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Eva Tydén

Swedish University of Agriculture

Anna Hillström

Swedish University of Agriculture

Emma Odelros

Mälaren Equine Clinic

Charlotte Kemna

Distriktsveterinärerna Öland

Malin Hammarskjöld

Swedish University of Agriculture

Ylva Hedberg Alm

ylva.hedberg.alm@slu.se

Swedish University of Agriculture

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High occurrence of *Parascaris* spp. infection and pulmonary eosinophilia in young Swedish trotting racehorses, without clear causal relationship

Eva Tydén¹, Anna Hillström², Emma Odelros³, Charlotte Kemna⁴, Malin Hammarskjöld¹, Ylva Hedberg Alm^{1*}

¹ Department of Animal Biosciences, Swedish University of Agriculture, Uppsala, Sweden; ² Clinical Pathology Laboratory, Swedish University of Agriculture, Uppsala, Sweden; ³ Mälaren Equine Clinic, Sigtuna, Sweden; ⁴ Distriktsveterinärerna Öland, Färjestaden, Sweden

* Corresponding author: ylva.hedberg.alm@slu.se; ORCID ID 0000-0001-8638-820X

Abstract

Background: Mild-moderate equine asthma (MEA) is common in young racehorses and can result in poor performance and economic loss. Different inflammatory phenotypes of MEA have been recognized, with increases in neutrophils, mast cells and/or eosinophils in bronchoalveolar lavage fluid (BALF). Currently, the aetiology of MEA is not fully understood. Although studies have indicated an allergic aetiology for eosinophilic MEA, *Parascaris* spp. infection has also been associated with increased levels of pulmonary eosinophils. Furthermore, a high prevalence of patent *Parascaris* spp. infection has been found in young Standardbred racehorses. The aims of the present study were therefore to, in young Swedish trotting racehorses, investigate (1) the occurrence of patent *Parascaris* spp. infection, (2) the occurrence of pulmonary eosinophilia using retrospective data from two equine hospitals in Sweden and (3) environmental and parasitological risk factors associated with pulmonary eosinophilia, using a questionnaire-based survey and parasite diagnostics.

Results: Overall, 15% of horses had a patent *Parascaris* spp. infection. There was a significant association between age and a patent *Parascaris* spp. infection, with 1-2-year-old horses more likely to excrete *Parascaris* eggs compared with 3-4-year-old horses ($p<0.05$). Further, Standardbred racehorse breed was significantly associated with a patent *Parascaris* spp. infection ($p<0.01$). Within the cold-blooded trotter breed, there was a significant but reverse association of age and *Parascaris* egg excretion, where no eggs were found in 1-2-year-old horses, and the four horses with patent infection were aged 3-4 years ($p<0.05$). The most common BALF abnormality found in all age-groups was a clinically significant increase in eosinophils. There was a significant negative correlation with age, with increased eosinophils found in 31.3% of two-year-olds, 10.8% of three-year-olds and 10.1% of four-year-olds ($p<0.01$). In horses diagnosed with eosinophilic pulmonary inflammation, managemental factors did not differ from healthy controls. Only one of ten horses with eosinophilic pulmonary inflammation was diagnosed with a patent *Parascaris* spp. infection, compared with two out of ten controls.

Conclusions: The present study demonstrated a high occurrence of patent *Parascaris* spp. infection and eosinophilic pulmonary inflammation in young Swedish Standardbred racehorses. However, to

conclude any possible aetiologic relationship between these two observations will require further studies.

Key words: broncho-alveolar lavage, eosinophils, mild-moderate equine asthma, *Parascaris* spp., Standardbred racehorse, trotting racehorse

69 **Background**

70 Mild-moderate equine asthma (MEA) is common in young racehorses, with as many as 80-90%
71 affected (1, 2). Although conflicting results exist as regards to the magnitude of contribution of MEA
72 to poor performance in racehorses, the potential economic impact of the disease is likely substantial
73 (3). Different inflammatory phenotypes of MEA have been recognized, with the disease showing mild
74 to moderate increases in either neutrophils, mast cells, eosinophils or a mixture of inflammatory cell
75 types, in broncho-alveolar lavage fluid (BALF) (4). Considering the heterogeneity of the
76 inflammatory response observed, there is likely also a heterogenic aetiology, with environmental
77 factors having received most attention (1, 5-7). Although atopic human asthma associated with
78 allergic sensitization is evidently related to airway eosinophilia with a Th2-cell driven airway
79 inflammatory response (8), horses with severe equine asthma predominately show a high neutrophil
80 count in BALF (9). However, MEA shows a more diverse inflammatory response, with mild increases
81 in eosinophils one of the recognized phenotypes (10). To date, there is insufficient knowledge as to
82 the immunologic mechanisms driving the different inflammatory phenotypes in MEA, including
83 eosinophilic airway inflammation, hindering targeted therapies and disease management (11). Studies
84 have shown an airway hyper responsiveness in young racehorses with pulmonary eosinophilia and
85 increased BALF eosinophilia to be associated with higher respirable particulate exposure, indicating
86 an allergic aetiology (6, 10). However, parasitic infections also elicit a prominent eosinophilic
87 inflammatory response, and both the lungworm *Dictyocaulus arnfeldi* and *Parascaris* spp. have been
88 associated with increased levels of pulmonary eosinophils (12, 13). Furthermore, although horses
89 develop an age-related immunity towards *Parascaris* spp., an unexpectedly high prevalence of this
90 parasite was found in young Standardbred racehorses, with as many as 20% of two-year-old
91 Standardbreds shown to be infected (14). Additionally, with increasing evidence of multi-resistant
92 populations of *Parascaris* spp., there is a risk of a concomitant rise in infection pressures in young
93 horse populations (15).

94 The aims of the present study were to, in young Standardbred and cold-blooded trotting racehorses,
95 investigate, in Sweden, (1) the occurrence of patent *Parascaris* spp. infection, (2) the occurrence of

increased eosinophils in BALF indicative of eosinophilic MEA using retrospective data from two equine hospitals and (3) possible environmental and parasitological risk factors associated with eosinophilic MEA, using a questionnaire-based survey, diagnostic anthelmintic treatment and faecal samples.

Results

Part 1: Occurrence of patent *Parascaris* spp. infection in young Standardbred and cold-blooded trotting racehorses

Participating horses

A total of 381 horses from 55 different training yards met the set inclusion criteria of breed and age and were included for faecal sampling (see Materials and Methods section). Nine of the 55 establishments were recruited via personal contacts, with the remaining 46 training yards recruited through the social media platform Facebook. The breed distribution was 79 cold-blooded trotters (20.7%) and 302 Standardbred racehorses (79.3%). Most farms consisted of either Standardbreds (n = 30) or cold bloods (n = 14), with some farms incorporating both breeds (n=11). The number of included horses was calculated to be 4.76% of the total number of 1–4-year-old racing trotting horses registered to be in training in Sweden as of October 2025 (16). The calculated margin of precision for the study to represent the total Swedish trotting population within the set age category was 4.96%, given a 95% confidence interval.

The age distribution of the included horses was as follows: 70 yearlings (18.4%), 120 two-year-olds (31.5%), 106 three-year-olds (27.8%) and 85 four-year-olds (22.3%). The mean and median ages for all participating horses were 2.5 years and 3.0 years, respectively. For Standardbreds, the mean and median ages were 2.6 years and 3.0 years, respectively, and for the cold-blooded trotters, 2.3 years and 2.0 years, respectively.

Patent *Parascaris* spp. infection

Faecal samples (n=381) were collected during the months of April (n=2), May (n=59), September (n=215), October (n=102) and November (n=3) in 2025. A selection of horses (n=22) had multiple faecal samples collected and if at least one of these samples was positive, the horse was counted as a positive individual. For the number and percentage of patent *Parascaris* spp. infection found dependent on age, breed and sex, see Table 1.

Table 1. Horses with a patent *Parascaris* spp. infection using faecal sample McMaster analyses during the months of April, May, September, October and November 2025.

	Positive samples (n)	Total number in the group	Percentage with patent <i>Parascaris</i> spp. infection
All horses	57	381	15.0
1-year-old	13	70	18.6
2-year-old	24	120	20.0
3-year-old	13	106	12.3
4-year-old	7	85	8.2
Cold-blood	4	79	5.1
Standardbred	53	302	17.6
Colt	21	127	16.5
Filly	26	173	15.0
Gelding	9	77	11.7
Unknown	1	4	25.0

There was a significant association between age and a patent *Parascaris* spp. infection, with 1-2-year-old horses significantly more likely to excrete *Parascaris* eggs in the faeces, compared with 3-4-year-old horses ($p<0.05$). Further, Standardbred racehorse breed was significantly associated with a patent *Parascaris* spp. infection ($p<0.01$). In addition, within the cold-blooded trotter breed, there was a significant but reverse association of age and *Parascaris* egg excretion, where no eggs were found in any of the one to two-year-old horses, and with the four positive horses aged three or four years ($p<0.05$). There was no statistically significant association between a patent *Parascaris* spp. infection and sex ($p=0.64$).

The 57 horses with faecal samples positive for *Parascaris* spp. eggs had an EPG of between 25-1550, with a mean of 191 EPG and a median of 100 EPG. Most horses (n=42 (73.7%)) had an EPG below 200 (Figure 1).

Part 2: Occurrence of increased eosinophils in broncho-alveolar lavage fluid indicative of mild-moderate asthma in young Standardbred and cold-blooded trotting racehorses

Retrospective data retrieval

A total of 273 records agreed with the set inclusion criteria (see Materials and Methods section). However, in four cases, the BAL results were missing from the records and in one case, the clinical record stated that the sample submitted was too old for analysis (> 24 hours), resulting in a total of 268 BALF results in the final inclusion. Of these, 15 cases were from hospital 1 (UDS) and 253 cases from hospital 2 (MHK), reflecting the hospitals' different client uptakes as regards to breed and discipline. Only ten of the 268 cases were cold-blooded trotters. The distribution across the different age categories were as follows: yearling = 1, two-year olds = 48, three-year olds = 120 and four-year olds = 99.

Inflammatory cell phenotypes

The distributions of inflammatory phenotypes using the stringent definition A for healthy horses (see Table 2) (4), for all horses and per age-group, are depicted in Figure 2a. For this definition, a total of 65.3% BAL samples, regardless of age, showed abnormalities. For all horses, an increase in a single cell type was seen in 117 cases (43.4%), distributed as follows: an increase in eosinophils in 47 cases (17.5%), an increase in mast cells in 47 cases (17.5%) and an increase in neutrophils in 23 cases (8.6%). Mixed cell types were distributed as follows: increases in both mast cells and eosinophils in 40 cases (14.9%), increases in both neutrophils and eosinophils in eight cases (3.0%), increases in both neutrophils and mast cells in six cases (2.2%), with an increase in all three cell types observed in four cases (1.5%).

Table 2. Reference values used for interpretation of the BAL analyses (4).

Descriptor	Healthy horses (definition A)	Indicative of mild equine asthma (definition B)
Total leukocyte count (per μ l)	≤ 530	
Neutrophils (%)	≤ 5	>10
Eosinophils (%)	≤ 1	>5
Mast cells (%)	≤ 2	>5

The distributions of inflammatory phenotypes using definition B for healthy horses consistent with MEA regardless of technique used (definition B, see Table 2) (4), for all horses and per age-group, are depicted in Figure 2b. For this definition, a total of 77 cases (28.7%), regardless of age, showed abnormalities. For all horses, an increase in a single cell type was seen in 66 cases (24.6%), distributed as follows: an increase in eosinophils in 35 cases (13.1%), an increase in mast cells in 22 cases (8.2%) and an increase in neutrophils in nine cases (3.5%). Mixed cell types were distributed as follows: increases in both mast cells and eosinophils in nine cases (3.4%) and increases in both neutrophils and eosinophils in two cases (0.7%).

Age and phenotype

Broncho-alveolar lavage fluid abnormalities using definition A were seen in 77.1% of samples from two-year olds, in 64.2% of samples from three-year olds and in 62.6% of the samples from four-year olds. Using definition B, abnormalities were detected in 50.0% of samples from two-year olds, in 24.2% of samples from three-year olds and in 24.2% of samples from four-year olds. Thus, age related differences were observed, with abnormal BALF more commonly seen in two-year olds and, for definition B, there was a significant association between age and abnormal BALF, with younger horses more likely to have an increase in any white cell blood type in the BALF as compared with older horses ($p<0.01$).

The distributions of MEA phenotypes for each age-category for both definition A and B are depicted in Figures 2a and 2b. For definition A, the most observed phenotype in two-year olds was an increase in eosinophils (29.2%), for three-year olds, an increase in both mast cells and eosinophils (18.3%) and for four-year olds, an increase in mast cells (25.3%). For definition B, the most common phenotype in all age-categories was an increase in eosinophils (31.3% in two-year-olds, 10.8%, in three-year-olds and 10.1% in four-year-olds).

Eosinophilic inflammation

There was a significant negative association with the presence of eosinophilic inflammation in the BALF with age, with a lower probability of eosinophilia with increasing age, apparent both for definition A ($p < 0.001$) and definition B ($p < 0.01$), as depicted in Figures 3a and 3b. There was no statistically significant association between increased eosinophils and increased mast cells for either definition ($p = 0.05$ (definition A), $p = 0.08$ (definition B)). No association between increased eosinophils in the BALF and season could be shown using either definition ($p = 0.9$ (definition A), $p = 0.1$ (definition B)).

Part 3: Prospective study of risk factors associated with eosinophilic mild-moderate equine asthma

Study population

Collection of suitable horses was performed during a 20-month period, and the final study population included a total of 20 horses, divided equally between cases (n=10) and controls (n=10). The average ages of the cases and controls were 3 years and 3 months and 3 years and 0 months, respectively. All control horses had the BAL performed during the month of May, apart from one that was sampled in August. Cases were sampled during the months of January and May through September. The age, breed, sex, BAL differential count, and faecal sample results are presented in Table 3.

Previous anthelmintic history showed that one horse, E6, had been treated with an anthelmintic drug with effect against *Parascaris* spp., specifically treatment with fenbendazole given three or four months prior to BAL sampling. In this horse, no faecal sample had been taken by the owner prior to treatment. The remaining horses had either not been treated or been treated with macrocyclic lactones or praziquantel, both drugs which are regarded to have no effect against *Parascaris* spp. (17). Six horses (three cases and three controls) had a faecal sample obtained prior to commencing the study, with no sample positive for *Parascaris* spp.

220 Table 3. Broncho-alveolar fluid cell distribution and *Parascaris* spp. eggs per gram faeces (EPG)
 221 results from racehorses aged one to four years old with increased eosinophils in broncho-alveolar-
 222 lavage fluid (BALF) (n = 10; E1-E10) or normal BALF (n = 10; C1-C10).

ID	Age	Breed	Sex	Month of	BALF result (%)			<i>Parascaris</i>	Faecal	<i>Parascaris</i>	
					BAL	<i>EOS</i>	<i>NEU</i>	<i>MC</i>	EPG	observation	EPG
						collection				sample 1	for worms
E1	3 y 0 m	SB	G	May	25	0	4	0	neg	0	
E2	4 y 9 m	SB	G	Jan	6	4	1	0	neg	0	
E3	3 y 9 m	SB	C	Jan	16	1	0	0	neg	0	
E4	3 y 3 m	SB	F	July	13	3	9	150	neg	0	
E5	4 y 3 m	SB	F	Aug	12	2	2	0	neg	missing	
E6	3 y 3 m	SB	F	Aug	28	2	3	0	neg	0	
E7	2 y 5 m	SB	C	Sep	10	2	1	0	neg	0	
E8	2 y 9 m	CB	G	Jan	24	1	2	0	neg	0	
E9	3 y 0 m	SB	G	May	12	2	13	0	neg	missing	
E10	2 y 2 m	SB	F	June	7	0	0	0	neg	0	
C1	3 y 2 m	SB	C	May	0	1	1	0	neg	0	
C2	3 y 0 m	SB	C	May	0	1	1	0	neg	0	
C3	3 y 0 m	SB	F	May	0	0	1	0	neg	0	
C4	3 y 0 m	SB	F	May	0	3	2	0	neg	50	
C5	3 y 0 m	SB	F	May	1	1	0	25	neg	0	
C6	3 y 0 m	SB	F	May	0	0	0	25	neg	0	
C7	2 y 0 m	SB	F	May	0	4	0	0	neg	0	
C8	2 y 2 m	SB	C	May	0	1	0	0	neg	0	
C9	4 y 1 m	SB	F	May	0	0	1	0	neg	0	
C10	3 y 2 m	SB	F	Aug	0	0	2	0	neg	0	

223 BAL(F): broncho-alveolar lavage (fluid); EOS: eosinophils; NEU: neutrophils; MC: mast cells; EPG: eggs per gram

224 Patent *Parascaris* spp. infection and BALF composition

225 The mean, median and range of the percentage of eosinophils in BALF found in the cases were as
226 follows: 15.3%, 12.5% and 6-28%. Two horses with increased eosinophils, E4 and E9, also had
227 increased number of mast cells (>5%). Only one of the cases, E4, had a patent *Parascaris* spp.
228 infection, with 150 EPG in the faecal sample collected at the time of the BAL. No case horse had
229 *Parascaris* eggs detected in the follow-up faecal sample obtained at 12 weeks after fenbendazole
230 treatment, nor any detected *Parascaris* spp. worms in the faeces during the first 72 hours post-
231 fenbendazole treatment.

232 Two of the control horses had a patent *Parascaris* spp. infection at the time of BAL sampling, C5 and
233 C6, both with 25 EPG in the faecal sample. Another control horse, C4, had a faecal sample positive
234 for *Parascaris* eggs, with 50 EPG, at 12 weeks after fenbendazole-treatment. No control horse had
235 *Parascaris* spp. worms detected in the faeces during the first 72 hours post-fenbendazole treatment.

236

237 Management factors and BALF composition

238 For a summary of management factors, previous faecal sample result and prior anthelmintic treatment,
239 see Suppl. Table 1. The few numbers of horses precluded the use of statistical analysis of the data.
240 Below is a descriptive summary of management of both cases and controls.

241 In both groups of horses, cases and controls, haylage was the most common roughage fed. All control
242 horses received their roughage in an elevated position, whilst 50% of the horses with increased
243 eosinophils in BALF were fed their roughage solely on the ground. Of the horses with abnormal
244 BALF, 50% were out at pasture 24 hours per day at the time of sampling, with three of the control
245 horses similarly out at pasture at sampling. Horses that were partly stabled were predominately
246 bedded on shavings, with only one horse with elevated eosinophils in BALF and three control horses
247 bedded on straw.

Signs of reduced performance and BALF composition

For a summary of trainer reported performance ability and clinical signs of cough, see Suppl. Table 2.

Of the horses with increased eosinophils in the BALF, seven horses had reported signs of reduced performance. Two additional horses, E7 and E8, had frequent coughing as the sole complaint, but were not currently in training, and thus their performance level had not been evaluated. One horse, E10, showed neither reduced performance nor signs of coughing. Of the horses with reduced performance, two coughed only at training (E6 and E9) and one showed infrequent coughing, but also at rest (E3). Four horses with elevated eosinophils (E2, E3, E5, E9) had raced prior to examination, and all but one of these horses had multiple winnings.

Of the horses with normal BALF, seven horses had reported signs of reduced performance, with the remaining three horses performing at the expected level when training, without signs of coughing (C2, C4, C6). Six of the horses with reduced performance coughed when exercised. Of the control horses, only one horse had started in a race prior to examination (C4).

Discussion

The present study aimed to investigate the occurrence of *Parascaris* spp. infection and pulmonary eosinophilia in young Standardbred and cold-blooded trotting racehorses and to determine a possible aetiologic relationship between these two disease processes. Results showed patent *Parascaris* spp. infection to be present in as many as 15% of 1–4-year-old trotting racehorses, with a 20% occurrence in 2-year-old Standardbreds. Immunity to *Parascaris* spp. starts to develop from around six months of age, and infection is thus expected to decrease significantly with increased age (18). Although this trend was confirmed in the present study, with a significant inverse relationship of *Parascaris* egg excretion and age, the overall occurrence in the studied age-group was substantially greater than that found in Thoroughbred racehorses of a similar age, where 4% of yearlings and 3% of 2–4 year-olds,

were found to excrete *Parascaris* spp. eggs (19). Similarly, Fritzen et al. (2010) found a *Parascaris* spp. prevalence of less than 3% in 1–3-year-old horses, breed unspecified (20). Our results are more in line with the Finnish data published by Hautala et al. (2019) where 34% of yearlings and 20% of 2-year-old Standardbred racehorses were found with a patent *Parascaris* spp. infection (14). This raises the question as to whether breed-related susceptibility exists, or if the high infection rate in Standardbreds could be related to the immune modulation and adaptation that occur in horses training and competing at a young age (21-24). The latter explanation would, however, not be supported by Fritzen et al. (2010), since Thoroughbred racehorses are also subjected to intense training as youngsters (19). Furthermore, in the present study, none of the sampled 1–2-year-old cold-blooded trotters had a patent *Parascaris* spp. infection, which could indeed suggest breed-related differences as regards to predisposition to infection. However, another plausible explanation of the observed breed differences could be management related. Several risk factors for *Parascaris* spp. infection have been identified, including large breeding farm origin, high movement frequency, use of deep litter systems, poor sanitation in stables and the use of horse manure to fertilize pastures, and could offer an alternative explanation to inherent breed differences in susceptibility to infection (14, 20). In the present study, 44 out of the 55 included farms were composed of either Standardbreds or cold-blooded horses, and it is thus possible that farms with different breed composition, also differed in management practices and farm size. Future studies with a focus on management practices in farms consisting of different horse racing breeds would thus be of interest.

Similarly to the occurrence of a patent *Parascaris* spp. infection, the present study demonstrated a significant negative correlation between the occurrence of eosinophilic pulmonary inflammation and age in young trotting racehorses. Increased eosinophilic cell counts in BALF predominated in 2-year-olds, with almost one third of sampled horses in this age category shown to be affected, regardless of cut-off level used. Further, an eosinophilic pulmonary inflammation of clinical significance, i.e. an eosinophilic differential count greater than 5% in the BALF, was the most common inflammatory phenotype seen overall in the entire study population. These findings are somewhat surprising, with a pure eosinophilic MEA phenotype in young racehorses in general considered rare (25). For example,

Nolen-Walston et al. (2013) found only four out of 98 studied racehorses (Thoroughbreds and Standardbreds), median age of three, to have an eosinophilic MEA phenotype (26). Likewise, Secombe et al. (2015) found an eosinophil increase to be the least common inflammatory phenotype, with only 6.1% of 2-6-year-old racehorses presenting with increased eosinophils ($\geq 1\%$) in BALF (27). In the latter study, a mastocytic inflammation was most common, significantly associated with the Thoroughbred breed, raising the question as to the influence of breed on inflammatory airway phenotype profile. However, the age range in Nolen-Watson's (2013) study also differed from our study, with a greater age span (2-8 years). Thus, age-related differences in mild-moderate asthma inflammatory phenotypes may also exist. To this end, using the more stringent definition for abnormal BALF, the present study showed a change in predominating inflammatory phenotype with increasing age, from eosinophilic in two-year-olds, to a mixed eosinophilic-mastocytic in three-year olds, and finally, a mastocytic phenotype in four-year-olds. This age difference in dominating inflammatory profile agrees with Hostetter et al. (2017), who studied BALF changes over time in normal foals, and found the eosinophil percentage to be highest in foals aged 6 months (median of 2.2%), and mast cell percentages highest in yearlings and adults (medians of 2.2% and 3.3%, respectively) (28). Interestingly, Moore et al. (1995) instead found a mild neutrophilia to be the dominating inflammatory profile in 3-year-old Standardbreds, although four of the studied 32 horses showed marked eosinophilia (29). Clearly, further studies are required to investigate factors affecting MEA inflammatory cell phenotypes in young racehorses.

The aetiology of eosinophilic MEA has to date not been fully elucidated. Mast cells and eosinophils are the hallmarks of a type 2 driven inflammatory reaction, and as such, these two cell phenotypes are often grouped together when classifying MEA in horses. In the present study, there was a tendency for a significant relationship between mast cell and eosinophilic phenotypes, with a combined increase in these two cell types the dominating profile observed in the three-year-old group.

Given that a strong type 2 immune response is observed both in parasitic infections and as a response to allergens (30), resulting in the recruitment of both eosinophils and mast cells, these two possible pathological mechanisms as regards to the pathogenesis of MEA have received some attention in the

literature (6, 10, 31-33). The final part of the present study therefore attempted to compare the parasitological status and management factors in horses with increased eosinophils in BALF to age- and breed matched controls.

As regards parasitological status, in the present study, only one of the ten horses with an eosinophilic inflammation had a patent *Parascaris* spp. infection at the time of BAL sampling. In addition, no horse developed a patent infection at 12 weeks post-sampling and no expelled round worms were observed after fenbendazole treatment. Although it is possible that expulsion of roundworms in the faeces was missed by some owners, these findings together do not support pulmonary larval migration of *Parascaris* spp. as a common aetiologic cause of eosinophilic pulmonary inflammation in young trotting racehorses. In contrast, Riihimäki et al. (2008a) found evidence pointing to parasitic infection in a subset of 3–4-year-old Standardbred racehorses with marked eosinophilia in BALF, where visible roundworms were observed in several horses following anthelmintic treatment and *Parascaris* spp. eggs present in pooled faecal samples (31). Furthermore, Riihimäki et al. (2008b) found an eosinophilic cell increase in BALF to be more common during the summer months, in contrast to a neutrophilic cell profile, which instead dominated during the winter season (32). It was speculated that this seasonal discrepancy could indicate differences in aetiology between these two inflammatory phenotypes, with parasite exposure during the summer months a possible contributor to the eosinophilic inflammatory response seen prevailing during this part of the year. Likewise, Secombe et al. (2015) found cell percentages to differ with season, again eosinophils dominating during the summer period (27). However, these authors concluded that the observed seasonal variation could be the result of pollen exposure. Regardless, in the present study, no significant association with eosinophilic pulmonary inflammation and season was found.

Conversely, rather than indicating a parasitic origin, other studies support an allergic hypersensitivity reaction as responsible for eliciting an eosinophilic pulmonary inflammation. For example, Ivester et al. (2014) demonstrated an increased occurrence of eosinophilic MEA in 1–3-year-old Thoroughbred racehorses exposed to a high concentration of respirable particulate matter, caused by feeding hay in an elevated position over time (6). In addition, Hansen et al. (2020) demonstrated a significant

correlation between eosinophils in BALF and allergen-specific IgE for mite and pollen (33), with Hare & Viel (1998) showing pulmonary eosinophilia in young Thoroughbred racehorses to be associated with increased airway response to a histamine challenge (10). These findings collectively suggest that eosinophilic MEA could be the result of a hypersensitivity reaction to inhaled allergens, a theory that agrees with the pathophysiology of eosinophilic asthma in humans, where allergen exposure is one of the primary aetiologic mechanisms (34). In the present study, management factors related to the stable environment, and thus possible dust and allergen exposure, did not appear to differ between horses with normal BALF and those with elevated eosinophils. Although no specific measurements of airborne microorganisms or respirable particulate matter was performed in the present study, both controls and cases presumably had a low exposure to such agents, given the management they received (5). To this end, most horses, both cases and controls, were fed haylage as opposed to dry hay, with as many as 50% of cases out at pasture 24 hours per day at the time of BAL sampling. Further, in the case group, most horses were fed their roughage on the ground, as opposed to an elevated position, which has been related to lower respirable and inhalable particulates (6). Moreover, only one of the case horses that was partly stabled, was bedded on straw, a bedding material associated with higher respirable dust concentrations in the environment compared with shavings (35). However, the number of horses included was too few for statistical comparisons of managerial factors.

Study limitations

Notably, the study has some limitations that require addressing. In part 1, single faecal samples were obtained over a limited time-period. Although age is the primary factor affecting *Parascaris* spp. egg excretion (18, 36), one study showed that, regardless of age, approximately 40% of variance in egg excretion could be explained by seasonal variation, with higher ascarid egg counts during the warmer months of May-August (37). Thus, the limited sampling period and the inclusion of horses regardless of previous anthelmintic treatment, may have underestimated the number of positive horses. In part 2,

any association of the BALF results and athletic performance was not investigated, with inclusion based solely on age and breed, and the majority of medical records did not specify the reason for performing the BAL. Further, because the study only included horses whose trainers or owners sought veterinary investigation, the results cannot be extrapolated to the general population of young trotting racehorses. Diagnosis of presumed MEA was based purely on abnormal cytology results in the BALF, and although this is the commonly used diagnostic method in clinical practice, pulmonary eosinophilia may not equate to MEA *per se*. Finally, in part 3, the number of included horses were too few to draw statistically sound interpretations as to possible aetiologic factors causing pulmonary eosinophilia in the investigated population.

Conclusion

In conclusion, the present study demonstrated a high occurrence of patent *Parascaris* spp. infection and eosinophilic pulmonary inflammation in young Swedish Standardbred racehorses. However, to conclude any possible aetiologic relationship between these two observations will require future prospective studies using a larger number of horses. Additionally, the development of markers of *Parascaris* spp. larval migration would be of considerable aid in discerning a possible causal association, instead of diagnosis relying on the presence of a patent *Parascaris* spp. infection.

Methods

Part 1: Occurrence of *Parascaris* spp. infection in young Standardbred and cold-blooded trotting racehorses

Participating horses

Inclusion criteria

404 Swedish Standardbred racehorses and cold-blooded trotters between 1-4 years of age.

405 Exclusion criteria

406 No exclusion criteria apart from breed and age, were applied. Thus, horses with a history of
407 anthelmintic treatment of a drug effective against *Parascaris spp.* were not excluded.

408 Recruitment, sampling and data collection

409 Faecal samples were collected during the months of April through November 2025. Trainers of
410 establishments suitable for study participation were recruited in two different ways: (1) through social
411 media (specifically via a Facebook group designed for persons with an interest in trotting horses
412 (“Travgruppen”), with 31,000 members active within the sport) and (2) through personal contacts. All
413 interested trainers were asked to contact the research group via e-mail and thereafter received, through
414 post, material for sample collection, including pre-stamped envelopes for transportation of the sample
415 (s) to the section for Parasitology, Department of Animal Biosciences, Swedish University of
416 Agriculture, Ultuna, Sweden, for analysis. In some instances, when personal communication had been
417 used for recruitment, the faecal samples were personally collected and taken to the university for
418 analysis by the research group. Information requested for each faecal sample included the horse’s
419 name, age, date of last anthelmintic treatment and drug given, as well as the owner’s name, e-mail
420 address, postal code and a signed consent form. Information of each horse’s sex and breed
421 (Standardbred or cold-blooded trotter) was retrieved through the official sport information site for
422 Swedish trotting racehorses (www.travsport.se). Participants not willing to declare the horse’s name,
423 were asked to provide breed, age and sex.

424 *Parascaris spp.* analyses

425 Faecal samples were analysed using the McMaster method with a minimum detection limit of 25 eggs
426 per gram faeces (EPG) (38). A total of six grams of faecal material were flotated using a saturated
427 NaCl solution (SG = 1.18). In cases where *Parascaris spp.* eggs were observed outside the McMaster
428 chambers markings, the result was set at the detection limit (25 EPG).

Statistical analyses

Statistical analyses of data were performed using Graph Pad Prism (version 10.6.1, 2025), utilising two different statistical tests, the chi-squared test and Fisher's exact test. The level of significance was set at $p < 0.05$. For exploring associations between age in Standardbred racehorses and sex, and the presence of patent *Parascaris* spp. infection, the chi-squared test was used. Due to fewer sampling points, Fisher's exact test was used for exploring associations between breed, age (in both breeds combined), age in cold-blooded trotters, and the presence of patent *Parascaris* spp. infection. For total age, horses were grouped into two categories, 1–2-year-olds and 3–4-year-olds, to ensure adequate group size for analysis.

The margin of error (precision) of the study, given a 95% confidence interval (CI), was calculated using the formula:

$$z \times \sqrt{\frac{p \times (1 - p)}{n}}$$

where z is the z -score associated with the CI (here 1.96), p is the sample proportion (we used 0.5 as it is the most conservative model) and n the total number of included horses.

Part 2: Occurrence of increased eosinophils in broncho-alveolar lavage fluid indicative of mild-moderate asthma in young Standardbred and cold-blooded trotting racehorses

Inclusion criteria

Two Swedish equine hospitals were enrolled in the study. Standardbred and cold-blooded trotting racehorses, between 1–4 years of age, that at presentation at either hospital had been subjected to a

broncho-alveolar lavage (BAL), from January 1, 2014, until December 31, 2024 (Standardbreds), or from January 1, 2015, until December 31, 2025 (cold-bloods), were included. Birth date was set as January 1 for all horses, since the actual birth date in most cases was unknown. Data retrieved were limited to the horse's age, month of sampling, and the result of the BAL analysis. Only the primary sample collected from each horse was included and any follow-up samples from the same patient were excluded from the final data set.

Broncho-alveolar lavage technique

Standard practice at both hospitals was to use the following broncho-alveolar lavage (BAL) technique: a 240 cm long Broncho-Alveolar-Lavage Catheter with balloon (10 cc) was passed through one of the nostrils and via the trachea past the bifurcation until resistance indicated the tube to be wedged in a distal bronchus. During passage of the BAL tube, local anaesthetic was infused to minimize discomfort. Once in place, the tube was cuffed with 10 ml of air. The volume of infused sterile saline for BALF collection, however, differed between hospitals, with an infused volume of sterile sodium chloride at hospital 1 of 300 ml and, at hospital 2, of 240 ml. The aspirated fluid was carefully mixed before being transferred to a sampling tube and submitted for analysis.

Broncho-alevolar lavage analysis

234 of the BAL samples from both hospitals were kept chilled at 4°C and analysed at the Clinical Pathology Laboratory at the University Animal Hospital, Swedish University of Agricultural Sciences within 24 hours of collection. Previous studies have demonstrated stability of eosinophils within this time frame (39). The total nucleated cell count was determined using an automatic haematology analyser (ADVIA 2120i Haematology system, Siemens Healthcare Diagnostics AB, Tarrytown, NY, USA). Cytology slides were prepared with cytocentrifugation (Thermo Scientific Cytopspin 4 centrifuge, Thermo Fisher Scientific, Waltham, Massachusetts, US) after diluting the BAL fluid 1:5 in albumin, and stained with May-Grünwald-Giemsa. A 200 differential count was performed and percentages of neutrophils, macrophages, lymphocytes, eosinophils and mast cells were recorded.

For 34 samples, the cytology slides were prepared within 24 hours of BALF collection at hospital 2 using the same method as above, but with total nucleated cell count determined using another automated haematology analyser (Sysmex XN-1000V, Sysmex Nordic ApS Filial, Landskrona, Sweden). The cytology slides were then analysed at the Clinical Pathology Laboratory at the University Animal Hospital, Swedish University of Agricultural Sciences.

Table 2 shows the cut-off values used for interpretation of the BAL analysis, one more stringent differentiating from healthy horses (definition A), with the second cut-off value considered clinically significant values regardless of method used for BALF collection (definition B).

Statistical analysis

All data sets were recorded in Microsoft Excel (Microsoft Office Professional Plus 2016). Statistical analyses were performed using Graph Pad Prism (version 10.6.1, 2025). For investigating any association between eosinophilic MEA phenotype and season, with the four seasons defined as: spring (March -May), summer (June-August), autumn (September-November) and winter (December-February), the Chi-squared test was applied. Logistic regression was utilized to determine associations between MEA (any phenotype) and age and eosinophilic MEA phenotype and age. Finally, Fisher's exact test was employed to analyse associations between increased eosinophils in BALF with concomitant increase in mast cells.

Part 3: Risk factors associated with increased eosinophils in broncho-alveolar lavage fluid

Prospective study data collection inclusion criteria

Via direct contact through e-mail, Swedish equine hospitals known to perform BAL were contacted and asked to inform owners of specified equine patients of the study, including asking for voluntary participation with written consent. Equine patients with the following criteria were sought for study participation: Standardbred and cold-blooded trotting racehorses less than five years of age and with a BAL result showing an eosinophilic differential count greater than five percent. Ten control horses,

matched by age and breed to the final case group, were included. These controls had undergone broncho-alveolar lavage (BAL) as part of a separate study, which confirmed normal BAL fluid (BALF) analysis.

Broncho-alveolar lavage technique and broncho-alveolar lavage analyses

For a description of the procedure for collection of BALF and BALF analysis, see 2.2.2 and 2.2.3. In this study part, all samples were kept chilled at 4°C and within 24 h of collection prepared and analysed at the Clinical Pathology Laboratory at the University Animal Hospital, Swedish University of Agricultural Sciences.

Faecal samples and anthelmintic treatment

All owners that agreed to study participation were asked to obtain a faecal sample from the participating horse and thereafter treat the horse with a single oral dose of fenbendazole (Axilur, 19% oral paste, MSD Animal Health, Sweden) according to the estimated body weight. The owners were also instructed to note any signs of *Parascaris* spp. worms in the faeces during the first 72 hours after anthelmintic treatment. A second faecal sample was obtained at least 12 weeks after anthelmintic treatment. All control horses followed the same procedure. For a timeline of the sampling procedure, see Figure 4. Faecal samples were analysed using the McMaster method with a minimum detection limit of 50 EPG (36). A total of three grams of faecal material were flotated using a saturated NaCl solution (SG = 1.18).

Questionnaire survey

All participating owners were asked to fill in a questionnaire (Table 4).

529 Table 4. Questionnaire data collected from participating cases and controls.

Information	Descriptor
Age	Years
Gender	Mare/Stallion/Gelding
Breed	Standardbred/Cold-blood
Signs of reduced performance	Yes/No
If yes, what best describes the reduced performance	Performance below expected level/ Reduction of previous performance level/ Markedly reduced ability to performs
How long has the horse showed reduced performance	Weeks/Months/1 year/More than 1 year
How quickly did the reduced performance appear	Suddenly (days)/Gradually (months)/Gradually (years)
Has any other diagnostics (apart from BAL) been performed because of the reduced performance	Yes/No
If yes, specify	Free text
Has the horse received any treatment because of the reduced performance	Yes/No
If yes, specify	Free text
Does the horse cough	No/Yes, infrequently/Yes, often/Yes, only when worked
Amount of turn-out	24 hours/Stabled at night, turned-out daytime/ Stabled daytime, turned-out at night
Type of roughage	Hay/Haylage
How is the roughage fed	On the ground/Elevated position (haynet/hayrack)
Type of bedding	Straw/Shavings/Peat/Other
Date of last faecal sample	Date
Result of last faecal sample	Free text
Date of last anthelmintic treatment	Date
Drug used at last treatment	IVM ^a /MOX ^b /PYR ^c /FBZ ^d /COMB ^e

530 ^aivermectin; ^bmoxidectin; ^cpyrantel; ^dfenbendazole; ^ecombination drug (ivermectin or moxidectin and praziquantel)

531

532 List of abbreviations

533 MEA: mild-moderate equine asthma

534 BAL: bronchoalveolar lavage

535 BALF: bronchoalveolar lavage fluid

536 EPG: eggs per gram faeces

537 Figure Legends

538 Figure 1. Scatter-plot depicting eggs per gram (EPG) of *Parascaris* spp. in individual faecal samples
539 in all positive horses per age category (1-4 years).

540 Figure 2a and b. Distribution of inflammatory cell phenotypes in broncho-alveolar lavage fluid
541 (BALF) collected from Standardbred trotters presenting at two different Swedish equine clinics over
542 an 11-year time period (January 1 2014-December 31 2024) according to definition A (eosinophils
543 >1%, mast cells >2%, neutrophils >5%) and definition B (eosinophils >5%, mast cells >5%,
544 neutrophils >10%).

545 Figure 3a and b. Box whisker plots showing the age distribution of horses with normal broncho-
546 alveolar lavage fluid (BALF) and horses showing an increase in numbers of eosinophils >1% (figure
547 3a) or > 5% (figure 3b).

548 Figure 4. Timeline depicting sample collection of horses presenting with increased eosinophils in
549 broncho-alveolar lavage fluid (BALF) and control horses with normal BALF. At enrolment, after the
550 performance of a bronchoalveolar lavage, a faecal sample for analysis of the presence of *Parascaris*
551 spp. eggs was taken from all horses. Owners were asked to fill in a survey response form and
552 thereafter treat their horse with a single oral dose of fenbendazole (7.5 mg/kg) according to body
553 weight. After fenbendazole treatment, owners were instructed to observe any excreted *Parascaris* spp.
554 worms in the faeces for a duration of 72 hours. After at least 12 weeks from the first faecal sample, a
555 follow-up faecal sample was obtained for analysis of the presence of *Parascaris* spp. eggs.

556 Additional Files Legends

Additional file 1: Supplementary Table 1. Management factors, previous faecal sample result and prior anthelmintic treatment in horses with elevated levels of eosinophils in bronchoalveolar-lavage fluid (BALF) and control horses with normal BALF.

Additional file 2: Supplementary Table 2. Trainer reported performance ability and clinical signs of cough in horses with elevated levels of eosinophils in bronchoalveolar-lavage fluid (BALF) and control horses with normal BALF.

Declarations

Ethics approval and consent to participate

Part 1: The study was approved by the Uppsala animal welfare ethics committee (D nr 5.2.18-22081/2023). Owner consent was required for study participation with all participating owners asked to sign an informed consent form prior to study inclusion. Part 2. According to the Swedish Board of Agriculture's regulations and general advice on laboratory animals (SJVFS 2019:9, case no. L150) the retrospective study described in this part did not require permission from the Central or Local Animal Experiment Ethics Committees. Informed written consent from owners of animals for the general use of medical records for research purposes, including results of laboratory tests and pathology reports, was collected at admission in all cases. Part 3. The study was approved by the Uppsala animal welfare ethics committee (D nr 5.2.18-22081/2023). Owner consent was required for study participation with all participating owners asked to sign an informed consent form prior to study inclusion. Ethical review and approval were waived for the use of questionnaire data in part 3, in accordance with relevant guidelines and regulations issued by the Swedish Board of Agriculture's regulations and general advice on laboratory animals (SJVFS 2019:9, case no. L150). All handling and use of human data were performed in accordance with relevant guidelines and regulations (The Swedish Ethical Review Act (2003:460)).

581 Consent for publication

582 All owners of part 1 and 3 of the study provided written informed consent for the use of data for
583 publication and were informed that the horses' identities would not be disclosed. Part 2 included only
584 retrospective data where owners at admission were asked to sign an admission form stating that
585 anonymised data can be used for future educational or research purposes.

586 Availability of data and materials

587 All data supporting the findings of this study are available within the paper and its Supplementary
588 Information. Subject to third party agreement (horse owner), individual patient records can be
589 available from the corresponding author on reasonable request.

590 Competing interests: None declared.

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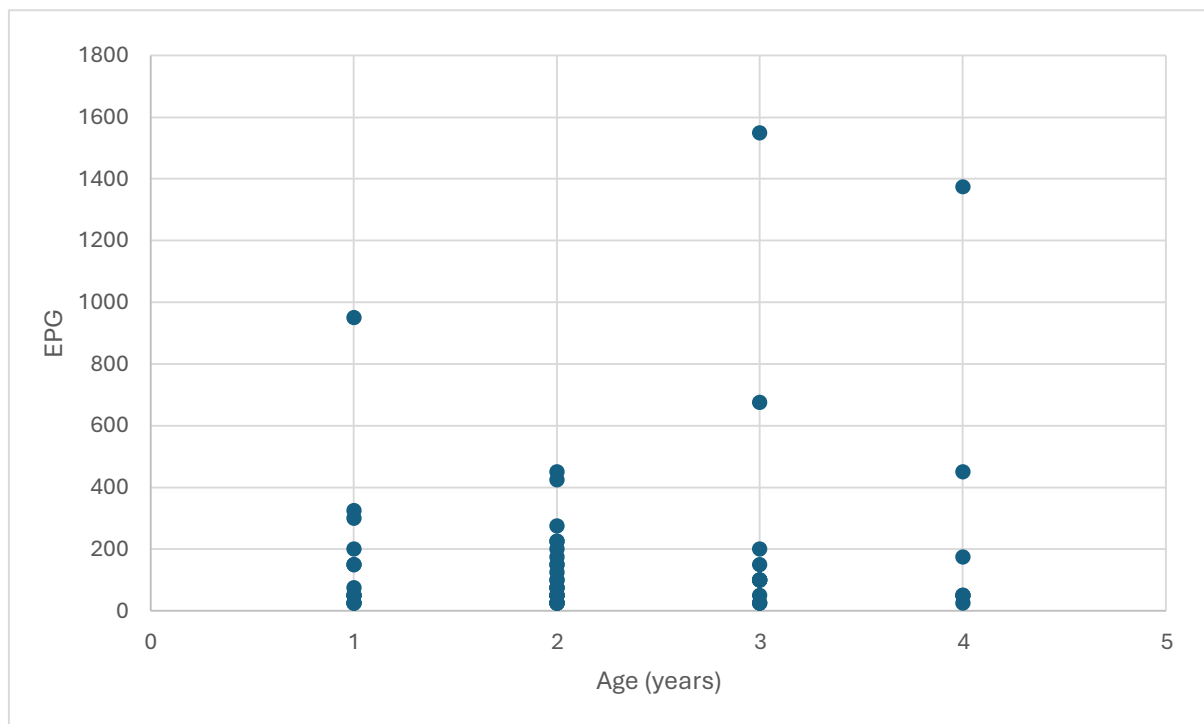
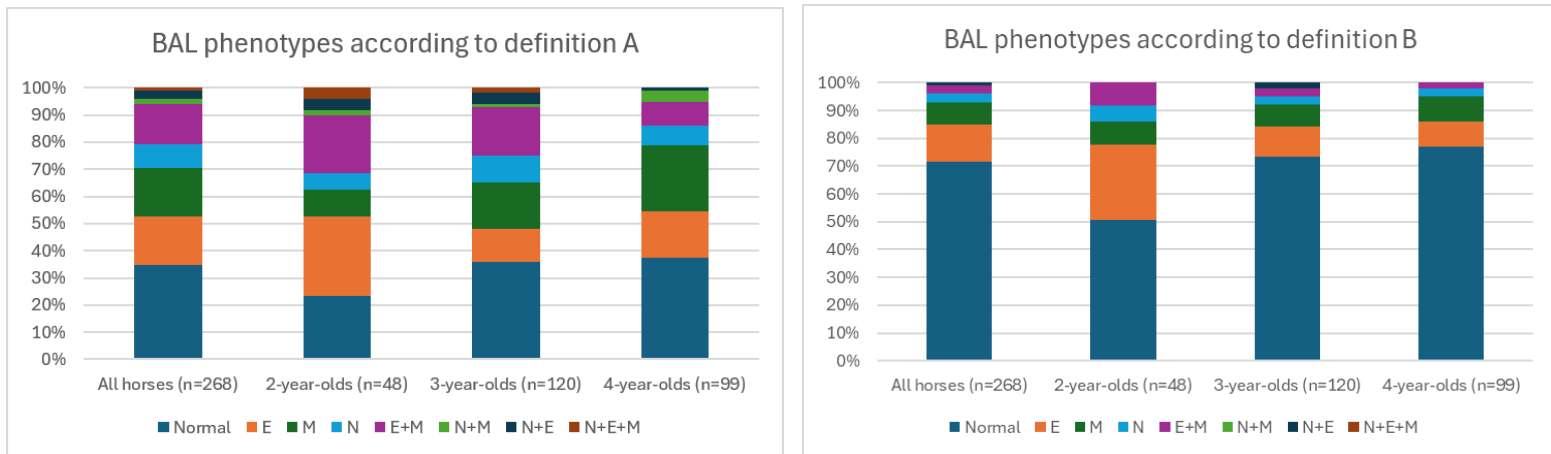


Figure 1. Scatter-plot depicting eggs per gram (EPG) of *Parascaris* spp. in individual faecal samples in all positive horses per age category (1-4 years).



E: eosinophilic; M: mastocytic; N: neutrophilic, E+M: mixed eosinophilic and mastocytic; N+M: mixed neutrophilic and mastocytic; N+E: mixed neutrophilic and eosinophilic; N+E+M: mixed neutrophilic, eosinophilic and mastocytic.

Figure 2a and b. Distribution of inflammatory cell phenotypes in bronchoalveolar lavage fluid (BALF) collected from Standardbred trotters presenting at two different Swedish equine clinics over an 11-year time period (January 1 2014- December 31 2024) according to definition A (eosinophils >1%, mast cells >2%, neutrophils >5%) and definition B (eosinophils >5%, mast cells >5%, neutrophils >10%). One yearling (not depicted) had increased eosinophils according to definition A, but was defined as normal with definition B.

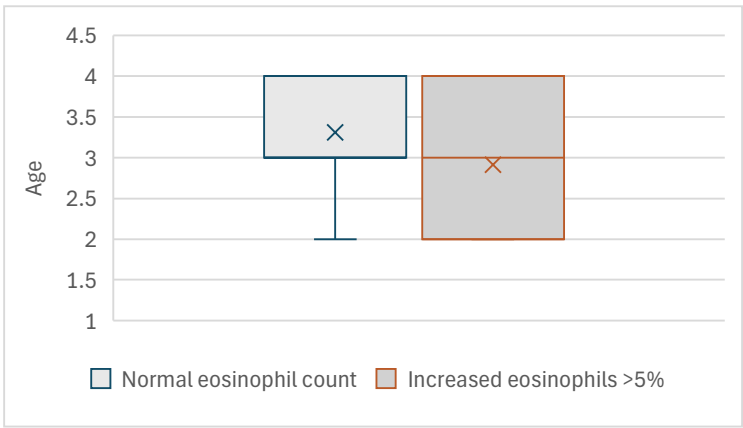
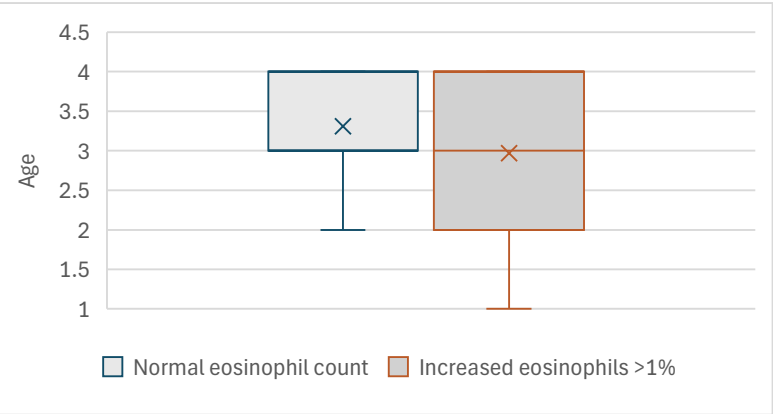


Figure 3a and b. Box whisker plots showing the age distribution of horses with normal BALF and horses showing an increase in numbers of eosinophils >1% (figure 3a) or > 5% (figure 3b).

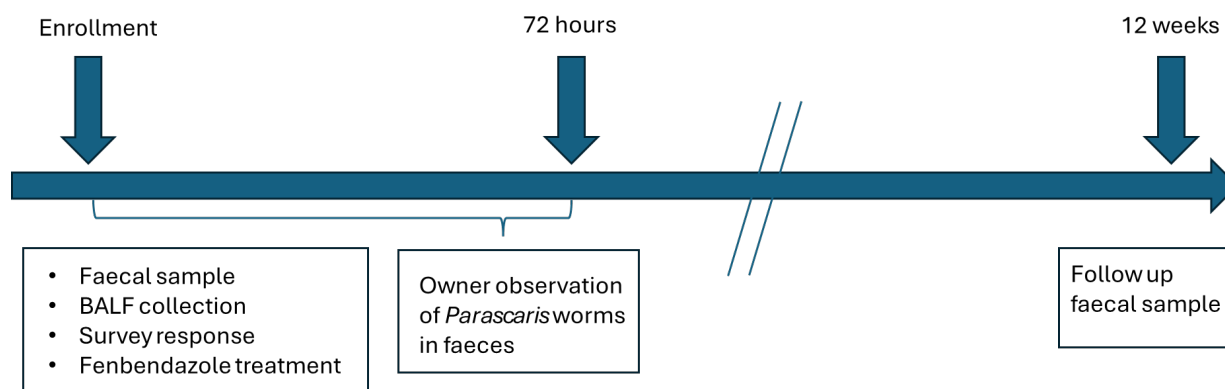


Figure 4. Timeline depicting sample collection of horses presenting with increased eosinophils in broncho-alveolar lavage fluid (BALF) and control horses with normal BALF. At enrolment, after the performance of a bronchoalveolar lavage, a faecal sample for analysis of the presence of *Parascaris* spp. eggs was taken from all horses. Owners were asked to fill in a survey response form and thereafter treat their horse with a single oral dose of fenbendazole (7.5 mg/kg) according to body weight. After fenbendazole treatment, owners were instructed to observe any excreted *Parascaris* spp. worms in the faeces for a duration of 72 hours. After at least 12 weeks from the first faecal sample, a follow-up faecal sample was obtained for analysis of the presence of *Parascaris* spp. eggs.

Supplementary Files

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- [Suppltable1.docx](#)
- [Suppltable2.docx](#)