Date: 22-12-14

Final report

Strongylus vulgaris: Approaches for non-chemical control and novel diagnostic opportunities

Project number: H-18-47-389

Project period:
2019-2021. Prolonged six month with the final report to 22-12-31.

Main applicant:
Eva Tydén, Swedish University of the Agricultural Sciences, eva.tyden@slu.se

Co-applicant(s):
All active co-applicants in the project:
Ylva Hedberg-Alm, UDS; Eva-Osterman-Lind, SVA; Miia Riihimäki, SLU, Tina Phil, University of Copenhagen

Main report (max. 10 pages)

Introduction

The parasitic worms of horses have developed resistance to anthelmintic drugs, which is a growing threat to the health of horses. Like antibiotics, anthelmintic drugs must be used restrictively to stay active for as long as possible. It is crucial for the future of the horse industry that long-term sustainable practices for parasite control, including grazing hygiene, are applied to slow down the development of anthelmintic resistance without at the same time leading to increased parasite damage.

Small and large strongyles

The great majority of horses in Sweden are leisure or sports horses that do not have access to extensive grazing areas. Parasite eggs are accumulated in the paddocks and there is a need for appropriate prevention methods to protect horses from parasite related diseases. The strongyle nematodes are the most important internal parasites of the horse worldwide. The strongyles are divided into two groups; i) small strongyles – the cyathostominae, and ii) large strongyles including the most pathogenic species, *Strongylus vulgaris*. These parasites are ubiquitous and live as adults in the large intestine of the horse. The life cycle of strongyle species is direct where eggs, which are passed out with faeces, develop into larvae on the pasture. Strongyles exhibit three sequential larval stages, first (L1), second (L2), and third (L3), where L3 is the infective stage. Thereafter the life-cycle is somewhat different between cyathostominae and *S. vulgaris*. The L3 of cyathostominae exsheath in the small intestine before they enter the large intestinal wall where they moult to L4 and subsequently enter the gut lumen as adults [1]. The life-cycle of *S. vulgaris* includes migration of larvae to the cranial mesenteric arteries where the

The project has been financed by:

STIFTELSEN HÄSTFORSKNING

larvae stay for several months and develop to L4 and subsequently to L5 before migrating downstream to enter the lumen as adults in the large intestines [2].

The pathogenicity of *S. vulgaris* is related to the migration of larvae in the mesenteric arteries where arteritis, hemostatic changes and thrombosis may cause thrombo-embolic colic with non-strangulating intestinal infarctions (NSII) [3]. Horses with NSII are difficult to manage clinically since they may not show typical symptoms or fulfill the criteria for colic surgery. Virtually all grazing horses are infected with cyathostominae, and low numbers of adult stages are considered harmless. However, a serious clinical syndrome termed larval cyathostominosis may occur following synchronized reactivation of inhibited larvae in the gut mucosa and subsequent mass emergence of L4 into the intestinal lumen [1].

The aims of this project

The horse is natural host to a large variety of parasites of which the stronyle nematode *Strongyle vulgaris* is the most pathogenic and most feared among horse owners worldwide. The unforeseen observation of an increased prevalence of the strongyle nematode *S. vulgaris* in Sweden constitutes a reestablished threat against equine health and the economy in horse management. However, owing to growing problems with multidrug resistance among the small strongyles, frequent anthelmintic treatment of all horse is not a sustainable solution. The three tasks are described below.

i. *Task I: Survival of infective S. vulgaris and cyathostominae larvae on pasture*
ii. *Task II: Alternative preventive method to control S. vulgaris and cyathostominae*
iii. *Task III: Novel markers for early diagnosis of infection with migrating S. vulgaris larvae*

Material and methods

Grass sampling and harvesting of strongyle larvae

All grass samples, performed in Task I and II, were collected from the pastures in the morning before 10 am. For each sample, a pinch of grass, approximately 0.5 cm in diameter, was cut with scissors close to the ground. Larvae were collected using the Baermann funnel method. From each sample the total number of L3 was counted. Grass samples were dried and weighed, and estimates of larvae per kilogram dry matter of grass were calculated (L3/kg DM). In addition, for the overwintering study, L3s were identified to genus level [17]. All studies were performed in Knivsta (latitude 59, longitude 17).

Task I: Survival of infective S. vulgaris and cyathostominae larvae on pasture:

On 14 May 2020, a pasture free from parasites was experimentally infected with faeces from ten horses naturally infected with cyathostominae and *S. vulgaris*. Prior to infection, the pasture was cut to a height of 10 cm. From each horse, two faecal pats, each weighing 2 kg, were placed in a row at a distance of one metre between pats. Grass samples were collected once per month from July 2020 until no larvae could be detected (April and May 2022). No samples were obtained in January-March 2021 or December 2021-March 2022 due to the ground being frozen and/or covered in snow. Each month, pinches of grass were collected as described above. Additionally, L3 were identified as cyathostominae or *S. vulgaris.*

Task II: Alternative preventive method to control S. vulgaris and cyathstominae: Harrowing and Predatory fungi

Harrowing: In the autumn of 2019, a parasite-free pasture was experimentally infected during a three-week period (5-20 May) with faeces from four horses shedding between 300-500 EPG. Faeces from the four included horses were collected in the morning and mixed thoroughly.

Thereafter, faeces from the mixture were weighed and placed on the pasture in 2 kg pats, at a distance of one meter within and between rows. An equal number of faecal pats were placed on pastures A and B. On 23 June, pasture A was harrowed twice using a Zocon W64 greenkeeper, with a six-meter width and four rows of stars. The date of harrowing was based on a weather forecast of a low risk of rainfall within the coming week. Pasture B was left untreated and served as the control. Grass samples were collected, as described above from pastures A and B in the morning approximately every second week from 7 July 2020 until 7 July 2021. No samples were collected in December to March due to snow/ice on the pastures. In total, grass samples were collected on 19 occasions during the study period.

Duddingtonia: In 2020, eight horses naturally infected with cyathostominae were divided into two groups. Four horses were fed with chlamydospores of *D. flagrans* with dose of 9×10^4 spores/kg/day. Faeces from both groups were collected on day 5, 10 and 15. Three pats per horse with a weight of 2.0 kg were placed on a parasite free pasture for evaluation of larval migration. Grass were harvested, as described above, (0-20 cm) around and under each faecal pats week 2, 4 and 6 post placement (7th July to 4th August). Grass plots for harvesting was outlined using a frame 70x70 cm. Larvae were harvested and the total amount of larvae were estimated.

Task III: Novel markers for early diagnosis of infection with migrating S. vulgaris larvae:

Clinical features to differentiate non-strangulating intestinal infarction (NSII) from idiopathic peritonitis: In a retrospective clinical study, medical records from cases diagnosed with NSII or idiopathic peritonitis from three equine referral hospitals in Sweden during 2017-2020 were reviewed. Information including demographic data, relevant medical history, and clinical- and laboratory parameters were obtained from patient records. Clinical and laboratory parameters, clinical progression and initial response to antimicrobial treatment as well as survival-rates were compared between horses diagnosed with NSII and horses diagnosed with idiopathic peritonitis.

Plasma-thromboelastography (TEG) and the Calibrated Automated Thrombogram (CAT) assay to identify hemostatic changes in horses with migrating *Strongylus vulgaris* larvae in the horse: Hemostatic changes in horses infected with *S. vulgaris* without clinical signs of disease and horses with thrombotic disease (NSII) secondary to *S. vulgaris* larval migration were investigated using plasma-TEG and CAT. Additionally, hemostatic changes in clinically healthy horses and horses with severe GI diseases with similar clinical signs and laboratory findings as horses with NSII (idiopathic peritonitis, enterocolitis and strangulating intestinal lesions) was also determined. All horses had a clinical examination, full hematology and biochemistry blood analysis including SAA and fibrinogen, *S. vulgaris* antibodies, and d-dimer. In addition, citrated plasma samples were analysed with plasma-TEG and CAT.

Results

Task I: Survival of infective S. vulgaris and cyathostominae larvae on pasture:

Both *S. vulgaris* and cyathostominae L3 were detectable in the grass for 17 months and two winter seasons are needed for elimination of L3 on a contaminated pasture. In detail, cyathostominae L3 in grass samples, larval yield increased significantly from July 2020 ($p < 0.01$) and August 2020 ($p < 0.05$) to September 2020, which was the month when the greatest average larval yield was found. There was no significant reduction in larval counts from December 2020 until sampling could commence after snow and ice in April 2021. A successive reduction in larval yield was observed over the spring months in 2021, and a significant reduction from the larval yields in September, October and December 2020 was found from

June 2021 onwards ($p < 0.01$). The last month when L3 were detected in any of the samples was November 2021. From December 2021 to March 2022, sampling was again impeded by ice and snow. Sampling in April and May 2022 produced zero larval yields in all samples (Figure 1A).

For *S. vulgaris* L3 in grass samples, L3 levels were substantially lower than those of cyathostominae, with the greatest monthly larval yield found in December 2020. A significant reduction in larval yield from the amount of larvae found in October and December 2020 was observed in November 2021, when *S. vulgaris* larvae were only found in one sample ($p < 0.05$). No significant reduction in larval counts was found from prior to freezing (December 2020) until sampling again commenced in April 2021 ($p > 0.9999$). When sampling commenced after the second winter, in April and May 2022, it showed zero larval yields (Figure 1B). For more detailed information see publication Osterman-Lind, et al (2022).

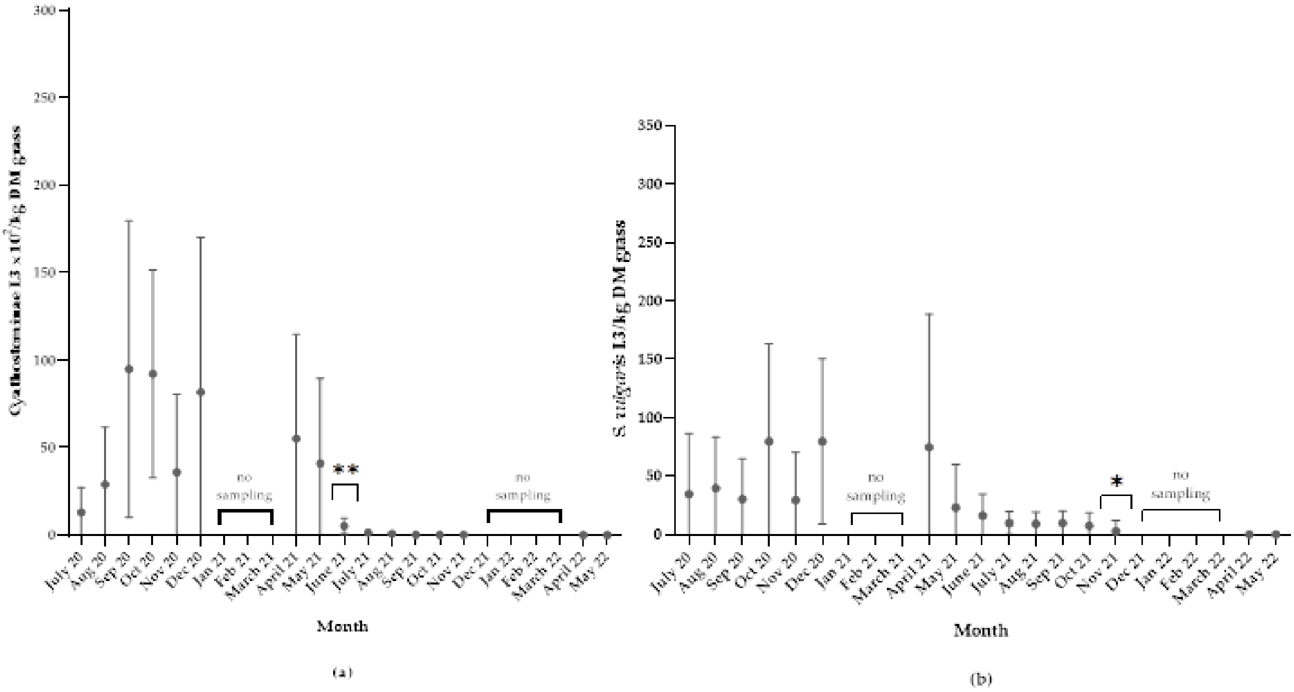

Figure 1 (a) and (b). Scatter-plot showing: (a) Cyathostominae larval yield (L3 x 102/kg DM grass) and (b) S. vulgaris larval yield (L3/ kg DM grass) during the overwintering study. Note the different scales on the y-axis for Cyathostominae (L3 x 102/kg DM) and S. vulgaris (L3/kg DM).

Task II: Alternative preventive method to control S. vulgaris and cyathostominae: Harrowing and Predatory fungi

Harrowing: Harrowing resulted in no overall significant difference in larval yield compared with that of the control field ($p = 0.977$). However, it did result in a significantly higher density of L3/kg DM grass in pasture A compared with pasture B in sampling week 18 ($p < 0.01$). In sampling week 26, a sudden rise in L3/kg DM grass was noted in the untreated pasture (B) ($p < 0.0001$), which appeared to correspond with an increase in rainfall in September, although no statistically significant correlation was found ($p = 0.584$). For the other sampling weeks, no significant differences in the number of L3/kg DM grass were observed between the harrowed pasture (A) and the untreated pasture (B). The mean (± SD) number of L3/kg DM grass over the whole study period was 2646 (± 4901) in the harrowed pasture (A) compared with 2880 (±5739) in the control pasture (B) (Figure 2). For more detailed information see publication Osterman-Lind, et al (2022).

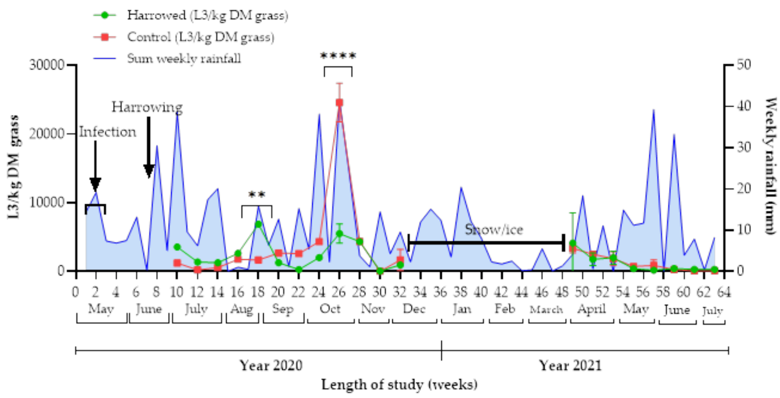

*Figure 2. Harrowing study, 2020-2021: Larval yield (mean L3/kg DM grass ± SD) in the harrowed pasture (pasture A) and control pasture (pasture B) and weekly rainfall (mm) in each sampling week (1-63). Time of infection and harrowing are indicated with arrows. No sampling was performed during weeks 33–48 due to the ground being frozen. ** p < 0.01; **** p < 0.0001. All error bars cannot be graphically shown due to very low variation between readings.*

Duddingtonia flagrans: The analysis of the effect of *D. flagrans* (Bioworma) on recovery of L3 larvae was performed under laboratory conditions. Recovery of larvae was compared between untreated control horses and horses fed with *D. flagrans* for one week and two weeks. The results are displayed in figure 3 A and show that horses that are fed with *D. flagrans* have a reduction in larvae. This implies that the larvae are trapped in the net of *Duddingtonia* and are not capable of migrating out from the faeces. To analyse if *D. flagrans* reduces the number of larvae on the grass an experimental plot study performed. Results show a slight reduction of L3 in the grass surrounding the faecal pats (Figure 3 B).

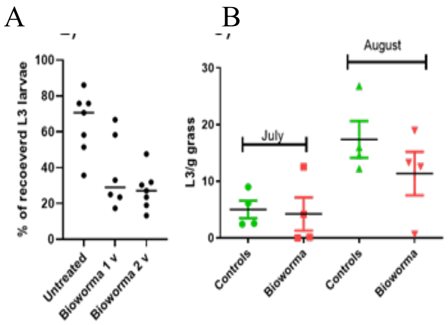

Figure 3 A. The results show that larvae is reduced in horses fed with Bioworma (*Duddingtonia)*.
Figure 3 B. Shows number of L3 in the grass surrounding the faecal pats. There is an indication of reduction of number of infective L3, indicating that larvae a trapped in the net produced by the *Duddingtonia* fungi.

Task III: Novel markers for early diagnosis of infection with migrating S. vulgaris larvae:

Clinical features to differentiate non-strangulating intestinal infarction (NSII) from idiopathic peritonitis: Horses with NSII (n = 20) were significantly more likely to present during the winter months with a poorer response to medical treatment within 48 hours. Cases of idiopathic peritonitis (n = 107) had a 100% survival rate with medical treatment, although one case required surgical correction of a colon displacement. In comparison, all confirmed NSII cases were non-responsive to antimicrobial treatment, with a survival rate to discharge of 50% after colon resection. Specific rectal findings and peripheral blood neutropenia were strongly associated with NSII. For more detailed information see Hedberg-Alm (2022)

Plasma-thromboelastography (TEG) and the Calibrated Automated Thrombogram (CAT) assay to identify hemostatic changes in horses with migrating Strongylus vulgaris larvae in the horse: NSII horses had a higher maximum amplitude (MA) in the plasma-TEG and an increased lagtime and time to peak (ttPeak) in the CAT assay compared to horses with enterocolitis as well as a higher MA in the plasma-TEG than horses with strangulating lesions. The *S. vulgaris* NSII negative group had a shorter R time (the time until the first evidence of a clot is detected) and K time (the time from the end of R until the clot reaches 20mm, that is, speed of clot formation), a significantly higher angle and MA in the plasma-TEG and a longer lagtime in the CAT assay compared to the clinically healthy horses.

Conclusions

Survival of infective *S. vulgaris* and cyathstominae larvae on pasture: Although the infective strongyle larval stage was able to survive one winter season, larvae numbers were significantly reduced by the next summer, suggesting that resting pastures for one year in a cold temperate climate will result in a substantially reduced infectivity level. For parasite-free pastures, however, they should be rested from grazing horses for a period of two years.

Alternative preventive method to control *S. vulgaris* and cyathstominae: The management practice of harrowing on a single occasion during the grazing season in a Nordic climate, after L3 have developed, did, however, not reduce the number of infective larvae, despite resting the pasture. The use of the nematophagous fungi *D. flagrans* as a feed additive shows potential as a means of lowering pasture parasite infectivity levels, but further studies to determine optimal dosage and effect under field conditions would be beneficial.

Clinical features to differentiate non-strangulating intestinal infarction (NSII) from idiopathic peritonitis: The results support medical treatment and a cautious approach to surgical decision in cases of peritonitis in countries such as Sweden where idiopathic peritonitis cases predominate. However, the results also suggest that, until specific markers for diagnosing *S. vulgaris* larval migration become available, clinicians working in countries where the parasite is endemic, should still be aware that horses presenting with septic peritonitis, particularly during the winter months, could suffer from intestinal infarction secondary to larval migration. In order to identify potential surgical cases, horses with septic peritonitis should be carefully evaluated, with particular emphasis on findings of rectal palpation and initial response to antimicrobial treatment.

Plasma-thromboelastography (TEG) and the Calibrated Automated Thrombogram (CAT) assay to identify hemostatic changes in horses with migrating Strongylus vulgaris larvae in the horse: Plasma-TEG and the CAT showed significant hemostatic changes in horses with *S. vulgaris* with and without clinical disease. Horses with *S. vulgaris* without clinical disease had hemostatic changes that can be interpreted as hypercoagulability compared to the clinically healthy horses. Horses with *S. vulgaris* with clinical disease (NSII) had hemostatic changes than can be interpreted as both hypo- and hypercoagulable compared to the other severe gastrointestinal diseases studied. The global hemostatic tests seem valuable in establishing the horse's hemostatic balance and hence aid in the assessment of the horse's risk of having thrombotic disease and potentially guide the initiation of treatment and the prognosis, regardless of gastrointestinal diagnosis.

Relevance for the practical horse sector incl. recommendations

The most relevant outcomes from this project to the horse sector:

- Harrrowing on a single occasion during dry weather in the summer: no positive effect and not to be used if the aim is to reduce parasite infectivity and should be discouraged.
- Pastures infected with *S. vulgaris* need to be rested to two years in order to be deemed free of the parasite and can be used on horse farms with positive *S. vulgaris* horses to help eradicate the infection.

- Resting pastures for one year can be used to reduce infectivity level (eg. small strongyles) and could be used as part of a pasture management program on horse farms.
- The use of the nematophagous fungi *D. flagrans* as a feed additive shows potential as a means of lowering pasture parasite infectivity levels, but further studies to determine optimal dosage and effect under field conditions would be beneficial.
- Equine veterinarians can use the results of the study to help decide if abdominal surgery is needed in peritonitis cases, by evaluating horses with careful rectal palpation, monitoring the initial 48 hours response to antibiotic treatment and take into account month of presentation.
- Future studies are needed including a larger number of horses with different GI diseases to better clarify the use of TEG and CAT to help with diagnosis of *S. vulgaris* associated disease, before implementation in clinical practice.

To be able to implement the results of pasture management, horse owners (and advisory equine veterinarians) need to be made aware of these alternatives to drug use. We have therefore produced an updated recommendation for Swedish equine owners and equine veterinarians highlighting the use of selective anthelmintic treatment and the importance of use of pasture management practices, such as faecal removal. The recommendation will be available on several equine and veterinary websites to download as well as available in a pamphlet form for distribution ex. via equine clinics, see results dissemination.

References

1. Ogbourne, (1972) *Observations on the free-living stages of strongylid nematodes of the horse*, Parasitology 64, 461-477 **2.** Urquhart et al (1996) *Veterinary parasitology, 2nd ed. Blackwell Science, United Kingdom*, 42-47 **3** Phil et al (2017) *Nonstrangulating intestinal infarctions associated with Strongylus vulgaris: Clinical presentation and treatment outcomes of 30 horses (2008-2016).*Equine Veterinary Journal, 50, 474–480 **4.** Matthews (2014) *Anthelmintic resistance in equine nematodes* International Journal Parasitology Drugs Drug Resistance, 4, 310-315 **5.** Nielsen et al (2006) *Prescription-only anthelmintics--a questionnaire survey of strategies for surveillance and control of equine strongyles in Denmark.* Veterinary Parasitology, 135, 47-55 **6.** Nielsen et al (2012) *Strongylus vulgaris associated with usage of selective therapy on Danish horse farms-is it reemerging?* Veterinary Parasitology, 189, 260-266

Part 3: Result dissemination

Scientific publications, *published*	*Author(s), year, title, journal, Vol, No, pp. (doi/link if applicable)*
	Hedberg-Alm Y, Tydén E, Tamminen LM, Lindström L, Anlén K, Svensson M, Riihimäki M. (2022) Clinical features and treatment response to differentiate idiopathic peritonitis from non-strangulating intestinal infarction of the pelvic flexure associated with *Strongylus vulgaris* infection in the horse. BMC Vet Res. Apr 23;18(1):149. doi: 10.1186/s12917-022-03248-x.
	Osterman-Lind, E.; Hedberg Alm, Y.; Hassler, H.; Wilderoth, H.; Thorolfson, H.; Tydén, E. (2022) Evaluation of Strategies to Reduce Equine Strongyle Infective Larvae on Pasture and Study of Larval Migration and Overwintering in a Nordic Climate. Animals 2022, 12, 3093. https://doi.org/10.3390/ ani12223093
Scientific publications, *submitted*	*Author(s), title*

Scientific publications, *manuscript*	*Author(s), title*
	M. L. Honoré, L. N. Nielsen, E. Týde2, Y. H. Alm, M. Riihimäki, , M. K. Nielsen, T. H. Pihl Plasma-thromboelastography and the Calibrated Automated Thrombogram assay identifies hemostatic changes in horses with migrating *Strongylus vulgaris* larvae in the horse. To be submittet 2022/23
Conference publications/ presentations	*Author(s), year, title, conference name, location and date, (link if applicable)*
	Ylva Hedberg Alm, 2021, Clinical features and treatment response to differentiate idiopathic peritonitis from non-strangulating intestinal infarction of the pelvic flexure associated with *Strongylus vulgaris* infection in the horse. International Conference of Equine infectious diseases. Digital
	E Tydén, 2022, Hur introducerar man en häst i en ny omgivning? Avmaskning. Hippokampus 2022. Available at SLU play
	E Tydén and Eva Osterman-Lind *Control of equine strongyle infections – the Swedish approach*, Keynote lecture at The 8th Conference of the Scandinavian-Baltic Society for Parasitology (SBSP) and the Annual Meeting of the European Veterinary Parasitology College (EVPC)Author(s), Copenhagen 10-11 October 2019 (Abstract, oral presentation)
Other publications, *media etc.*	*Title, year/date, place of publication (link if applicable)*
	Ylva Hedberg-Alm, Eva Tydén, Miia Riihimäki Lena-Mari Tamminen Lisa Lindström Karin Anlén Maria Svensson, 2022, Kliniska aspekter och behandlingssvar för att särskilja idiopatisk peritonit från tarminfarkt i flexura pelvina orsakad av *Strongylus vulgaris* infektion hos häst. Svensk veterinärtidning, nr. 5, s 32-37.
	Nationella rekommendationer "Hästens mag-tarmparasiter – att förebygga och behandla" Publicerat på flertalet webbsidor exempelvis Vidilab https://www.vidilab.se/wp-content/uploads/2022/11/Hästparasiter-web-FINAL.pdf och UDS.
	Stockholm International Horse Show, Eva Osterman-Lind – synliggörande av de nya nationella rekommendationerna, SVA, november 2022.
	April 2023 kommer avnämare från hästnäringen att bjudas in till ett hybrid event (IRL på SLU eller streamas via webinarium) där de nationella avmaskningsrekommendationerna ska presenteras samt resultat från det här forskninsgprojektet. Vi beräknar ca 500 deltagare.
Oral communication, *to horse sector, students etc.*	*Title, year/date, group presented to (link if applicable)*
	Eva Tydén Förebyggande parasitkontroll hos häst. Nätverksträff för BMA 13 oktober 2022
	Ylva Hedberg Alm Alternative approaches to control of strongyle parasites in horses: Nordic perspectives. Hästforskarträff på SLU. 5 oktober 2022.
	Ylva Hedberg Alm Clinical features and treatment response to differentiate idiopathic peritonitis from non-strangulating intestinal infarction of the pelvic flexure associated with *Strongylus vulgaris* infection in the horse. Hästforskarträff SLU 2021
	Ylva Hedberg Alm International Conference of Equine infectious diseases 2021 digital Clinical features and treatment response to differentiate idiopathic peritonitis from non-strangulating intestinal infarction of the pelvic flexure associated with *Strongylus vulgaris* infection in the horse
	E Tyden, Eva Osterman-Lind Betesplanering och träckprovsanalyser Hästkunskapsdagen 9 november 2019 https://www.lansstyrelsen.se/uppsala/kalenderhandelser---uppsala/2019-08-28-hastkunskapsdagen-2019.html

	E Tydén and Eva Osterman-Lind *Control of equine strongyle infections – the Swedish approach*, Keynote lecture at The 8th Conference of the Scandinavian-Baltic Society for Parasitology (SBSP) and the Annual Meeting of the European Veterinary Parasitology College (EVPC)Author(s), Copenhagen 10-11 October 2019 http://csbsp8evpc2019.eu/PROGRAM.html
Student theses	*Author/Student, co-authors/supervisors, year, title, type of thesis (doi/link if applicable)*
	Veterinary student Asp Tauni, Elinor, 2021, Fodertillskott med rovsvampen Duddingtonia flagrans (BioWorma®) som komplement till avmaskning hos häst. https://stud.epsilon.slu.se/16873/
	Veterinary student Hillevi Hassler, 2021,Harvning som beteshygienisk åtgärd : ett komplement till avmaskning för att minska mängden infektiösa cyathostominaelarver på bete?
	Veterinary student Sjödell, Nina, 2020. Bukhinneinflammation hos häst : en retrospektiv jämförelse av *Strongylus vulgaris* orsakad peritonit och idiopatisk peritonit. https://stud.epsilon.slu.se/15889/ .
	Veterinary student Julia Ling Frilevande larver av Strongylus vulgaris : en experimentell studie. https://stud.epsilon.slu.se/15714/
	Wilderoth, Hanna, 2019. Förebyggande åtgärder för bekämpning av blodmask. https://stud.epsilon.slu.se/14615/
Other	PhD thesis: Marie Louise Honoré Lytzhøft-Olsen, 2022, Elucidating hemostatic aberrations in horses with migrating *Strongylus vulgaris* larvae with and without clinical disease. Time: 29 Apr. 2022, 10:00-13:00. Place: Københavns Universitet, Room 8.51.D105, Building 51, Agrovej 8, 2630 Tåstrup