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No correlation between eosinophilic pulmonary inflammation and patent Parascaris spp. infection in foals with natural parasite exposure

Eva Tydén
Swedish University of Agriculture
Anna Hillström
Swedish University of Agriculture
Zala Zgank
Menhammar stuteri
Mårten Lagerlund
Swedish University of Agriculture
Amanda Örnerstig Forsberg
Horse Clinic, University Animal Hospital, Swedish University of Agriculture
Ylva Hedberg Alm
ylva.hedberg.alm@slu.se

Swedish University of Agriculture

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Abstract

Background

Infection with the equine roundworm, *Parascaris* spp., is very common in young foals, with a prevalence of 31–58%, and although an age-related immunity develops, as many as 20% of 2-year-old Standardbred racehorses may also be infected. The parasite migrates through the lung parenchyma as part of its 80–104-day long life cycle, with necropsy studies showing this pulmonary larval migration to cause pronounced eosinophilic inflammation in pulmonary tissues. Furthermore, young racehorses may be diagnosed with mild-moderate equine asthma (MEA), with one phenotype of this disease showing an abnormal increase in eosinophils in broncho-alveolar lavage fluid (BALF). Although studies have indicated this eosinophilic inflammation to be caused by a type-2 hypersensitivity reaction to respirable particulate matter, the possible contribution of *Parascaris* spp. pulmonary migration to eosinophilic MEA in this cohort of horses is currently unknown. The aim of the present study was to determine whether there is an association between *Parascaris* spp. larval migration and elevated eosinophils in BALF by performing monthly BAL and faecal samples in a group of foals naturally infected with *Parascaris* spp.

Results

All foals (n = 10) had at least one faecal sample positive for *Parascaris* spp. during the study period and increased eosinophils in BALF were observed on at least one sampling occasion in seven foals. However, only two foals had an eosinophil count above 5%, the level considered of clinical significance for MEA in adult horses. In these two foals, a concurrent patent *Parascaris* spp. infection was observed on three out of the total six sampling occasions with elevated eosinophils. However, neither foal had a patent *Parascaris* spp. infection at two to three months after the first sign of eosinophilic pulmonary inflammation, which would have been expected, considering the parasite's life cycle.

Conclusions

The present study failed to demonstrate a clear association between *Parascaris* spp. infection and elevated eosinophils in BALF in foals naturally exposed to *Parascaris* spp. infection. However, since parasite diagnostics currently relies on detection of patent infection, the future development of pre-patent biomarkers is required to definitively exclude a causal relationship between *Parascaris* spp. infection and eosinophilic MEA.

Background

Eosinophilic inflammation of the lung, as demonstrated by increased numbers of eosinophils in broncho-alveolar lavage fluid (BALF), has been described in the rare and serious manifestation of idiopathic chronic eosinophilic pneumonia, but more commonly, as one of the inflammatory phenotypes of mild-

moderate equine asthma (MEA) (1–4). With as many as 80–90% of young racehorses diagnosed with MEA, together with its probable association with poor performance, the disease is of considerable importance, as regards both the economic impact and the welfare of the affected animals (3, 5). To date, the aetiology of eosinophilic MEA has not been definitively determined, with most attention given to a possible pulmonary hyperreactivity and allergic response to inhaled particulate matter from the environment (2, 3). However, parasite infections have long been known to elicit an eosinophilic immune response, with eosinophils being the primary cell type involved in inactivation and suppression of helminth infections (6). This must raise the question as to whether parasite larval migration through pulmonary tissue, as occurs with *Parascaris* spp. infections, could also be involved in the eosinophilic pulmonary inflammation observed in young racehorses.

Infection with the roundworm *Parascaris* spp. is common in foals, with a prevalence of between 31–58% demonstrated in various countries, including Sweden (7–11). Although an age-related immune response towards *Parascaris* spp. occurs from around six months of age, as many as 20% of two-year-old Standardbred trotters were shown to be infected with the parasite (12, 13). The parasite has a direct life cycle, where horses become infected by ingesting eggs from a contaminated pasture, with the infective larval stage, L_2, subsequently released as the eggs enter the stomach and small intestine. After penetrating the wall of the small intestine, and passing through the liver, the larvae reach the lungs around two weeks after egg ingestion (14). Correspondingly, Nicholls et al. (1978) showed a marked eosinophilia in the interlobular and subpleural areas of the lung including eosinophilic alveolitis, bronchitis and bronchiolitis at 14 days post-infection. It is also at this time that possible respiratory signs are seen in affected foals, with mucopurulent nasal discharge and cough observed in experimentally infected foals at 13–23 days post-infection (15). To date, the possible contribution of *Parascaris* spp. infection as a cause of pulmonary eosinophilia in young racehorses is unknown, and no studies to our knowledge have investigated the prevalence of increased eosinophils in BALF in a horse population with known *Parascaris* spp. exposure and infection.

The aim of the present study was therefore to investigate a possible association between eosinophilia in BALF and the presence of *Parascaris* spp. infection in foals with known parasite exposure.

Results

Clinical examination and respiratory signs

All foals were bright, alert and responsive during the clinical examinations. Body temperature was obtained rectally on the first sampling occasion on farm 1 and on the first two sampling occasions on farm 2. At all other sampling occasions, body temperature was obtained by scanning the foals' temperature microchips implanted in the neck region. A significant correlation between rectal temperature and microchip readings in yearlings has been previously demonstrated (16). The mean body temperature was 37.9, with a range of 36.3–38.6 ℃, and considered within normal limits for the foals' age ranges of 6.5–15 months (16). Furthermore, no abnormal auscultations of the thorax were recorded.

Some foals displayed mild serous or mucous nasal discharge with a total of three foals showing moderate nasal discharge (IDs 1:2, 1:4, 1:5) on one sampling occasion each. One of these foals (ID 1:2) had moderate mucous nasal discharge on the fifth sampling occasion, together with spontaneous cough and a positive response to manual compression of the larynx. Because the endoscopic examination of the trachea in this foal resulted in pronounced coughing, this foal was not subjected to BAL on that occasion, due to the procedure likely to cause severe distress in the animal. Several foals had palpable lymph nodes, but all lymph nodes were considered within normal limits for the age range of the foals. For full details of the clinical examination, see Suppl. Table 1.

Systemic inflammatory markers

All fibrinogen levels were within the normal reference range (1.8-4.0 g/L) apart from one sample with a value of 4.1 g/L (ID 2:2). Four foals (IDs 1:1, 1:2, 1:5, 2:2) had on five sampling occasions mildly increased serum amyloid A (SAA) levels, ranging from 5.5–87.0 mg/L (normal reference < 5.0 mg/L). Blood samples were missing on three occasions, due to the foal not available for sampling (2:5), euthanasia due to lameness (2:3) and the foal being too stressed for sampling (2:1).

Endoscopic examination of pharynx and trachea

The tracheal mucous grades for each foal on each sampling occasion are shown in Table 1 and Table 2. On farm 1, the tracheal mucous grades ranged from 0–4, with an average mucous grade across all sampling occasions in all foals of 1.2. The most common mucous grade was grade 1 (n = 7), followed by grade 0 (n = 13), grade 2 (n = 5), grade 3 (n = 3) and grade 4 (n = 1). On farm 2, the tracheal mucous grades ranged from 0–3, with an average mucous grade across all sampling occasions in all foals of 1.3. The most common mucous grades were grade 0 and grade 2 (n = 8, respectively), followed by grade 1 (n = 7) and grade 3 (n = 4). Due to poor quality video or use of saline flush during the examination, two video recordings (one on each farm) could not be evaluated. Spearman’s rank correlation coefficient showed no significant correlation between tracheal mucous grade and percentage of eosinophils in the BALF cell differential count (r=-0.137, p = 0.3).

Table 1

Bronchoalveolar lavage fluid eosinophil differential cell counts, tracheal mucous grade and *Parascaris* spp. EPG (eggs per gram faeces) in faecal samples on farm 1, with monthly samples obtained for a duration of six months during 2024–2025.

ID	Parameter	Sampling occasion					
		1	2	3	4	5	6
1:1	Eosinophils in BAL (%)	1	0	0	0	0	2
	Tracheal mucous grade*	1	2	1	3	3	2
	Parascaris EPG	3000^{FBZ}	150^{FBZ}	0	0	0	0
1:2	Eosinophils in BAL (%)	2	1	0	1	N/A	2
	Tracheal mucous grade*	1	1	0	1	2	2
	Parascaris EPG	200^{FBZ}	0	0	0	0	0
1:3	Eosinophils in BAL (%)	0	1	0	0	0	0
	Tracheal mucous grade*	1**	1	1	0	3	4
	Parascaris EPG	0	50	50	0	100^{FBZ}	0
1:4	Eosinophils in BAL (%)	1	1	0	0	2	0
	Tracheal mucous grade*	1	0	0	0	1	1
	Parascaris EPG	200^{FBZ}	0	0	0	0	50
1:5	Eosinophils in BAL (%)	0	0	0	0	0	1
	Tracheal mucous grade*	0**	0	1	-***	2	1
	Parascaris EPG	600^{FBZ}	0	0	0	0	0

* Grades 0–5 (according to Coutil et al., 2016); ** poor video quality: *** not diagnostic quality; FBZ: treatment with fenbendazole

Table 2

Bronchoalveolar lavage fluid eosinophil differential cell counts, tracheal mucous grade and *Parascaris* spp. EPG (eggs per gram faeces) in faecal samples on farm 2, with monthly samples obtained for a duration of six months during 2025. An extra sampling occasion was performed in two foals (2:1 and 2:5).

ID	Parameter	Sampling occasion						
		1	2	3	4	5	6	7†
2:1	Eosinophils in BAL (%)	7	14	20	9	5	22	3
	Tracheal mucous grade*	-***	2**	2	3	0	2	N/A
	Parascaris EPG	50	50	0	0	0	0	50
2:2	Eosinophils in BAL (%)	0	0	3	1	0	3	N/A
	Tracheal mucous grade*	1	2	3	2	2	3	N/A
	Parascaris EPG	500^{FBZ}	300^{FBZ}	0	0	0	0	N/A
2:3	Eosinophils in BAL (%)	1	10	8	2	1	N/A	N/A
	Tracheal mucous grade*	0	0**	0	1	0	N/A	N/A
	Parascaris EPG	700^{FBZ}	100	0	0	0	N/A	N/A
2:4	Eosinophils in BAL (%)	0	0	0	1	0	0	N/A
	Tracheal mucous grade*	1	1	1	1	0**	2	N/A
	Parascaris EPG	1200^{FBZ}	0	50	0	0	0	N/A
2:5	Eosinophils in BAL (%)	N/A	0	2	1	0	N/A	1
	Tracheal mucous grade*	N/A	0	1	2	0	3	N/A
	Parascaris EPG	N/A	50	350^{FBZ}	0	250^{FBZ}	450^{FBZ}	300^{PYR}

* Grades 0–5 (according to Coutil et al., 2016); ** poor video quality: *** not diagnostic quality; FBZ: treatment with fenbendazole; PYR: treatment with pyrantel; † Extra sampling occasion for 2:1 and 2:5; N/A: not applicable

Broncho-alveolar lavage

The total number of cells in the BALF was below the reference level set for total nucleated cell counts in healthy horses (≤ 530 cells/μL) (17) in all but two samples, both from foal 2:2, where the total nucleated cell counts were 560 cells/μL and 590 cells/μL. The cell count differential was normal in one sample (560 cells/μL) with an increase in eosinophils (3%) in the other sample (590 cells/μL). The only nucleated cell to show an abnormal increase in any of the BAL samples was eosinophils, and no samples showed abnormally elevated neutrophils or mast cells, using either definition outlined in the ACVIM Equine Asthma Consensus Statement (Table 3) (17). The eosinophilic percentages in the BALF on each sampling occasion for each foal are displayed for farm 1, in Table 1 and for farm 2, in Table 2. Mildly increased eosinophils based on the stringent cut-off level for healthy horses of greater than 1% eosinophils, was observed on at least one sampling occasion in seven of the foals. However, only two foals, IDs 2:1 and 2:3, had an eosinophil differential count above 5%, the level considered of clinical significance for mild-moderate equine asthma in adult horses (17). Foal 2:1 had eosinophilic differential cell counts above this threshold on 5/6 sampling occasions, with values ranging from 7–22%. Foal 2:3 had eosinophilic cell counts above the 5% threshold on 2/5 sampling occasions, with eosinophils counts of 8% and 10%.

Table 3
Reference values used for interpretation of the BAL analyses (17).

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

Patent *Parascaris* spp. infection

All foals had a patent *Parascaris* spp. infection during the study period (Tables 1 and 2). On farm 1, *Parascaris* spp. positive faecal samples were demonstrated on nine occasions, and on farm 2, on 12 occasions. During the first half of the study period (sampling occasions 1–3), including both farms, 17 samples showed a patent *Parascaris* spp. infection, compared with four positive faecal samples during the latter half (sampling occasions 4–6). The foals were aged from 6.5–12 months during the first half of the study period, and from 9.5–15 months during the study period´s second half. The overall mean EPG of the positive samples was 410 EPG, with a range of 50-3000 EPG. On farm 1, all foals were treated with fenbendazole on at least one occasion, according to this farm's anthelmintic treatment policy of treating at a *Parascaris* spp. EPG ≥ 100, with one foal (1:1), treated on two occasions. On farm 2, four of the five foals were treated with fenbendazole on one (2:3 and 2:4), two (2:2) or three (2:5) occasions. Additionally, foal 2:5 was treated with pyrantel, due to a continuing patent *Parascaris* spp. infection, despite two consecutive fenbendazole treatments.

Eosinophilic pulmonary inflammation and patent *Parascaris* spp. infection

As stated above, two foals (2:1 and 2:3) had BALF with eosinophil differential cell counts above 5% on a total of seven sampling occasions, specifically five samples for foal 2:1 and two samples for foal 2:3. On these sampling occasions, faecal samples were positive for *Parascaris* eggs on three sampling occasions, specifically on one occasion for foal 2:1 and on both occasions for foal 2:3 (Table 2). During the remaining four sampling occasions with significantly elevated eosinophils, these foals' faecal samples were negative for *Parascaris* spp. eggs. None of these foals had a patent *Parascaris* spp. infection with egg excretion at two to three months after the first sign of significant eosinophilic pulmonary inflammation. Spearman's rank correlation coefficient showed no significant correlation between EPG level and percentage of eosinophils in the BALF cell differential count ($r=-0.129$, $p = 0.3$).

Missing samples

Samples were missing from three different foals on one or more occasions, for various reasons. On farm 1, BAL was not performed on sampling occasion five in foal 1:2 due to distress, coughing and tracheal hyperreactivity during endoscopy. On farm 2, foal 2:5 was not available for sampling on the first sampling occasion, and the BAL sample obtained in this foal on sampling occasion 6, was not of diagnostic quality, containing respiratory epithelial cells. Due to lameness, foal 2:3 was euthanized after sampling occasion 5; the euthanasia was performed by the owners own veterinarian and unrelated to the study. An extra sampling occasion (BAL and faecal samples only) was performed in foal 2:1 and foal 2:5 (Table 2). In addition, due to distress in the foal, blood samples were not obtained from foal 2:1 on sampling occasion 6.

Discussion

The results of present study showed that a mild pulmonary eosinophilia was common in foals naturally exposed to *Parascaris* spp. infection. Specifically, an eosinophilic differential cell count of 2–3% in the BALF was observed in 70% of foals/yearlings aged between 6.5–15 months on at least one sampling occasion. However, a marked eosinophilic elevation of a magnitude considered of clinical significance in adult horses diagnosed with MEA (> 5%) (17) was rare and only present in two out of the ten sampled foals. Furthermore, there was no clear association between *Parascaris* spp. pulmonary larval migration and eosinophilic pulmonary inflammation in these two foals, with neither foal exhibiting a patent infection at the expected time, i.e. 2–3 months after the demonstrated eosinophilic elevation in the BALF (18).

Interestingly, none of the foals, regardless of mild or marked eosinophilic elevation in the BALF, showed overt respiratory signs such as coughing or abnormal pulmonary auscultations. Additionally, although endoscopic findings showed mild mucous accumulation in the trachea to be common, there was no significant association between tracheal mucous grade and eosinophilic cell differential count. Of note, none of the BAL samples showed abnormal elevation of other inflammatory cell types, e.g. mast cells or neutrophils, cells that are also commonly associated with MEA in adult horses. The cut-off values for

normal differential cell counts in the BALF used were that of adult horses (17) and consistent with the results of the present study, published data indicate that eosinophil levels in normal foals could be higher than adults, with a mean eosinophil percentage of 2.2% found in six-month-old foals subjected to BAL (19). This possible age-related difference in normal cell differential counts in BALF could offer a possible explanation for the lack of abnormal respiratory signs associated with mild pulmonary eosinophilia in foals. However, because of their young age, any possible effect that the elevated pulmonary eosinophils in the studied population could have on performance remains unknown.

Exposure to *Parascaris* spp. in the foals in the present study, grazing on infected pastures, would be expected to result in a continuous intake of infected eggs, with different larval and mature parasite stages present in each foal simultaneously. Thus, should larval pulmonary migration result in clinically significantly elevated eosinophils in BALF, presumably the chances of finding such an elevation with monthly BAL sampling would be expected to be very high. Considering the *Parascaris* spp. life cycle, with larvae reaching the lung at around two weeks post-infection, and a pre-patent period ranging from 80–104 days, the peak in eosinophilic pulmonary inflammation in the studied foals would have been expected to occur at two to three sampling occasions prior to the sampling occasion when eggs were detected in the faeces (18, 20, 21). In the present study, neither of the two foals with clinically significant eosinophilic elevation in the BALF had a positive faecal sample within this time-period, when considering the first BAL sample with eosinophilic inflammation. Collectively, the findings in the present study do not appear to support *Parascaris* spp. larval migration as a cause of elevated eosinophils in BALF, despite the prominent eosinophilic pulmonary inflammation after experimental *Parascaris* spp. infection demonstrated in early necropsy studies (21).

Our findings support previous reports of an age-related decline in patent *Parascaris* spp. infections. Patent infections were more common during the first half of the study period, when the foals were 6.5–12 months old, than during the latter half, when they were 9.5–15 months old.

This is in line with studies showing an age-related immunity and lower probability of patent infection in older foals (12). Normally, egg excretion will begin at 3–4 months of age, with peak levels at five months (22) and a second peak of egg excretion at 8–10 months (23). Additionally, patent infection occurred only in 50% of foals experimentally infected at 6–12 months of age (12). Thus, although seasonal variations in the magnitude of egg excretion were demonstrated in one study (22), the consensus is that age is the major factor influencing *Parascaris* spp. patency.

Although our findings suggest that pulmonary larval migration of *Parascaris* spp. may not be associated with a marked increase in BALF eosinophils, several limitations should be considered, and a potential association cannot be excluded. First, the foals were naturally exposed to *Parascaris* spp., and consequently, neither the exact timing of infection nor the infection dose was known. This made it difficult to relate changes in BALF eosinophils directly to the timing and intensity of larval migration. Second, because of the invasive nature of the BAL procedure, relatively old foals were included in the study. The foals were 6.5–10 months old at the start of the study and 11.5–15 months old at the final

sampling. As immunity to *Parascaris* spp. develops progressively with age, older foals are less likely to develop patent infections, even though exposure and larval migration may still occur. Consequently, faecal detection of *Parascaris* spp. eggs may not reliably reflect ongoing or recent larval migration in this age group (24). This limits our ability to determine whether the absence of a marked eosinophilic response in BALF was related to limited larval exposure or to the pulmonary response itself.

Currently, the only diagnostic method available to confirm *Parascaris* spp. infection is analysis of faecal samples for the presence of parasite eggs and a patent infection. However, pre-patent diagnostic methods for detecting parasite infections have been developed for some parasites. For example, a serological test for antibodies against the equine tapeworm, *Anoplocephala perfoliata*, is now commercially available (25) and in humans and pigs, ELISA assays for detecting ascaris-specific antibodies have been developed (26). Further, over recent years, microRNA have received attention both in human and veterinary medicine as potential stable biomarkers of various disease processes (27–29). Interestingly, annotation of the microRNAome of another equine parasite species with larval tissue migration, *Strongylus vulgaris*, was recently published, and marks an important step in the goal for pre-patent detection of parasite infections (30). Future research to develop stable biomarkers, such as parasite-specific miRNAs, for migrating equine parasites, including *Parascaris* spp., will be of substantial benefit for correct diagnosis and early intervention of possible pathological manifestations of parasite larval migration.

Conclusion

Although the only pulmonary inflammatory phenotype demonstrated in foals naturally infected *Parascaris* spp. was an eosinophilic inflammation, no clear association between pulmonary larval migration and elevated eosinophils in BALF could be shown. An experimental design allowing knowledge of the exact time of parasite infection and/or the future development of reliable pre-patent biomarkers for *Parascaris* spp. infection are likely required to definitively exclude a possible contribution of *Parascaris* spp. infection to elevated eosinophils in BALF in young horses.

Materials and methods

Animals and housing

Two Standardbred trotter stud farms were recruited for the study and written informed consent was obtained prior to commencement of sampling. From each farm, a total of five foals were included (Table 4).

Table 4
Age and sex of included foals from two Standardbred trotter stud farms.

Farm 1 – foal ID	**Age at start of study**	**Sex**
1:1	10 months	Filly
1:2	6.5 months	Filly
1:3	7 months	Filly
1:4	7 months	Filly
1:5	6.5 months	Filly
Farm 2 – foal ID	**Age at start of study**	**Sex**
2:1	8 months	Colt
2:2	9 months	Colt
2:3*	8 months	Colt
2:4	8.5 months	Filly
2:5**	8 months	Filly
* Euthanized prior final sampling occasion ** Not included on first sampling occasion		

Farm 1

Farm 1 consisted of an approximate total of 130 Standardbred racehorses, including foals and yearlings, with around 28–45 annual foalings. All foals were kept in an outdoor housing system with access to an outdoor shelter and paddock, from the time of study commencement until April. Thereafter, mares and foals were kept on 24-hour turn out at pasture. Straw was used for bedding and feed consisted of haylage fed from a hayrack with additional concentrates and minerals. Horses from farm 1 visited other equine establishments approximately twice annually.

Farm 2

Farm 2 consisted of an approximate total of 40–60 Standardbred racehorses, including foals and yearlings, with around 12–20 annual foalings. All foals were kept indoors in loose boxes at night with outdoor turnout in paddocks for 15–20 hours daily from the time of study commencement until April. Thereafter, mares and foals were kept on 24-hour turn out at pasture. Straw was used for bedding and feed consisted of haylage fed from a hayrack (outdoors) or on the ground (indoors), with additional concentrates and minerals. Horses from farm 2, the older horses visited other equine establishments, especially for competing, on a weekly or monthly basis.

Anthelmintic routines and resistance status

On farm 1, there was documented resistance of *Parascaris spp*. to macrocyclic lactones, pyrantel and fenbendazole (31, 32). The farm did not implement any routine anthelmintic treatment of foals against *Parascaris spp*. Instead, regular faecal samples were obtained from all foals from two to three months of age and treatment with fenbendazole was given if a sample showed ≥ 100 EPG of *Parascaris spp*. Prior to the study, treatment with fenbendazole had been given to foal 1:1 on 17 June 2024 and to foals 1:2 and 1:5 on 27 August 2024.

On farm 2, all foals were treated with fenbendazole without prior faecal samples twice, at 10–11 weeks of age and at 19–21 weeks of age. During the study period, treatment with fenbendazole was given if the faecal sample showed > 100 EPG of *Parascaris spp*. The resistance status of farm 2 was unknown.

Clinical examination

All included foals were sampled on six separate occasions, approximately four weeks apart. On farm 1, sampling was performed from December 17 2024 until May 6 2025, and on farm 2, from January 30 2025 until June 16 2025. On each occasion, the foals were subjected to a clinical examination, including body temperature measurement, auscultation of the heart and lungs, assessment of the mucous membranes, submandibular lymph nodes, presence of any nasal discharge or spontaneous cough, and response to manual elicitation of a cough response.

Blood sampling

All blood samples were collected by jugular venipuncture via a vacutainer system into EDTA tubes and serum separator tubes (BD, Franklin Lakes, NJ, USA) for plasma and serum analysis, respectively. Serum samples were allowed to clot at room temperature and EDTA samples kept on wet ice until centrifugation. All blood samples were separated by centrifugation at 2,000 *g* for 15 min and the plasma or serum fraction thereafter immediately collected, batched, stored on wet ice until transferred to storage at -70°C before further analysis.

Analysis of inflammatory markers

Analysis of fibrinogen was performed on thawed aliquots of plasma, using the clinical chemistry analyser DxC 700 AU (Beckman Coulter AB, Bromma, Sweden) and the reagent Diazyme Fibrinogen (Diazyme Laboratories, DiaSystem Scandinavia AB, Huskvarna, Sweden), at the Clinical Pathology Laboratory at the University Animal Hospital, Swedish University of Agricultural Sciences. Analysis of serum amyloid A (SAA) was performed on thawed aliquots of serum, using the clinical chemistry analyser DxC 700 AU (Beckman Coulter AB, Bromma, Sweden) and the reagent Reagens Eiken VET-SAA (Eiken Chemical Co, Amsterdam, Netherlands), at the Clinical Pathology Laboratory at the University Animal Hospital, Swedish University of Agricultural Sciences.

Endoscopic examination

After sedation with an intravenous injection in the jugular vein of 3 or 4 mg of detomedine per foal (Domosedan 10 mg/ml, Orion Pharma Animal Health, Danderyd, Sweden) and 3 or 4 mg of butorphanol per foal (Butomidor 10 mg/ml, Salfarm Scandinavia AB, Helsingborg, Sweden), dosage depending on size and age of the foal, an endoscope (1,4 m, EV-7828P, Scandivet, Enköping, Sweden) was passed through one of the nostrils for videoendoscopic examination of the pharynx and trachea. All examinations were video recorded and later analysed for tracheal mucous grading (grades 0–5 according to Coutil et al., 2016) by two

separate operators blinded as regards to the identity of the foals.

Broncho-alveolar lavage

A 10 mm in diameter, 240 cm long Broncho-Alveolar-Lavage Catheter with balloon (50 cc) (Scandivet AB, Enköping, Sweden) 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, 10 ml of mepivicain (Mepidor, 20 mg/ml, Salfarm Scandinavia AB, Helsingborg, Sweden) diluted in 40 ml of sterile saline was infused to minimise discomfort. Once in place, the tube was cuffed with 10 ml of air. Thereafter 3x100 ml of sterile saline was infused through the tube and immediately recovered. The sample was thoroughly mixed before 10 ml was transferred into a plastic sample vial and a further 8 ml transferred to a second plastic sample vial containing 2 ml of chilled albumin. Both samples were kept on wet ice for transportation and thereafter refrigerated at 4 ℃ until analysis the following day, within 24 hours of collection.

Broncho-alevolar lavage analysis

Broncho-alveolar-lavage samples were analysed at the Clinical Pathology Laboratory at the University Animal Hospital, Swedish University of Agricultural Sciences within 24 hours of collection. 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.

Faecal samples

A faecal sample from each foal on each sampling occasion was obtained either from the stable or, if the foal had not defecated, manually from the rectum. Faecal egg counts (FECs) were performed using a modified McMaster technique (33). In brief, 3 g of each faecal sample were flotated using saturated NaCl solution (SG = 1.18 g/cm^2) resulting in a multiplication factor of 50.

Statistical calculations

Spearman's rank correlation coefficient was calculated in GraphPadPrism (version 10.6.1, 2025), for the association of eosinophilic differential cell count in BALF and tracheal mucous score and the association of eosinophilic differential cell count in BALF and *Parascaris spp*. EPG.

Abbreviations

MEA
mild-moderate equine asthma
BAL
broncho-alveolar lavage
BALF
broncho-alveolar lavage fluid
EPG
eggs per gram faeces

Declarations

Ethics approval and consent to participate

The study was approved by the Uppsala animal welfare ethics committee (Dnr 5.8.18–03404/2025). All owners provided written consent for the use of data for publication and were informed that the foals' identities would not be disclosed.

Competing interests:

None declared.

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Author Contribution

E.T. and Y.H.A. wrote the main manuscript text; Y.H.A. prepared the tables and original data files; Y.H.A. and M.L. blindly evaluated the endoscopy videos; E.T., Y.H.A., A.Ö.F., Z.Z., M.L. performed the practical data and sample collection, handling and preparation; A.H. analysed and interpreted the BALF samples; M.L. participated in providing text and sample analysis; Y.H.A. performed statistical analysis; all authors reviewed the manuscript.

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Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

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