# Risk factors for equine intestinal parasite infections and reduced efficacy of pyrantel embonate against *Parascaris* sp.

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## Abstract

Gastrointestinal parasites, *Parascaris* sp. and strongyles, are common in young horses worldwide and control of these parasites is challenged by increasing anthelmintic resistance. Our aim was to identify risk factors for these infections as well as to assess the efficacy of fenbendazole (dose 7.5mg/kg) and pyrantel embonate (dose 19 mg/kg) against *Parascaris* sp. We also evaluated association between owner observed symptoms and patent infections with these parasites. Fecal samples were collected from 367 young horses in Finland and a questionnaire study was conducted. Fecal egg counts were performed by Mini-FLOTAC® method.

Univariable logistic regression models using patent infection status (Yes/No), separately for *Parascaris* sp. and strongyle infections as an outcome were run initially to screen potential risk factors collected by the questionnaire. After the initial screening, multiple logistic regression models were constructed and run to account for correlated data structure, risk factors and potential confounders simultaneously.

Two significant risk factors for a patent *Parascaris* sp. infection were found: breeding farm size (p=0.028) and frequency of horse movements (p=0.010). Horses originating from large breeding farms were more likely (OR=2.47, 95% confidence interval (CI) 1.10 -5.51) to shed *Parascaris* sp. eggs upon relocation to training stables compared to horses originating from small breeding farms. Horses living in farms with frequent horse movements to other premises had higher odds (OR=3.56, 95% CI: 1.35 -9.39) of a patent *Parascaris* sp. infection compared to farms with less frequent horse movements.

Risk factors for patent strongyle infection included age ($p<0.001$) and season ($p=0.017$). Horses were less likely (OR=0.27, 95% CI: 0.10 - 0.66) to shed strongylid eggs during the spring compared to the winter.

Horses excreting over 200 ascarid eggs per gram were included in the anthelmintic efficacy trial. A mean FECR less than 90% was interpreted as presence of anthelmintic resistance. The mean FECR was 98.5% (95% CI: 95.8 - 100) and 68.0% (95% CI: 52.7 - 83.3) in the fenbendazole (n=31) and pyrantel (n=26) treatment groups, respectively.

In conclusion, we identified two new risk factors for patent *Parascaris* sp. infection; breeding farm size and frequency of horse movements. Reduced efficacy of pyrantel against *Parascaris* sp. was observed for the second time in Europe. A relatively high *Parascaris* sp. prevalence in yearlings (34%) and two-year-olds (20%) was observed, which has not been reported earlier. An association between symptoms and a patent *Parascaris* sp. infection was observed in foals.

**Keywords: Anthelmintic resistance, *Parascaris*, strongyle, risk factor, pyrantel, efficacy, egg count**

**Highlights**

- High horse movement frequency is a risk factor for *Parascaris* sp. egg shedding
- Horses originating from large breeding farms are more likely to shed ascarid eggs
- Reduced efficacy of pyrantel against *Parascaris* sp. was observed
- Proportion of horses shedding strongylid eggs was lowest during the spring time

## 1. Introduction

*Parascaris* sp. and strongyle parasites infect horses worldwide. *Parascaris* sp. infections are mainly seen in foals as horses develop age-related immunity against this parasite, by six months of age marked resistance has usually developed (Clayton and Duncan, 1979a). Foals become infected by ingesting infective eggs and the life cycle of the hatched larvae involves hepatotracheal migration before reaching patency about 90-110 days post infection (Clayton and Duncan, 1979b). Ascarid fecal egg counts (FEC) peak at about four months of age (Bellaw et al., 2016; Donoghue et al., 2015; Fabiani et al., 2016). Two studies conducted on untreated foals have also demonstrated that following a clear decline in egg counts after the first peak there is another smaller peak in ascarid egg counts between eight and ten months of age (Donoghue et al., 2015; Fabiani et al., 2016). Strongyle parasites are common in all age groups but FECs are higher in young horses compared to horses over four years old (Kornaś et al., 2010; Kuzmina et al., 2016; Nielsen et al., 2018; Scheuerle et al., 2016).

*Parascaris* sp. infection causes respiratory symptoms, reduced weight gain and lethargy (Clayton and Duncan, 1978). Severe cases may lead to death resulting from intestinal impaction or rupture (Nielsen, 2016). Small strongyles may cause weight loss, colic and occasionally severe diarrhea called larval cyathostomosis (Love et al., 1999; Peregrine et al., 2005). However, often these parasitic infections are asymptomatic and information about the clinical signs associated with infections is lacking.

Anthelmintic resistance among equine internal parasites is a well-recognized and fast spreading problem worldwide (Matthews, 2014; Peregrine et al., 2014; von Samson-Himmelstjerna, 2012). There are three anthelmintic drug classes commonly available for *Parascaris* sp. and strongyle control in horses; the benzimidazoles, the tetrahydropyrimidine pyrantel and the macrocyclic lactones (ivermectin and moxidectin). For several decades, horses have been regularly treated with these anthelmintics, often many times per year (Comer et al., 2006; Lloyd et al., 2000; Matthee et al., 2002; Mellor et al., 2001; O'Meara and Mulcahy, 2002; Osterman Lind et al., 2007; Robert et al., 2015). This overuse of dewormers has most likely enhanced the development of anthelmintic resistance (Kaplan, 2004). Fenbendazole and pyrantel embonate are commonly used to control *Parascaris* sp. infections in foals. Anthelmintic treatments for foals are typically applied in regular intervals without preceding diagnosis, and the efficacy of the selected anthelmintic is rarely assessed (Becher et al., 2018; Bolwell et al., 2015; Relf et al., 2014). *Parascaris* sp. resistance to macrocyclic lactones was first reported in 2002 in the Netherlands (Boersema et al., 2002) and now appears widespread (Alanazi et al., 2017; Armstrong et al., 2014; Lyons et al., 2008; Näreaho et al., 2011). Resistance to pyrantel (PYR) in *Parascaris* sp. populations is less common, but has been described in the USA (Lyons et al., 2011), Australia (Armstrong et al., 2014) and recently, for the first time in Europe, in Sweden (Martin et al., 2018). Fenbendazole (FBZ) resistance among *Parascaris* sp. is reported from Australia (Armstrong et al., 2014), Saudi Arabia (Alanazi et al., 2017) and the United States (Lyons et al., 2011).

Reported risk factors for *Parascaris* sp. infection include fertilizing pastures with horse manure and keeping foals on deep litter instead of regularly cleaned stables (Aromaa et al., 2018; Fritzen et al., 2010). Several management factors are shown to influence strongyle egg shedding such as time since last deworming (Fritzen et al., 2010; Levy et al., 2015), cleaning pastures (Tzelos et al., 2017), daily access to pasture within 30 days of sampling (Nielsen et al., 2018) and group rotation on grazing (Relf et al., 2013).

The aims of this study were to 1) identify risk factors for *Parascaris* sp. and strongyle infections, 2) evaluate association between clinical signs reported by owners and patent infections with these parasites, and 3) evaluate FBZ and PYR efficacy against *Parascaris* sp. on horse farms in Finland.

## 2. Materials and methods

### 2.1. Study population

An open invitation for horse owners to participate in the study was disseminated via Finnish horse sport magazines, Facebook groups for Finnish horse owners and the University of Helsinki web site from April 2017 until March 2018. Owners were asked to fill in a questionnaire and to submit fecal samples from their horses meeting the following inclusion criteria; healthy, minimum of four months but less than three years of age and boarded in Finland. Fecal samples were collected between April 2017 and May 2018. The participants were instructed to wait a minimum of two months after the latest anthelmintic treatment before sample collection.

To analyze the collected data, horses were grouped into three categories; under one-year-olds, yearlings and two-year-olds. However, in the multiple regression model, the age of the horse was presented in months.

All horses were privately owned and the owners volunteered to the study by signing an informed consent. Horses naturally infected by *Parascaris* sp. with ascarid eggs per gram (EPG) counts over 200 were included in the anthelmintic efficacy trial.

### 2.2. Questionnaire study

An online questionnaire survey was carried out to identify risk factors for *Parascaris* sp. and strongyle infections as well as clinical signs in the study population observed by the horse owners. A few horse owners and trainers preferred to fill out a paper copy of the online questionnaire, which was allowed.

Questionnaires collected information on both horse and farm levels. A separate questionnaire was filled out for each horse. Questions covered the identity information of the horse, anthelmintic treatment frequency within the last year as well as anthelmintic product used, the latest deworming date, origin of the horse and stable management practices. Participants were also asked if they had observed their horse(s) exhibiting any of the following signs within two months prior to sampling; cough, nasal discharge, retarded growth, diarrhea, bad hair coat or any other sign. At the farm level, the questions covered parasite control strategies, general management practices, number of horses on the farm and horse movements. The national equine register database (open access Heppa-software, Finnish Trotting and Breeding Association) was used to fill in some missing data such as birth dates, sex and breed of the horses.

The questionnaire translated from the original language (Finnish) is provided as a supplementary file.

### 2.3. Fecal sample collection and egg counts

Most fecal samples were collected from fresh droppings by the farm manager and a few were rectally collected by a veterinarian. Samples were packed into tightly sealed plastic bags, kept refrigerated when possible, transported to the lab and analyzed within three days from collection.

Fecal egg counts (FEC) were performed by Mini-FLOTAC® (University of Naples Federico II, Naples, Italy) technique with a minimum detection limit of five eggs per gram (Cringoli et al., 2017). Five grams of feces were weighted and mixed with 45 ml of saturated $MgSO_4$ solution having specific gravity of 1.25. Parasite eggs were microscopically identified and counted with 100 x magnification.

### 2.4. Anthelmintic efficacy trial

Efficacy of two orally administered anthelmintics, FBZ (Axilur® Vet 18,75%, MSD Animal Health) at single dose of 7.5mg/kg and PYR (Strongid®-P Vet 44%, Zoetis) at single dose of 19 mg/kg (equals 6.6 mg base/kg) were tested against *Parascaris* sp. in horses between 4 and 23 months old. As horse farms are small in Finland, limiting the opportunity to recruit multiple horses from each farm to the trial, we recruited all horses meeting the inclusion criterion of FEC over 200 ascarid EPG, even if it was only a single horse at the given farm.

Anthelmintic efficacy was evaluated by Fecal Egg Count Reduction Test (FECRT), where FEC calculated 4 to 0 days prior to the anthelmintic administration was compared to FEC calculated 10 to 17 days post administration for each horse individually. Individual horses were grouped to FBZ, PYR and control group and mean fecal egg count reductions (FECR) per each group were calculated. A mean fecal egg count reduction (FECR) less than 90% in the two treatment groups was interpreted as presence of anthelmintic resistance (Morris et al., 2019), and for each group 95% confidence intervals of the means were calculated.

On each farm, the recruited horses were randomly allocated to the FBZ treatment group, the PYR treatment group or the control group. A control group is important in ascarid efficacy studies because of a strong confounding effect of an age-dependent immunity (Morris et al., 2019). Horses from the same farm were ranked by age from the youngest to the oldest and blocked to groups of three before randomization. In farms with only one or two horses to participate, the randomization was still done in to one of the three treatment groups. A random number generator was used to assign horses. However, to minimize the risk of adverse health effects, such as intestinal obstruction, three foals were assigned to the FBZ group without randomization due to high ascarid FECs and poor body condition. In general, horses under 12 months of age were not assigned to the control group since all the horses were privately owned and adverse health effects were to be avoided. Two horses were allocated to the control group without randomization since a veterinarian was not present to administer a treatment at the time of the first sampling, but the owner was willing to participate in the study. After the initial randomization six out of seven horses in the control group still had ascarid FEC over 200 EPG at the end of the study period and were then assigned to one of the treatment groups. Also three horses originally allocated to PYR group still had FEC over 200 EPG for ascarid eggs after finishing the study period and were then assigned to FBZ group. Additionally, one foal was first allocated to FBZ group, and then two months later met the inclusion criterion again, and was allocated to PYR group.

To estimate the correct anthelmintic dose, every horse was measured by a commercial girth tape (Boehringer Ingelheim Vetmedica, Vetcare, Salo, Finland), with 10% added to the weight estimation. The given anthelmintic dose was then rounded up to the next 50 kg based on the scale on the application syringe. All horses were medicated by a veterinarian or one of the authors.

### 2.5. Statistical Analysis

Stata software, version MP 15.1, (*StataCorp LCC, 2018)* was used for statistical analysis. P-values < 0.05 were considered to indicate statistically significant results.

Initially, association between each potential risk factor (all categorical) collected by the questionnaire and a patent *Parascaris* sp. infection (Y/N) as an outcome was evaluated by running univariable logistic regression models, accounting for farm effect. A similar univariable analysis was conducted to assess a possible association of patent strongyle infection and covariates collected by the questionnaire.

The horse-level variables considered in the analysis included age, breed, sex, and origin of the horse as well as sampling season, the latest anthelmintic product used, size of the breeding farm (*i.e*., number of foals born per year at the farm of origin), bedding material, frequency of manure removal from the horse shed, use of deep litter, frequency of deep litter change, accessibility to the summer pasture, living conditions and group size in open housing. To analyze the data, horses were grouped into three categories; under one-year-olds, yearlings and two-year-olds. Additionally, bedding material was divided into four categories; 1) straw or straw pellet, 2) peat, 3) straw combined with peat and 4) wood shavings or pellets. Accessibility to the summer pasture had three categories; 1) the horse had access to pasture, 2) the horse did not have pasture access, and 3) it was not known if the horse had access to pasture. Living conditions (at the time of sampling) included three categories; 1) individual box stall, 2) pasture, and 3) freestall housing. The group size in freestall housing was divided to three categories; 1) less than 5 horses, 2) 5 to 12 horses and 3) more than 12 horses. To observe the seasonality in egg shedding, three categories were created to describe different seasons in Finland. Pasture season included samples taken from June to September, winter included samples collected from October to March and spring included samples taken in April and May.

The farm-level variables included the use of FECs, anthelmintic treatment frequencies by different age groups, farm size, number of horses under two-years of age in the farm, availability of pastures, pasture cleaning, manure use for fertilizing pastures, horse movements, introduction frequency of new horses to the farm, and presence of imported horses. Use of FECs was divided to two categories; FECs had been previously used on the farm, or FECs had not been used previously. Yearly anthelmintic treatment frequencies for different age groups were divided to three categories; 1) less than five treatments, 2) five to six treatments and 3) more than six treatments. Number of horses younger than two years at the farm was divided into three categories 1) 1 to 3, 2) 4 to 9, and 3) 10 to 28 horses. Availability of pastures was categorized as pastures available at the farm and pastures not available. Introduction frequency of new horses to the farm and horse movement frequency to other premises was categorized to frequent and rare. ‘Frequent’ meant weekly or monthly actions and ‘rare’ meant movements occurring yearly or more rarely. The ‘imported horses’ variable was categorized into farms with imported horses and farms without imported horses.

The association between patent *Parascaris* sp infection and symptoms (cough, nasal discharge, retarded growth, diarrhea or bad hair coat) observed by the participating horse owners within two months prior to sampling was assessed by logistic regression accounting for farm effect, using symptoms as an outcome. A similar analysis was run to assess the association between symptoms and patent strongyles infection. Occurrence of owner observed symptoms was coded Y/N for statistical analysis. If the owner had observed any of the listed symptoms within the two last months 'Y' was recorded, otherwise 'N' was recorded. In addition, the association between owner observed symptoms and patent parasitic infection was assessed separately in each age category.

Results of the efficacy trial were analyzed by calculating the mean FECR per group (FBZ, PYR and control). If the individual post treatment FEC was higher than the pretreatment FEC, the reduction was interpreted as zero when calculating the group mean FECR.

### 2.5.1. Multiple logistic regression

Using the patent infection status (Y/N) as the outcome, all variables that passed the initial screening in the univariable logistic regression with $p<0.2$ were included in developing a full model. Age and breed were included to the model to account for their confounding effect. The model was built by forward selection. We selected the most significant variables shown by univariable screening and added one variable at the time to the model as long as all variables in the final model remained significantly associated with the outcome. Correlated data structure (observations from horses within a same farm) was accounted for in the modelling by including farm as a random effect.

Due to a small sample size, no interactions were evaluated.

## Results

Fecal samples were analyzed from 367 horses representing 95 farms. Most horses (n=303) were Standardbreds, and the remainder included Finnish cold blooded horses (n=31), Finnish warmbloods (n=23), ponies (n=5), Hanoverian horses (n=2), mixed breeds (n=2) and an Icelandic horse (n=1). Standardbreds represented three age categories; under one-year-olds (n=87), yearlings (n=161) and two-year-olds (n=55).

Other breeds represented only two age categories; under one-year-olds (n=55) and yearlings (n=9). Majority of the fecal samples were collected during the winter (n=239), but some were obtained also during the spring (n=57) and pasture season (n=71).

Farm sizes varied from small stables with only two horses to a large stud farm with up to 120 horses. Mean and median number of horses per farm were 16 and 10, respectively. Nearly half (48%) of the farms had less than 10 horses.  Mean and median number of sampled horses per farm were 4 and 2, respectively. Maximum number of sampled horses per farm was 33, and 44% of farms had only one horse participating in the study.

Questionnaire responses covered 89% (n=327) of sampled horses. All the respondents did not reply to every given question. They might not have known the answer for a specific question or the question was not applicable for them.

According to the questionnaire responses, young horses were given anthelmintics more frequently than older horses. The mean yearly treatment frequencies per age group are presented in Table 1.

*Parascaris* sp. eggs were found in 47% (45/95) and strongylid eggs in 87% (83/95) of all farms. Patent *Parascaris* sp. infection prevalence was 50% in horses younger than one year, 34% in yearlings, and 20% in two-year-olds. Strongylid eggs were excreted by 63% of horses under one year, 84% of yearlings and 67% of two-year-olds. Mean *Parascaris* sp. and strongylid fecal egg counts per age group are presented in Figure 1. Tapeworm eggs were found in 5% (n=17) of all samples and *Eimeria leuckarti* oocysts were detected in 1% (n=4) of analyzed samples. *Strongyloides westeri* eggs were found in one foal.

At least one symptom was observed by the study respondents in 17% of the horses in the youngest and the oldest age group, and in 34% of the yearlings. There was no association between strongylid egg shedding and owner observed symptoms. A significant association of owner reported symptoms and patent *Parascaris* sp. infection was only observed in the youngest age group (p=0.019). In this age group, 84% of those having symptoms were shedding ascarid eggs. A majority of the egg shedders in the youngest age group was, however, asymptomatic as only 31% of the *Parascaris* sp. shedders and 22% of the strongyle shedders were observed with symptoms.

Risk factors, and related p-values for patent *Parascaris* sp. and strongyle infection, identified by running univariable logistic regression accounting for farm effect are presented in Table 2. Season was significantly associated with patent strongyle infections (p=0.017) but not with *Parascaris* sp. egg shedding (p=0.496).

Results from the multiple logistic regression model indicated that age (p=0.020), breed (p=0.009), breeding farm size (p=0.028) and frequency of horse movements (p=0.010) were significantly associated with a patent *Parascaris* sp. infection. Horses originating from large breeding farms, where more than four foals are borne per year, had higher odds of a patent *Parascaris* sp. infection compared to horses bred by small breeding farms. Horses boarded on farms with frequent horse movements, having their horses visiting other premises weekly or monthly, had higher odds of a patent *Parascaris* sp. infection compared to farms with less frequent horse movements. Increasing age significantly decreased the odds of a patent *Parascaris* sp. infection. Standardbreds had four times higher odds of a patent *Parascaris* sp. infection compared to other breeds enrolled in the study. Results from the final model are presented in Table 3.

Significant risk factors for patent strongyle infection identified in the univariable analyses by logistic regression accounting for farm effect included age (p<0.001) and season (p=0.017). Horses were less likely (OR= 0.27, 95% CI: 0.10 - 0.66) to shed strongylid eggs during the spring months compared to the winter months. On the other hand, there was no difference in strongylid egg shedding between pasture season and winter. The multiple logistic regression model did not identify significant risk factors for patent strongyle infection.

In the anthelmintic efficacy trial samples were analyzed from 20 farms having between 1 and 12 horses participating in the study. The mean FECR for ascarid eggs was 98.5% (95% CI: 95.8 - 100) in the FBZ group (n=31) and the treatment was considered efficacious. The mean FECR for ascarid eggs in the PYR group (n=26) was 68.0% (95% CI: 52.7 - 83.3) which indicated resistance. In the control group (n=7) the

FECR for ascarid eggs was 20.9% (95% CI: 0 - 54.9). Individual FECs for the horses participating in the efficacy trial are presented in Figure 2.

**Discussion**

This study identified two significant risk factors for a patent *Parascaris* sp. infection; breeding farm size and a frequency of horse movements. To our knowledge, breeding farm size or horse movements have not been reported earlier as risk factors for *Parascaris* sp. infection. Horses originating from large breeding farms were more likely to shed *Parascaris* sp. eggs also after moving to training stables compared to horses originating from small breeding farms. Also, horses living on farms with frequent horse movements to and from other premises had higher odds of a patent *Parascaris* sp. infection compared to farms with less frequent horse movements. Both of these findings could be explained by higher infection pressure and higher likelihood of anthelmintic resistance in large breeding farms and farms with frequent horse movements. In addition, horses kept in operations with frequent horse movements may be more stressed than horses kept in operations with more stable social groups. Stress may cause immunosuppression and predispose horses to parasitic infections (Saville et al., 2001).

Seasonal differences in *Parascaris* sp. and strongyle infections have been studied by several authors (Bucknell et al., 1995; Fabiani et al., 2016; Nielsen et al., 2018; Ogbourne, 1975; Poynter, 1954; Rehbein et al., 2013; Relf et al., 2013). A study conducted in UK in 1950s showed a decrease in strongylid egg counts during the winter and an increase during the summer (Poynter, 1954). This phenomenon is explained by the seasonal variation of small strongyle infections, more individuals are maturing in summer and autumn compared to winter and spring (Ogbourne, 1975). A recent study in the United States also observed that horses in the South-East region of the country had higher odds of strongylid egg presence during the summer and autumn than in winter and spring (Nielsen et al., 2018). In contrast, other studies have not observed any seasonal variation in strongylid egg shedding (Rehbein et al., 2013; Traversa et al., 2010). In our data, horses were less likely to shed strongylid eggs during the spring months compared to the winter and there were no difference in egg shedding between pasture season and winter. A recent study conducted in the UK had similar observations; there was increased likelihood of higher levels of strongylid egg shedding during winter compared to spring (Relf et al., 2013). Our study did not show any seasonal differences for *Parascaris* sp. egg shedding which has also been concluded earlier (Rehbein et al., 2013). This finding agrees with observations that ascarid egg shedding patterns are primarily driven by age, and not seasonality (Donoghue et al., 2015; Fabiani et al., 2016).

In our study, the highest prevalence of *Parascaris* sp. egg shedding (50%) was observed in horses aged less than one year while the highest prevalence of strongylid egg shedding (84%) was observed in yearlings. These findings are in line with earlier studies (Fritzen et al., 2010; Hinney et al., 2011; Kornaś et al., 2010; Relf et al., 2013). In contrast, a study conducted in Sweden observed a peak in the strongylid egg shedding later, at the age of two and three years (Osterman Lind et al., 1999). Again, *Parascaris* sp. egg shedding appears tightly regulated by age whereas strongylid egg shedding is also influenced by season. It is well known that climatic conditions have a great impact on free living strongyle stages and strongyle pasture infectivity (Nielsen et al., 2007). Conversely, very little is known about environmental factors impacting *Parascaris* sp. infection pressure levels.

In our study, the *Parascaris* sp. egg shedding prevalence was 34% among yearlings and 20% among two-year-olds. These prevalences are higher than reported in several other studies. A study conducted in Germany found *P. equorum* eggs in only 2.87% of the samples collected from horses aged 1-3 years old (Fritzen et al., 2010), and a study on UK studs observed 4% prevalence of *P. equorum* shedders in yearling Thoroughbreds (Relf et al., 2013). A majority of the horses (83%) in our study were Standardbreds and it is possible that this breed is more susceptible to gastrointestinal parasite infections than other breeds. However, it should be kept in mind that Standardbreds are put in training already as yearlings, which is earlier than horses in many sport horse breeds. The early training may be a stress factor for horses and depress their immunity against parasitic infections.

We found a significant association of owner reported symptoms and patent *Parascaris* sp. infection in foals but this was not observed in the older age groups. Symptoms were recorded based on horse owner observations and therefore, the results may be biased depending on the owners' ability to recognize clinical symptoms in their animals. However, this finding emphasizes the importance of controlling *Parascaris* sp. in foals, as this is the age group where the clinical signs associated with the infection are observed by the owners.

Sex has been associated with both strongyle and *Parascaris* sp. infections. Several studies have shown females being infected more often or having higher EPG or worm counts than males (Fabiani et al., 2016; Francisco et al., 2009; Hinney et al., 2011; Nielsen et al., 2018). In contrast, there are also studies showing that geldings are more often infected with strongyles than intact males or females (Bucknell et al., 1995; Kornaś et al., 2015). In our study, sex was not associated with patent *Parascaris* sp. or strongyle infection. Our study population consisted of young horses that were managed in a similar way independent of their sex. Sex differences observed in earlier studies, to some extent, may be explained by different management strategies for males and females.

Fertilizing pastures with horse manure is a shown risk factor for *P. equorum* infection (Fritzen et al., 2010). However, this was not observed in our study.  Earlier studies have observed that good stable hygiene obtained by daily cleaning of stalls is associated with reduced *Parascaris* sp. infections (Aromaa et al., 2018; Fritzen et al., 2010). In our study, daily manure removal was associated with lower prevalence of *Parascaris* sp. egg shedders, however, it did not stay in the final model with the other variables included in it.

We recruited horses from farms of all sizes and collected samples of gastrointestinal parasite populations in different farm environments. Although the number of sampled horses per farm is low, the study provided insights of the anthelmintic resistance situation in *Parascaris* sp. in Finland. Overall, FBZ still appears efficacious, while PYR is losing efficacy. This is the second time pyrantel resistant *Parascaris* sp. has been reported in Europe and our results are very similar to the recently published study from Sweden (Martin et al., 2018).

In conclusion, this study brought new information of *Parascaris* sp. and strongyle epidemiology in young horses. Two new risk factors for patent *Parascaris* sp. infection were identified. Horses boarded in farms with frequent horse movements are more likely to shed *Parascaris* sp. eggs than horses kept in farms with less horse movements, and horses originating from large breeding farms are more likely to shed *Parascaris* sp. eggs compared to horses coming from smaller breeding farms. Horses were less likely to shed strongylid eggs during the spring compared to the winter. Failed efficacy of pyrantel against *Parascaris* sp. was observed for the second time in Europe. Our study demonstrated a relatively high *Parascaris* sp. prevalence in yearlings and two-year-olds, which has not been reported earlier. This was the first attempt to capture the association of owner observed clinical symptoms and *Parascaris* sp. and strongyle infection. A significant association of recorded symptoms and a patent *Parascaris* sp. infection was observed in horses aged less than one year.

**Conflict of interest statement**

The authors declare no conflicts of interest.

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**Fig 1**. Mean *Parascaris* sp. (black columns) and strongylid (white columns) fecal egg counts per age group presented in eggs per gram (EPG) at the first sampling

**Fig 2**. Individual pre (black columns) and post treatment (grey columns) fecal ascarid egg counts in the fenbendazole treatment group (A), pyrantel embonate treatment group (B) and untreated control group (C).

Table

**Table 1** Annual anthelmintic treatment frequencies in different age groups in Finnish horse farms (n=95) according to the questionnaire study.

| Age | Average /year | Range |
|---|---|---|
| Under one-year-olds | 5.4 | (0-12) |
| Yearlings | 4.3 | (2-12) |
| Two-year-olds | 3.9 | (1-9) |
| Three-year-olds | 3.3 | (1-6) |
| Adults | 2.6 | (1-4) |

Table

**Table 2** Factors associated to *Parascaris* sp. and/or strongylid egg shedding in initial screening tested by running univariable logistic regression, accounting for farm effect.

| Variable | Horses shedding *Parascaris sp.* eggs (%) | p-value | Horses shedding strongylid eggs (%) | p-value |
|---|---|---|---|---|
| Age[a] | | 0.331 | | <0.001 |
| < 1yo (n=142) | 50 | | 63 | |
| yearlings (n=171) | 34 | | 84 | |
| 2 yo (n=54) | 20 | | 65 | |
| Breed | | 0.219 | | 0.161 |
| standardbred trotter (n=303) | 40 | | 77 | |
| other breed[b] (n=64) | 27 | | 56 | |
| Breeding farm size | | 0.028 | | 0.200 |
| less than four foals per year (n=90) | 27 | | 73 | |
| more than three foals per year (n=96) | 61 | | 69 | |
| not known (n=181) | 31 | | 75 | |
| Changing frequency of deep litter bedding | | 0.040 | | 0.610 |
| more than once a year (n=36) | 33 | | 81 | |
| once a year (n=67) | 73 | | 67 | |
| Living conditions at the time of sampling | | 0.029 | | 0.199 |
| individual box stall (n=184) | 28 | | 76 | |
| pasture (n=10) | 30 | | 90 | |
| freestall housing (n=128) | 57 | | 65 | |
| Farm size | | 0.211 | | 0.935 |
| less than six horses (n=26) | 23 | | 77 | |
| six to twenty horses (n=113) | 33 | | 75 | |
| over twenty horses (n=176) | 47 | | 70 | |
| Frequency of new horses arriving to the farm | | 0.086 | | 0.690 |
| weekly or monthly (n=165) | 48 | | 72 | |
| yearly or more rarely (n=149) | 33 | | 74 | |
| Horse movements[c] | | 0.006 | | 0.940 |
| Horses are visiting other premises frequently (n=41) | 71 | | 73 | |
| Horses are visiting other premises rarely (n=272) | 35 | | 73 | |
| Manure removal from stalls/sheds | | 0.001 | | 0.116 |
| daily (n=225) | 29 | | 74 | |
| less than daily (n=82) | 68 | | 76 | |
| Sampling season | | 0.496 | | 0.017 |
| spring (n=57) | 40 | | 58 | |
| pasture season (n=71) | 25 | | 75 | |
| winter (n=239) | 41 | | 76 | |

[a] '< 1yo' refers to horses less than 365 days old, 'yearling' includes horses from 365 days old to 730 days old, '2 yo' refers to horses between 731 and 1095 days old.
[b] other breeds included Finnish cold blooded horses (48%), Finnish warmbloods (36%) and various breeds of riding horses
[c] frequently means visits weekly or monthly. Rarely means visits yearly or more rarely.

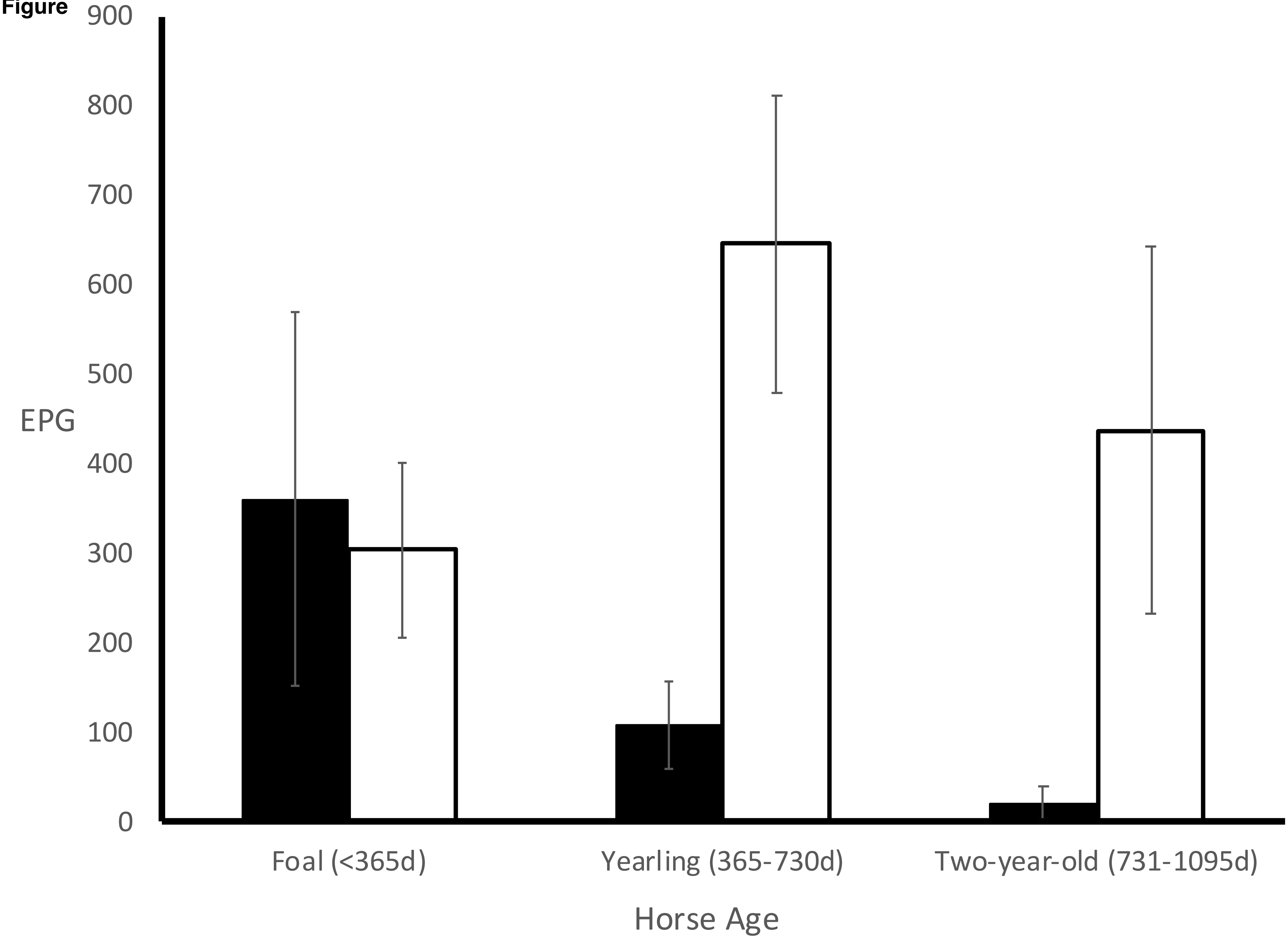
**Table**

**Table 3** Factors associated to *Parascaris* sp. egg shedding in multi variable model.

| **Variable** | | **Odds Ratio** | **95% Confidence Interval** | **p-value** |
|---|---|---|---|---|
| Age in months | | 0.943 | (0.90, 0.99) | 0.020 |
| Breed | standardbred trotter | 3.56 | (1.38, 9.16) | 0.009 |
| | other breed[a] | (reference) | | |
| Breeding farm size | more than three foals per year | 2.47 | (1.10, 5.51) | 0.028 |
| | not known | 1.52 | (0.72, 3.22) | 0.277 |
| | less than four foals per year | (reference) | | |
| Horse movements | horses are visiting other premises weekly or monthly | 3.57 | (1.35, 9.39) | 0.010 |
| | horses are visiting other premises yearly or more rarely | (reference) | | |

[a] other breeds included Finnish cold blooded horses (48%), Finnish warmbloods (36%) and various other breeds of riding horses

**Figure**


EPG
900
800
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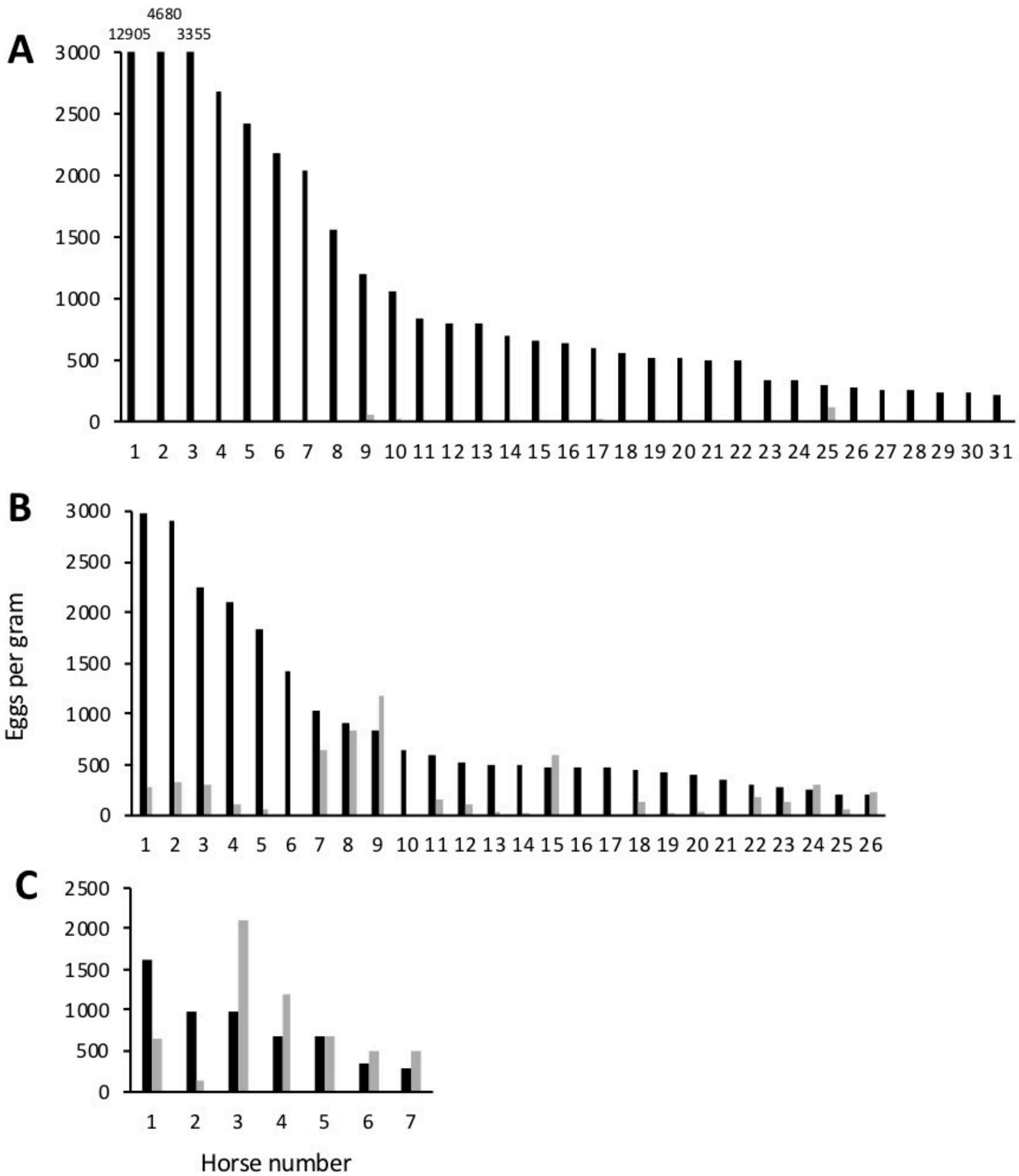
Foal (<365d)
Yearling (365-730d)
Two-year-old (731-1095d)
Horse Age

Figure



A
B
C
12905
4680
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3000
2500
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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
1 2 3 4 5 6 7
Eggs per gram
Horse number

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***Conflict of Interest**

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

‘Risk factors for equine intestinal parasite infections and reduced efficacy of pyrantel embonate against *Parascaris* sp.’

**Author Contribution Statement**

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KH conceptualized the study, administered the project, and drafted the first manuscript. All authors contributed to designing the study and preparing the manuscript. PRS supervised the statistical analysis and data interpretation. OK assisted with executing the study. All authors approved the final manuscript.