farms), of which 2 farms solely used this anthelmintic to treat *Parascaris* spp. infections in foals. Ivermectin (or moxidectin) is used most frequently and implemented in the anthelmintic policy of 30 farms (83% of farms). Most farms used two different types of anthelmintics.

Moment of first treatment also varies on each farm. 20 farms first dewormed foals before or on 4 weeks of age, while 16 farms first treated foals with an anthelmintic drug after 6 weeks of age.

Table 6: Shown in this table are how many different anthelmintic drugs farms use as anthelmintic policy. The proportion of farms expressed in percentages is shown in the third column. The number of farms using fenbendazole and pyrantel is also included in this table.

Number of different anthelmintica	Farms	Number of farms	Proportion of all farms expressed in percentages
No anthelmintica	Farm 7, 19	2	5,6%
1 type of anthelmintica	Farm 1, 2, 3, 12, 16, 22, 27, 29, 30, 31, 33, 35	12	33,3%
2 types of anthelmintica	Farm 4, 5, 6, 8, 9, 10, 11, 13, 18, 20, 21, 23, 25, 26, 32, 36	16	44,4%
3≥ types of anthelmintica	Farm 14, 15, 17, 24, 28, 34	6	16,7%
Fenbendazole	Farm 2, 3, 4, 5, 14, 17, 34, 36	8	22%
Pyrantel	Farm 1, 6, 8, 9, 10, 11, 13, 14, 15, 18, 20, 21, 24, 25, 26, 27, 28, 31, 32, 34	20	56%
Total	-	36	100%

Housing management

All participating farms reciprocated the questionnaire regarding housing management and are shown in table 7. Most farms house foals together with their mares both on the meadow and in the stables, depending on day or night time. Six farms house foals and their mares solely outdoors. Of the 36 farms housing foals thirteen farms drag meadow once or several times a year. The majority of farms (72%) provide anew meadow for equids, generally every two to three months. However, most farms (32 or 89%) don't remove feces from the pasture. Farm 18 cleaned feces from the pasture once a month, farm 24 once a week, farm 35 once a year and farm 34 twice a year. On six farms (17%) cows graze on the meadow prior to horses and on two farms (6%) sheep are housed on the meadow during absence of equids. One farm has both sheep and cows grazing together on the pasture, prior to pasturing of the horses.

Thirteen farms (43%) daily clean the stables housing foals and their mares. Fourteen farms (47%) clean the stables weekly and five farms (17%) clean the stables monthly or even after a longer period of time. Additionally, most farms use water to clean their stables once a year instead of using a disinfectant. Five farms house foals and mares in a deep litter housing facility.

During our research (and two years prior to our research) farm owners didn't observe any foals showing clinical signs that could be associated with an *Parascaris* spp. infection.

Table 7: The reciprocated questionnaire of all 36 participating farms are shown in this table and compromised by housing management including outdoor or indoor housing, pasture management including frequency of dragging meadow, new meadow, cleaning meadow and other species on the meadow and stable management including the frequency of cleaning and disinfection of stables.

Farms	Stable or meadow	Dragging meadow	New meadow	Cleaning meadow	Other species	Frequency cleaning stables	Disinfecton of stables
1	Meadow	No	Yes, 2-3 months	No	No	-	-
2	Both	No	Yes, 2 months	No	No	Weekly	Once every 10 years, halamid

3	Both	No	Yes, 2 months	No	No	Weekly	Twice a year, water
4	Meadow	Yes	Yes, 2-3 months	No	No	-	-
5	Both	Yes	Yes, 2-3 months	No	No	Daily	Once a year, water
6	Meadow or paddock	Yes, 3 times a year	Yes, 3 times a year	No	Yes, sheep	Monthly or longer	Never
7	Both	No	Yes, 2-3 months	No	No	Weekly	Once a year, water
8	Both	Yes, during hot summers	Yes	No	No	Weekly	Once a year, water
9	Both	No	Yes, every month	No	Yes, sheep	Daily	Once a year, halamid
10	Both	No	Yes, 2-3 months	No	No	Daily	Thrice a year, halamid
11	Meadow	No	Yes, thrice a year	No	No	-	-
12	Meadow	No	Yes, 2-3 months	No	Yes, sheep and cows	-	-
13	Both	No	Yes	No	Yes, cows	Weekly	Yes, once a year
14	Both	Yes, four times a year	Yes, once a year	No	No	Weekly	Once in 2-3 years, water
15	Both	Yes	No	No	No	Daily	No
16	Meadow	Yes, twice a year	No	No	Yes, one cow	-	-
17	Both	Yes	Yes, 2 months	No	No	Weekly	Yes, once a year, water
18	Both	No	Yes, depending on grass	Yes, once a week	No	Daily	No
19	Both	No	Yes, depending on grass	No	No	Daily	No
20	Both	No	No	No	No	Weekly	Yes, once a year, water
21	Both	Yes, once a year	Yes, every 2 weeks	No	No	Monthly or longer	Yes, once a year, water
22	Both	No	No	No	No	Monthly or longer	Yes, once a year, water
23	Both	No	Yes, every 2 weeks	No	No	Daily	No
24	Both	Yes, once a month	No	Yes, once a month	No	Daily	Yes, once a year, water
25	Both	No	No	No	No	Daily	Yes, once a year, water
26	Both	No	No	No	No	Daily	Yes, once a year, water
27	Both	Yes, 2-3 times a year	Yes, depending on grass	No	No	Weekly	Yes, once a year, halamid

28	Both	Yes, every 5 years	Yes, every week	No	No	Daily	Yes, once a year, water
29	Meadow	No	No	No	No	-	-
30	Both	No	No	No	Yes, cows	Weekly	Yes, once a year, water
31	Both	Yes	Yes, every month	No	No	Daily	Yes, twice a year, water
32	Both	No	Yes, depending on grass	No	No	Daily	Yes, once a year, water
33	Both	No	Yes, depending on grass	No	Yes, cows	Weekly	No
34	Both	No	Yes, 2 months	Yes, yes twice a year	Yes, cows	Weekly	Yes, once a year, water
35	Both	Yes, once a year	Yes, depending on grass	Yes, once a year	Yes, cows	Weekly	No
36	Both	No	No	No	No	Monthly or longer	Yes, once a year, water

Chi squared tested parameters

The Chi square test is executed to find a possible correlation between the frequency of cleaning stables on each farm and the number of infected foals. The null hypothesis is that proportions are equal, however the alternative hypothesis is that observed proportions are more or less than the expected values. Five farms have a deep litter housing and the frequency of cleaning stables on each farm is shown in the table on the previous page(s) (18 - 19). The expected P value for this contingency table is 0,002 (df 4), though the outcome of the chi square test as calculated with Excel is 17. The observed value is much higher than the expected value, so a positive correlation between increasing the frequency of cleaning the stables and a lower number of infected foals with Parascaris spp. has been demonstrated. Furthermore, deep litter is a risk for increasing the infection pressure for *Parascaris* spp..

Table 8: Chi square test contingency table (5x2) assessing the possible correlation between frequency of cleaning the stables and the number of infected foals on each farm.

Foals	Daily	Weekly	≥Monthly	Deep litter
Positive foals	33	47	6	26
Negative foals	80	56	13	12

Discussion

FECRT's results

Although efficacy of fenbendazole against *Parascaris* spp. for 42 foals was 100%, in four fenbendazole efficacy was below 90% (efficacy percentage of 0, 0, 79, 84 and a mean efficacy of 41%). In these foals fenbendazole achieved reduced efficacy and indications for resistance have been demonstrated. Though no specific data is available on FECRT thresholds for *Parascaris* spp., populations were considered to be susceptible when reduction of egg count were >90%, as stated by the AAEP guidelines established for strongyles (Armstrong, 2014). Other studies also recommended to use 90% as cut-off value (Bauer et al., 1986; Coles et al., 1992; Kaplan et al., 2004; Armstrong et al., 2014; Alanzi et al., 2017; Martin et al., 2018). The established FECRT of the four foals was respectively 79%, 84% and 0%.

The egg count of two of these foals with reduced efficacy fourteen to twenty-one days post anthelmintic treatment was in fact higher than the first established egg count, resulting in a FECRT of 0%. An explanation for this high egg count post treatment with Panacur® are false positive results, which could derive from coprophagy. However a validation study only found 5% of all samples to be false positives, whereas 30% were false negatives (Nielsen et al., 2010), so treatment failure is more likely.

Another possible explanation is variability in egg shedding and non-uniform distribution of eggs (Nielsen, 2015). The modified McMaster technique was performed at the KLIF laboratory of Utrecht University prior to our field study, to practice his technique, though variability in egg shedding, non-uniform distribution of eggs and possible egg loss while performing this technique couldn't be eliminated. In this study the geometric mean EPG pre-treatment was 1669, with a standard deviation of 1900 and the geometric EPG post-treatment was 60 with a standard deviation of 301, demonstrating that egg counts are very variable within this study.

The FECRT results of the other two foals showing reduced efficacy after treatment could be because of partially effective treatment or acquired age immunity. One foal was 6 months of age and the other foal was almost 5 months of age on the day of FEC to establish FECRT's, so age immunity could have been developed to some extent within these foals. However, age immunity doesn't is explain the positive egg count after treatment with fenbendazole. Furthermore, age variation among these foals makes treatment failure or partially effective treatment more likely.

In conclusion, in these four foals fenbendazole treatment failed and reduced efficacy of fenbendazole to *Parascaris* spp. should be suspected. However, treatment failure could also be ascribed to missed spilled treatment, considering fenbendazole is significantly more fluid than other anthelmintic pastes. Furthermore, factors other than reduced efficacy of fenbendazole could also lead to these results, including FECRT variability, age of foals and management on farms. A follow-up study should be performed to re-evaluate the efficacy of fenbendazole on these farms, by establishing FECRT's, since AR is a herd problem (Nielsen et al., 2019).

These four foals infected with *Parascaris* spp. that demonstrated reduced efficacy of fenbendazole against *Parascaris* spp., were treated with pyrantel subsequent to establishing FECRT's. Three of the four foals had a negative egg count fourteen to twenty-one days after treatment with pyrantel. Some published studies arise concerns for multiple drug resistance. Armstrong (2014) reported evidence of multiple drug resistance on two of the five farms. Though indications for multiple drug resistance to fenbendazole and pyrantel on farms included in this study was absent.

AR is a herd problem, since foals housed on each farm are exposed to the same populations of *Parascaris* spp. (Nielsen et al., 2019). In this study 20 farms participated in

FECRT's for fenbendazole and on 2 farms a mean efficacy of less than 90% has been established (50% and 80%). Alanzi et al (2017) found resistant populations of *Parascaris* spp. present on six of the eleven farms. Armstrong (2014) demonstrated possible resistance to fenbendazole on 2 of the 4 farms. Interestingly, the farms harbouring foals demonstrating reduced efficacy, didn't generally use fenbendazole as treatment for *Parascaris* spp. in foals. Farm 9 used pyrantel and moxidectine, farm 20 and 25 used ivermectine and pyrantel and farm 33 solely used moxidectine. A possible explanation is movement of foals, but most farms included in this study are breeding farms, ensuring very limited movement of foals. Hence, it's considered more likely that farm owners have used all three different type of anthelmintic drugs in the past years, since they frequently rotate between different types of treatment.

FECRT limitations and altered inclusion criteria

To evaluate resistance of *Parascaris* spp. to fenbendazole genetic material must be retrieved from *Parascaris* spp. worms. Adult worms can be retrieved from the small intestine of infected equids, though equids should be killed for this technique, since Parascariasis doesn't have a high mortality rate, so this technique isn't morally accepted and other options should be contemplated. Retrieving *in vivo* eggs from equids is possible for performing FECRT's, though it's only an indication for anthelmintic efficacy. Other alternatives are *in vitro* techniques with L3 larvae or eggs that could develop into larvae after incubation. Several single nucleotide polymorophisms (SNP) in the B tubulin genes of adult worms have been associated with fenbendazole resistance in other other parasites. However, the mechanism causing AR has not yet been discovered for ascarid species. Tyden et al (2014) developed a PCR for genotyping SNP in codon 167, 198 and 200 for the tubulin gene. They retrieved *Parascaris* spp. eggs after treatment with fenbendazole from a farm with reduced efficacy to fenbendazole, which was measured by establishing FECRT's, and incubated the eggs 14 days at 28 degrees for development into larvae. Though, no SNP known to cause reduced efficacy of fenbendazole in other parasites were observed in larvae of possible resistant populations of *Parascaris equorum*. Nevertheless, the diversity of isotype patterns among nematodes indicates other specific SNP could be causing reduced efficacy to fenbendazole in ascarid species and more research could provide molecular markers for early detection of resistance (Tyden et al., 2014; F. Martin, 2021).

Since these in vitro tests have not yet been validated for assessing AR for *Parascaris* spp., for this study other techniques had to be considered. FECRT is the practical and golden standard for evaluating efficacy of anthelmintic drugs *in vivo*. (Vidyashankar et al., 2012) As previously stated specific FECRT cut off values for *Parascaris* spp. have not yet been validated, so the AAEP guidelines for strongylid parasites have been used to assess the results.

However, defining AR solely by *in vivo* measurements is difficult (Kaplan, 2002). Hence, the definition 'anthelmintic resistance' is not used, but in this study efficacy was established and therefore 'reduced efficacy' has been found. Though, many factors other than reduced efficacy could have affected these FECRT results (Coles et al., 2006). To establish FECRT pre- and post-treatment egg counts were established by the modified McMaster technique, because it is relatively simple and can be executed on farms within ten minutes per sample (Vadlejch et al., 2011). However, FECRT is only suitable for detecting eggs shed by adult female worms, whereas larval stages can only be detected by serological diagnostics.. Nielsen et al (2010) found positive and negative predictive values of 0.95 and 0.66 for the modified McMaster technique. In other words, a positive result is very likely to be true, but a negative fecal egg count doesn't exclude an infection with this equid nematode. Occasionally in this study we noticed that foals with a negative egg count were actually infected with *Parascaris* spp.. Owners of two farms observed *Parascaris* spp. in the feces of foals within a few days after administrating anthelmintic drugs.

The specific stadia of these *Parascaris* spp. nematodes are unknown, though they didn't produce eggs or only a very limited amount (below the detection limit). Though, these foals weren't included in our research, due to a negative egg count during our first visit. Ideally diagnostics for detecting prepatent infections should be established. Besides pathological and respiratory signs may occur during the migrational phase and earlier detection would be of clinical importance (Burk et al., 2014). Serological analysis for strongyles are available, but have not yet been developed for *Parascaris* spp. (Burk et al., 2014). However, studies also suggest that blood tests are unreliable, since most foals get infected during their life time or obtain antibodies via the colostrum. (Nielsen et al., 2015; Burk et al., 2015) The transabdominal ultrasound technique suggested by Nielsen et al (2016*)* isn't suitable for routine diagnosis, because it's laborious and exorbitant.

The guidelines recommend including foals with an egg count of >200 for FECRT (Reinemeyer, 2009). However, initial field research showed the majority of foals didn't harbour such a high egg count. For this reason foals with an EPG of 100 or higher were included in this study to be treated and because of this more foals for FECRT could be included, similar to previous studies assessing fenbendazole resistance (Armstrong, 2014; Alanzi et al.,2017).

The low number of positive FEC's for Parascaris spp. could be explained by the young age of foals during initial field research and the long prepatent period of the nematode. Some foals were probably infected, but didn't shed eggs in the feces at the time. Therefore 23 foals were measured twice during a specific period of time (see appendix for specific foals) and thirteen foals were included for treatment after the second FEC. The majority of foals with a positive egg count were sixteen weeks (or four months of age). Peak shedding at four months is also described in other studies (Lindgren et al., 2008; Fabiani et al., 2016). The inclusion criteria required foals to range from ten weeks to five months, as recommended by Karman (2020) in her master thesis and resulted in including mostly breeding farms in our research.

Another possible explanation could be that foals were treated with anthelmintic drugs four weeks prior to our farm visit. Ideally periods of ≥8 weeks without anthelmintic treatment prior to our farm visits were implemented, as recommended by Nielsen at al. (2019). However, participating farms were reluctant to adopt extended dosing intervals regarding risk of infection with *Parascaris* spp..

Increasing the number of FECRT's could increase reliability of established FECRT's (Vidyashankar et al., 2012). In this study 292 fecal samples were collected and 46 FECRT's were performed. Karman and Groen included 297 foals included on 11 farms and 24 FECRT's within their master thesis. Armstrong (2014) included 91 foals for FECRT, Alanzi (2017) included also 46 foals and Karman included 24 foals that were treated with fenbendazole. The guidelines also recommended including a minimum of 6 foals on each farm for FECRT (Coles et al., 2006). However, the mean number of foals on each farm included for FECRT for fenbendazole in this study is 2,3. Reducing the number of FECRT's could result in unreliable results, since the result of one foal affects the group result to a greater extent. Unfortunately increasing the number of foals on each farm wasn't possible due to farm management in the Netherlands. Foals are moved from breeding farms (or other farms housing foals) to farms that offer larger group housing, after weening at approximately 5 or 6 month of age. The inclusion criteria of this study excluded foals older than 5 months, so these type of farms weren't accepted to participate in our field study.

Fecal samples were collected on the day of treatment and fourteen to twenty-one days after treatment as recommended by the guidelines. Most fresh fecal samples were collected from the ground immediately after foals defecated. Though on some participating farms feces was collected from stable beddings harbouring foals. The consistency, shape and amount of feces was observed similar to Lyons et al (2011), though feces of the mare instead of the foal could have

been collected for the FEC. 271 fecal samples were examined with the McMaster technique within a few hours after collection. Twenty-one fecal samples were collected by farm owners and send to VMDC. As recommended by the AAEP guidelines these samples were preserved in a refrigerator and analyzed in the KLIF laboratory at the Department of Biomolecular Health Sciences-I&I after fourteen to twenty-one days after treatment. Possible egg loss after these fourteen to twenty-one days could have affected FEC's, though *Parascaris* spp. eggs are very resilient and most eggs survive for up to one year. Owners were instructed to collect foal samples and send these to VMDC immediately, or store the samples in the refrigerator until then. However, human error could have occurred and feces not belonging to the foal or incorrect storage could have affected FEC's.

At last, sensitivity of the egg counting technique could also lead to variable FECRT results. A post-treatment EPG is expected to be low, so counting one or two eggs could determine the result of the FECRT. Additionally, inevitable egg loss during procedure increases the probability to count zero eggs. In this study the McMaster had a limit of detection of 25 EPG as recommended by Kaplan and Nielsen (2010) and the AAEP guidelines, though lowering the limit for detection by increasing the number of McMaster slides per fecal sample could significantly affect FECRT's outcome (Vidyashankar et al., 2012). Karman and Groen wielded limit of detection of 17 EPG. The limit of detection used in the study by Van Doorn (2007) is unfortunately unknown. Alanzi et al. (2017) wielded a detection limit of 20 EPG and Armstrong et al. (2014) had a minimum of 10 EPG. We recommend future studies to perform FEC's with five McMaster slides, to achieve a detection limit of 10 EPG, hence increasing the probability of egg counts being truly zero after treatment and generating more accurate FECRT results.

Prevalence within this study

The prevalence of *Parascaris* spp. eggs found in this study is 38,9%, with a 95% confidence interval of 33,3% to 44,5%. The prevalence of *Parascaris* spp. in foals has been assessed in various studies worldwide and varies from 22.4% to 80% (Armstrong et al., 2014). A study previously executed in The Netherlands, including 332 horses, detected positive foals on 26 of the 43 farms and reported a prevalence of 27% (van Doorn et al., 2007). Boersema (2002) found that 10 of the 25 fecal samples had a positive EPG and reported a prevalence of 40%. In 2020 Karman executed her master thesis on the efficacy of fenbendazole in the Netherlands and found 72 of 297 foals to be positive, i.e found a prevalence of 24%.

One evident explanation for the high(er) prevalence within this study is that twenty-three samples were measured twice, which resulted in including thirteen more foals for our research. Another possible explanation for the higher prevalence found in this study could be the age of included foals. Karman recommended including foals of three to five months of age, since they included foals of 3 to 12 months of age. Stud farms harbour foals <6 months of age, so acquired age immunity has not or partially developed. Besides infection pressure on these farms is also higher (Fritzen, 2010). This results in a higher prevalence of *Parascaris* spp. within this study.

Moreover, the sensitivity of the egg counting technique within this study was lower than the sensitivity used in the previous master thesis on this subject by Karman and Groen, so prevalence would be even higher when a lower limit of detection was used (Fritzen, 2010).

Furthermore housing conditions, farm management and anthelmintic policy can affect prevalence, which will be further described in the next section 'questionnaire'.

Questionnaire

The results of the questionnaire clarify that every farm has a different policy with regards to anthelmintic drug usage, though extensive treatment is present on most farms. Furthermore, farms didn't implement various management measurements to provide efficient parasite control. What also stood out was that farm owners didn't observe any foals showing clinical signs that could be associated with an *Parascaris* spp. infection, but this could easily be appointed to the high frequency of anthelmintic treatment.

Anthelmintic policy

Most farms have a scheduled anthelmintic policy, that includes frequent administration of anthelmintic drugs to foals out of fear for equid infections with *Parascaris* spp.. Furthermore, most veterinarians continue to advise farm owners to implement anthelmintic treatment every 8 weeks. Under these circumstances surviving Nematoda will be evidently exposed to anthelmintic drugs during their life cycle. (Clayton and Duncan, 1977). Allowing some worms to survive and increasing the number of susceptible refugia of *Parascaris* spp. populations should be the main focus for decreasing the selection pressure and delaying the development of AR (Leathwick et al., 2012), which could be established by extending the interval between two treatments (Leathwick et al., 2017).

Overall, ivermectin was used most frequently on farms within this study, but generally two different types of anthelmintica were used. It's interesting that despite worldwide reported resistance to ivermectine, most farm owners didn't stop using this anthelmintic drug. Farms also didn't perform FECRT's to establish the efficacy of the anthelmintic drugs used. Pyrantel was used on 20 farms, whereas fenbendazole was used on 8 farms within this study, since pyrantel is still effective against small strongyles. However, resistance of *Parascaris* spp. to pyrantel has been reported more frequently and besides fenbendazole doesn't result in small intestinal impaction post treatment (Nielsen, 2015). Furthermore, Leathwick (2017) demonstrated that rotation of anthelmintic drugs didn't prevent the development of resistance and frequent treatment with anthelmintica resulted in the development of resistance nonetheless.

Most foals within this study were treated on (or before) four weeks of age. Leathwick (2017) shows that under these circumstances 96% egg reduction is achieved. However, some level of refugia are welcome, since few susceptible populations surviving treatment increases the overwhelming selection pressure for *Parascaris* spp.. A subsequential study from Leathwick (2017) demonstrated that treating foals on day 60 and on day 120 with fenbendazole or pyrantel could contribute to slowing the development of resistance. In addition, a combination product containing both pyrantel and fenbendazole, if administered only two times a year, could delay the resistance development to all actives, though this type of product has not yet been developed (Leathwick et al., 2017). However, resistance development of *Parascaris* spp. to both drugs could simultaneously occur.

Furthermore, older horses could be treated with an anthelmintic only after a positive diagnostic result (including CSF, McMaster or modified Stoll methods), though AR following treatment in equids >6 months of age didn't develop rapidly in a modelling study (Leathwick, 2017). Unfortunately this cannot be implemented for horses <6 months, since most foals will get infected and untreated foals are a health risk (Scala et al., 2021).

Concluding, one specific anthelmintic strategy that can be successfully implemented on each farm isn't achievable. Rather anthelmintic policy should be set up on farms individually and in consultation with a veterinarian. The different species of parasites present in the environment, the prevalence of those species and the level of resistance among these populations should be considered. (Scala et al., 2021)

Housing management

The Chi squared test results emphasize the importance of farm management to reduce the infection pressure of *Parascaris* spp. among young foals. The frequency of cleaning stables influences prevalence of *Parascaris* spp. infections significantly, and daily cleaning of stables is recommended to diminish infection pressure. This is supported by a study executed in Italy, that showed daily management of indoor housing facilities harbouring foals and mares can contribute to effectively control *Parascaris* spp. infections (Scala et al., 2021). Additionally, farms reported to clean the stables annually with high pressure, most farms used water and only few farms used disinfectantia like halamid. Though, it's unknown how to eliminate *Parascaris* spp. eggs from the external environment effectively (Nielsen, 2016).

Furthermore, this study demonstrated that deep litter housing poses a risk for increasing infection pressure, as previously reported by other studies (Fritzen, 2010).

Besides housing management, outdoor rearing significantly affects prevalence of *Parascaris* spp. infections (Scala et al., 2021). Some studies indicate that the most important management measure is removing feces from the pasture. Cleaning feces from the pasture two times a week could reduce the infection pressure significantly (Fritzen, 2010). However, most farms drag the meadow, essentially spreading the feces and *Parascaris* spp. eggs across the pasture, instead of removing it. Fertilizing pasture with horse manure has been reported to be associated with higher prevalence of *Parascaris* spp., so dragging the pasture could potentially result in infecting more foals. On the other hand, dragging meadow could lead to more UV exposure and several studies indicate that UV-light could damage and even kill *Parascaris* spp. eggs (Shalimov, 1935; Neufahrt, A., 1960). However, removing feces from the pasture would be a more efficient management measure for controlling the infection pressure of ascaris species.

Most farms provide other pasture for equids every three to four months, but mostly for practical reasons and not to contribute to parasite control. Furthermore, larvated eggs can survive in the external environment for up to ten years, so it's questionable if this is a sufficient and feasible method on farms in the Netherlands (Fritzen, 2010).

Altogether various farm factors influence egg counts and thus FECRT, so it's difficult to evaluate to which extent one sole variable affects the outcome. Furthermore, veterinarians should inform farm owners on the importance of management measurements for providing efficient parasite control.

Conclusion

In conclusion, this study demonstrated reduced efficacy of fenbendazole to *Parascaris* spp. in four foals of between ten weeks to five months of age on Dutch farms during the summer months of 2021. Fenbendazole achieved 100% efficacy in 42 foals. However, a follow up study is recommended by establishing FECRT's for fenbendazole on these farms (AAEP guidelines, 2021; Reinemeyer, 2012; Veronesi et al., 2009) with a lower limit of detection by increasing the number of McMaster slides (Nielsen et al., 2019).

Furthermore, the results from the questionnaire showed that farm owners are over reliant on the use of anthelmintic drugs to control parasite infections. Allowing farms to continue this extentive use of anthelmintica will inevitably result in resistance to all available drug classes. Since resistance to all drug classes have been reported and no other anthelmintic drugs will be introduced, the focus should be on delaying the development of resistance (Sangster, 1999), by allowing some worms to survive and for susceptible worms to contribute to subsequent generations refugia. Increasing the interval between treatments and implementing management measurements could control the infection pressure and also provide beneficial effects on delaying the development of resistant *Parascaris* spp. populations. Furthermore, the efficacy of fenbendazole, by establishing FECRT's, should be monitored annually on farms in the Netherlands.

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References

Clayton, H. M., & Duncan, J. L. (1977). Experimental Parascaris equorum infection of foals. Research in veterinary science, 23(1), 109–114

H.M. Clayton, J.L. Duncan (1978), Clinical signs associated with parascaris equorum infection in worm-free pony foals and yearlings, Veterinary Parasitology, 4(1), 69-78

Nielsen, M.K., L. Mittel, A. Grice, M. Erskine, E. Graves, W. Vaala, R.C. Tully, D.D. French, R. Bowman, R.M. Kaplan. (2016). AAEP Parasite Control Guidelines. American Association of Equine Practitioners, Lexington.

Reinemeyer C. R. (2009). Diagnosis and control of anthelmintic-resistant Parascaris equorum. Parasites & vectors, 2(2), S8.

Reinemeyer, C. R. (2012). Anthelmintic resistance in non-strongylid parasites of horses. Veterinary parasitology, 185(1), 9-15.

Clayton and J.L. Duncan (1979), The migration and development of Parascaris equorum in the horse, International Journal for Parasitology, 9(4), 285-292

Martin, F., Höglund, J., Bergström, T. F., Karlsson Lindsjö, O., & Tydén, E. (2018). Resistance to pyrantel embonate and efficacy of fenbendazole in Parascaris univalens on Swedish stud farms. Veterinary Parasitology, 264, 69–73

Bishop, I Scott, EK Gee, CW Rogers, WE Pomroy, IG Mayhew. (2014) Sub-optimal efficacy of ivermectin against Parascaris equorum in foals on three Thoroughbred stud farms in the Manawatu region of New Zealand. New Zealand Veterinary Journal 62(2), 91-95

Srihakim S, Swerczek TW (1978). Pathologic changes and pathogenesis of Parascaris equorum infection in parasite-free pony foals. American Journal of Veterinary Research, 39(7):1155-1160

Lyons, E.T., and S. C. Tolliver Prevalence of parasite eggs (Strongyloides westeri, Parascaris equorum, and strongyles) and oocysts (Emeria leuckarti) in the feces of Thoroughbred foals on 14 farms in central Kentucky (2003), Springer-Verlag

Armstrong, S. K., Woodgate, R. G., Gough, S., Heller, J., Sangster, N. C., & Hughes, K. J. (2014). The efficacy of ivermectin, pyrantel and fenbendazole against Parascaris equorum infection in foals on farms in Australia. *Veterinary Parasitology*, 205(3-4), 575-580.

Alanazi, A. D., Mukbel, R. M., Alyousif, M. S., AlShehri, Z. S., Alanazi, I. O., & AlMohammed, H. I. (2017). A field study on the anthelmintic resistance of Parascaris spp. in Arab foals in the Riyadh region, Saudi Arabia. Veterinary Quarterly, 37(1), 200-205

Boersema, J. H., Eysker, M., & Nas, J. W. M. (2002). Apparent resistance of Parascaris equorum to macrocylic lactones.

Laugier C, Sevin C, Ménard S, Maillard K. (2012). Prevalence of Parascaris equorum infection in foals on French stud farms and first report of ivermectin-resistant P. equorum populations in France. *Vet Parasitol*, 13;188(1-2):185-9.

Molento M, Antunes J, Bentes RN. (2008) Anthelmintic resistance in Brazilian horses. *Vet Rec.*

Vadlejch J, Petrtýl M, Zaichenko I, Cadková Z, Jankovská I, Langrová I, Moravec M. (2011) Which McMaster egg counting technique is the most reliable? *Parasitol Res*.

Malekpour, S. H., Rakhshandehroo, E., & Yektaseresht, A. (2019). Molecular characterization of β-tubulin gene associated with benzimidazole resistance in larvae of field isolates of Parascaris (Nematoda: Ascarididae). Journal of Parasitic Diseases, 43(4), 672-678.

Kaplan RM, Klei TR, Lyons ET, Lester G, Courtney CH, French DD, Tolliver SC, Vidyashankar AN, Zhao Y. (2004) Prevalence of anthelmintic resistant cyathostomes on horse farms. J *Am Vet Med Assoc.*

Doorn, van D.C.K. , Lems S., Weteling A., Ploeger H. W. , Eysker M. (2007) Utrecht University, Departement of Infectious Diseases, division of Clinical Infectiology. *WAAVP*

M.K. Nielsen. (2016). Evidence-based considerations for control of parascaris spp. infections in horses. *Equine veterinary education. 28(4),* 224-231.

J.V. Fabiani, E.T. Lyons, M.K. Nielsen. (2016). Dynamics of Parascaris and Strongylus spp. parasites in untreated juvenile horses.

Gould, J. C., Rossano, M. G., Lawrence, L. M., Burk, S. V., Ennis, R. B., & Lyons, E. T. (2013). The effects of windrow composting on the viability of Parascaris equorum eggs. Veterinary parasitology, 191(1-2), 73-80.

Shalimov, L. G. (1935). The influence of the ultra-violet light on the development of the eggs of parasitic worms: Parascaris equorum syn. Ascaris megalo-cepahla, Enterobius vermicularis and Strongylus eqtnnus. Transactions on the Dynamics of Development, 10, 447-461

Nicholls, J. M., Clayton, H. M., Pirie, H. M., & Duncan, J. L. (1978). A pathological study of the lungs of foals infected experimentally with Parascaris equorum. Journal of comparative pathology, 88(2), 261-274.

Tydén, E., Dahlberg, J., Karlberg, O., & Höglund, J. (2014). Deep amplicon sequencing of preselected isolates of Parascaris equorum in β-tubulin codons associated with benzimidazole resistance in other nematodes. Parasites & vectors, 7(1), 1-8

Gao, J. F., Zhang, X. X., Wang, X. X., Li, Q., Li, Y., Xu, W. W., Gao, Y., & Wang, C. R. (2019). According to mitochondrial DNA evidence, Parascaris equorum and Parascaris univalens may represent the same species. Journal of helminthology, 93(3), 383–388.

Cribb, N. C., Cote, N. M., Bouré, L. P., & Peregrine, A. S. (2006). Acute small intestinal obstruction associated with Parascaris equorum infection in young horses: 25 cases (1985–2004). New Zealand Veterinary Journal, 54(6), 338-343

Kaplan, R. M. (2004). Drug resistance in nematodes of veterinary importance: a status report. Trends in parasitology, 20(10), 477-481.

Campbell, W. C. (1990). Benzimidazoles: veterinary uses. Parasitology Today, 6(4), 130-133.

Leathwick, D. M., Sauermann, C. W., Geurden, T., & Nielsen, M. K. (2017). Managing anthelmintic resistance in Parascaris spp.: A modelling exercise. Veterinary parasitology, 240, 75-81.

Lindgren, K. Ljungvall, Ö. Nilsson, Ljungström, O. B.-L. Lindahl, C. Höglund, J. (2008). Parascaris equorum in foals and in their environment on a Swedish stud farm, with notes on treatment failure of ivermectin.

Sangster, N. C. (1999). Pharmacology of anthelmintic resistance in cyathostomes: will it occur with the avermectin/milbemycins? Veterinary Parasitology, 85(2-3), 189-204

Drudge JH, Lyons ET, Tolliver SC: Critical tests of the benzimidazole anthelmintic, fenbendazole, in the horse. VM/SAC 70:537-540, 1975.

Scala, A., Tamponi, C., Sanna, G., Predieri, G., Meloni, L., Knoll, S. Sedda, G., Dessi, G., Grazia, M., Cappa, M., Varcasia, A. (2021). Parascaris spp. eggs in horses of Italy: a large-scale epidemiological analysis of the egg excretion and conditioning factors. Parasites Vectors, 14(1), 1756-3305.

College ter Beoordeling van Geneesmiddelen. (approached 2022, January 13). Diergeneesmiddeleninformatiebank. https://www.diergeneesmiddeleninformatiebank.nl/nl

Plumb's Veterinary Drugs. (approached 2021, July 18). Fenbendazole - Plumb's Veterinary Drugs (uu.nl)

Appendix

Attachment 1: 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) made by suspending 1280 grams of sucrose in 1000 ml of warm tap water. The specific gravity of the flotation medium was confirmed by a densimeter.
- 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.

Attachment 2: Questionnaire

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: spoelwormen@gmail.com
Mocht dit voor u lastig zijn of voor als er onduidelijkheden zijn graag contact opnemen met:
Gina Visser: 06-12461292 of Michelle Schellekens: 06-13325959
Onderzoeksbegeleider: Dr. D.C.K. (Deborah) van Doorn (Universiteit Utrecht)
*= Graag doorstrepen wat niet van toepassing is.

Algemene bedrijfsgegevens:
Bedrijfsnaam:
Adres, plaatsnaam:
Contactpersoon (E-mail / Telefoonnummer):
Paardenweegschaal aanwezig op uw bedrijf: ja / nee
Behandelend dierenarts:
Telefoonnummer behandelend dierenarts:
Totaal aanwezige veulens juni / juli / begin augustus 2021:

Graag de gegevens van de veulens hieronder invullen in de tabel. Mocht u een eigen overzicht hebben kunt u die ervoor in de plaats sturen (overzichten etc kunnen naast het emailadres ook gestuurd worden via WhatsApp)

Belangrijk om in te vullen voor het plannen van een afspraak:
- Geboortedata veulens (minimaal 10 wk tot maximaal 5 mnd leeftijd)
- Datum laatste ontworming van de veulens (minimaal 4wk, liefst 6wk of langer geen ontworming voor het 1e bezoek).

Naam veulen	Geboortedatum	Geslacht (H / M)	Datum ontworming, reeds gegeven + naam ontwormingsmiddel	Geplande toekomstige ontwormingsdatum + naam ontwormingsmiddel

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? Is dit schema hetzelfde schema als in 2019 en 2020, zo niet hoe zag het schema er toen uit?

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?

12.b Wat zijn de verschillende leeftijden van de gehuisveste veulens?

12.c. 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. Wordt de weide of paddock *wel / niet* gesleept gedurende het verblijf van de veulens in het weiland?

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

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

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

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

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

-

Attachment 3: Table of all foals and farms included in this study

Bedijf	Veulen	Leeftijd	EPG pre-treatment	Geslacht	Groep F/P
1	1	83	0	1	0
1	2	82	0	0	0
1	3	74	0	1	0
2	4	131	0	0	0
2	5	157	125	1	1
2	6	148	1675	1	1
2	7	89	0	1	0
2	8	73	0	1	0
3	9	131	25	1	0
3	10	138	0	1	0
3	11	76	0	0	0
3	12	113	2675	0	2
3	13	68	0	1	0
3	14	93	0	0	0
3	15	97	400	0	2
3	16	124	0	1	0
3	17	81	0	0	0
4	18	107	400	0	2
4	19	108	175	1	1
4	20	97	0	1	0
4	21	108	0	0	0
4	22	88	0	0	0
5	23	113	0	1	0
5	24	110	0	1	0
6	25	99	3325	1	2
6	26	81	200	0	1
6	27	79	225	1	2
6	28	115	100	0	2
6	29	99	0	1	0
6	30	86	0	1	0
6	31	138	0	1	0
6	32	119	0	1	0
6	33	103	2475	0	2
6	34	113	5100	0	2
6	35	114	3700	0	2
7	36	148	1925	1	1
7	37	122	2250	0	1
7	38	136	2500	0	2
7	39	138	1625	0	2
7	40	109	1750	0	2
7	41	90	550	0	2
7	42	120	975	0	2

8	43	134	0	0	0
8	44	127	0	1	0
8	45	115	0	1	0
8	46	104	0	0	0
8	47	72	0	0	0
9	48	75	0	1	0
9	49	84	0	1	0
9	50	79	0	1	0
9	51	88	0	0	0
9	52	100	0	0	0
9	53	100	0	0	0
9	54	98	0	1	0
9	55	96	2700	1	2
9	56	96	0	0	0
9	57	93	350	0	1
9	58	76	175	1	1
9	59	74	0	1	0
9	60	70	0	0	0
9	61	70	0	0	0
10	62	70	25	0	0
10	63	96	1525	1	2
10	64	102	1650	0	2
10	65	87	175	0	2
10	66	104	2150	1	2
10	67	79	0	1	0
10	68	75	0	1	0
10	69	100	350	1	2
10	70	86	0	1	0
10	71	76	0	0	0
10	72	76	0	0	0
11	73	121	100	1	1
11	74	155	0	0	0
11	75	113	225	0	2
11	76	110	800	0	2
11	77	110	600	1	2
11	78	111	0	1	0
11	79	107	275	1	1
11	80	110	1625	0	2
11	81	102	0	0	0
11	82	86	0	1	0
11	83	90	0	1	0
11	84	90	1000	1	2
11	85	76	0	1	0
11	86	77	0	1	0
11	87	89	0	0	0
11	88	74	0	0	0

12	89	77	0	0	0
12	90	72	0	1	0
13	91	102	100	1	2
13	92	82	0	0	0
13	93	138	0	0	0
14	94	136	25	0	0
14	95	137	600	1	2
14	96	119	375	1	1
14	97	97	0	1	0
14	98	72	0	1	0
15	99	125	775	0	2
15	100	125	900	0	2
15	101	99	675	1	2
15	102	96	200	1	2
15	103	81	0	1	0
15	104	79	0	1	0
15	105	91	150	0	2
16	106	105	0	0	0
16	107	124	0	1	0
16	108	113	0	1	0
16	109	83	0	1	0
16	110	82	0	1	0
16	111	78	0	1	0
16	112	94	0	0	0
16	113	83	0	1	0
17	114	100	0	0	0
17	115	92	0	1	0
17	116	92	225	1	1
17	117	91	50	0	0
17	118	82	0	0	0
17	119	82	0	1	0
17	120	80	0	1	0
17	121	76	0	1	0
18	122	94	0	1	0
18	123	90	0	0	0
18	124	101	0	0	0
19	125	96	0	0	0
19	126	83	0	1	0
19	127	99	0	1	0
19	128	129	2775	0	2
19	129	125	6775	0	1
19	130	122	4275	0	2
19	131	112	7500	0	1
19	132	102	1550	0	2
19	133	88	500	0	1
19	134	74	0	0	0

19	135	85	100	0	1
19	136	138	925	0	1
19	137	132	275	0	2
20	138	74	25	0	0
20	139	105	0	1	0
20	140	100	450	1	2
20	141	122	4400	1	2
20	142	119	575	1	1
20	143	84	0	0	0
20	144	90	50	1	0
20	145	71	0	0	0
20	146	100	800	0	2
20	147	96	5625	1	1
20	148	94	1675	0	1
20	149	96	1750	0	1
20	150	71	0	1	0
20	151	70	0	1	0
20	152	73	225	1	1
20	153	92	0	0	0
21	154	94	0	0	0
21	155	101	0	0	0
21	156	132	0	1	0
21	157	96	0	1	0
22	158	70	0	1	0
22	159	71	0	0	0
22	160	90	0	1	0
22	161	101	0	0	0
22	162	112	0	1	0
22	163	96	0	1	0
22	164	94	0	1	0
22	165	80	0	1	0
22	166	98	0	0	0
22	167	133	875	0	2
22	168	126	925	0	1
22	169	151	675	1	2
22	170	82	25	0	0
22	171	133	2725	0	1
22	172	137	700	1	2
23	173	85	0	0	0
23	174	73	0	0	0
23	175	75	0	0	0
23	176	106	0	1	0
23	177	79	0	1	0
23	178	79	0	0	0
23	179	76	0	0	0
23	180	140	0	1	0

23	181	94	0	1	0
23	182	99	0	0	0
24	183	109	2475	1	1
24	184	79	0	0	0
24	185	167	0	1	0
24	186	67	0	0	0
24	187	86	0	1	0
24	188	77	0	1	0
25	189	139	1375	0	1
25	190	162	2775	0	1
25	191	74	0	1	0
25	192	102	0	0	0
25	193	135	525	0	1
25	194	81	0	0	0
25	195	102	0	0	0
25	196	140	0	0	0
25	197	142	500	0	1
25	198	111	0	0	0
26	199	148	1000	0	2
26	200	112	2525	1	1
26	201	111	0	0	0
26	202	80	4425	0	1
26	203	74	0	0	0
26	204	91	0	0	0
26	205	85	0	0	0
26	206	85	0	0	0
26	207	80	0	1	0
26	208	68	0	1	0
27	209	78	0	0	0
27	210	90	200	1	1
27	211	116	200	1	2
27	212	125	0	1	0
28	213	126	5400	1	2
28	214	117	200	0	1
28	215	87	0	0	0
28	216	69	0	1	0
28	217	77	0	1	0
29	218	77	0	0	0
29	219	86	0	0	0
29	220	85	0	1	0
29	221	74	0	1	0
30	222	138	875	1	1
30	223	135	125	0	2
30	224	115	5175	0	1
30	225	113	1125	1	2
30	226	89	0	0	0

30	227	84	0	1	0
30	228	77	0	0	0
31	229	75	0	1	0
31	230	72	0	0	0
31	231	74	0	1	0
31	232	92	0	1	0
31	233	94	0	1	0
31	234	98	0	1	0
31	235	73	0	1	0
31	236	106	0	1	0
31	237	104	0	0	0
32	238	152	0	0	0
32	239	149	0	0	0
32	240	136	0	1	0
32	241	135	0	1	0
32	242	121	0	0	0
32	243	113	0	0	0
32	244	105	0	0	0
32	245	92	0	0	0
32	246	88	0	0	0
32	247	86	0	0	0
32	248	85	0	0	0
32	249	75	0	0	0
32	250	71	0	0	0
33	251	87	1875	1	1
33	252	71	0	0	0
33	253	113	2450	1	1
33	254	110	0	1	0
33	255	117	4750	1	1
33	256	103	1975	1	1
33	257	100	0	0	0
33	258	97	0	0	0
34	259	134	275	1	1
34	260	125	0	1	0
34	261	134	0	0	0
34	262	175	225	0	2
34	263	108	0	0	0
34	264	56	0	1	0
34	265	111	0	1	0
34	266	106	0	1	0
34	267	149	0	0	0
34	268	117	0	1	0
34	269	148	9325	0	2
34	270	147	0	0	0
34	271	146	125	0	1
35	272	72	0	1	0

35	273	158	3450	1	1
35	274	164	825	0	1
35	275	166	0	1	0
35	276	96	6400	1	1
35	277	128	225	0	1
35	278	169	0	1	0
35	279	131	0	0	0
35	280	119	375	1	1
36	281	120	1475	1	2
36	282	104	300	1	2
36	283	97	100	1	1
36	284	107	1825	1	2
36	285	92	175	0	2
36	286	105	8350	0	2
36	287	94	1150	0	1
36	288	111	1400	0	2
36	289	97	1450	1	1
36	290	114	1425	1	1
36	291	128	200	0	1
36	292	92	1525	0	1