Final report

Selective Treatment for the Control of Strongyles in horses - Risks and Opportunities

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Part 1: Detailed summary
Short description of objective, method, main results, importance for the horse sector, and recommendations. The summary should be written in Swedish or Norwegian

Den stora blodmasken, *Strongylus vulgaris*, anses vara hästens farligaste parasit. Hästens parasiter har utvecklat resistens och i likhet med antibiotika måste avmaskningsmedel användas restriktivt för att förbli verksamma. Sedan 2007 rekommenderas därför selektiv avmaskning (SAT) av hästar, vilket i praktiken innebär att alla hästar träckprovundersöks och endast de individer som utskiljer en viss mängd parasitägg avmaskas och/eller om *S. vulgaris* påvisas. När det gäller analys av ägg från cyathostomer och *S. vulgaris* går det inte att skilja dem morfologiskt; undersökning med odling eller PCR krävs för diagnostik av *S. vulgaris*. Att enbart använda sig av äggräkning som grund för avmaskning kan medföra en risk för återinträde av *S. vulgaris* eftersom även lågutskiljande hästar kan vara infekterade med denna parasit. Det finns en farhåga att *S. vulgaris* har ökat i Sverige och även att parasitrelaterade skador har ökat under de senaste åren. Syftet med det här projektet var att undersöka (i) förekomsten av *S. vulgaris* och identifiera riskfaktorer, (ii) om ökad parasitförekomst är kopplad till ökning av parasitrelaterade skador och (iii) för första gången i Skandinavien effekten av mockning som beteshygienisk åtgärd för att minska parasitsmitta.

I den första delstudien undersöktes förekomst av *S. vulgaris* och riskfaktorer förknippade med infektion tio år efter införandet av SAT. Träckprover och en enkät om avmaskningsrutiner samlades in från 106 hästgårdar från söder till norr. Resultaten visade att stor blodmask har ökat i Sverige. Vi ser en förekomst på 61% på gårdsnivå jämfört med 15% 1999. Det fanns dock

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inget samband mellan förekomst av *S. vulgaris* och antal blodmaskägg, ålder på hästen eller geografiskt område (söder-norr). Vi identifierade en ökad risk för *S. vulgaris* på gårdar som enbart diagnostiserade parasiter genom äggräkning, men det förelåg inte en ökad risk om gårdarna kombinerade äggräkning med analys för *S. vulgaris*. Det viktigaste budskapet från den här studien är att alltid inkludera analys för *S. vulgaris* i SAT, speciellt hos hästar som utsöndrar inga eller få ägg eftersom den gruppen annars lämnas obehandlade.

I delstudie två var målet at undersöka samband mellan infektion av *S. vulgaris* och kolik hos svenska hästar. Den farliga fasen i *S. vulgaris* infektion är de migrerande larverna i blodkärlen som försörjer tarmen. Larverna ger upphov till inflammation av kärlen, framförallt främre krösroten, med trombbildning som följd och kolik. Larverna ger upphov till inflammation av kärlen, framförallt främre krösroten, med trombbildning som följd och kolik. Studien var en fall-kontroll studie som löpte över ett år och utfördes på universitetsdjursjukhuset i Uppsala. Fall var hästar som uppvisade symtom från mag-tarmkanalen och dessa matchades med kontroller, som var hästar som besökte kliniken under samma vecka men av andra orsaker, till exempel hälta eller tandundersökning. Totalt ingick 137 fall och 137 kontroller. Träckprover analyserades med odling och PCR för *S. vulgaris* och serumprover analyserades med ELISA för att påvisande av antikroppar. Hästägarna besvarade även en enkät om avmaskningsrutiner. Vi fann inga signifikanta skillnader i antikroppstitrar, äggutskiljningsnivåer eller avmaskningsrutiner mellan fallhästar och kontrollhästar. Åtta av de 137 fallen behövde kirurgisk behandling och i ett fall påvisades skador i tarmen kopplade till migrerande *S. vulgaris* larver. Fyra fall-hästar avlivades på grund av dålig prognos och under obduktion diagnostiserades två hästar med *S. vulgaris*-skador kopplade till infarkt i tarmen orsakade av migrerande larver. Värt att notera var att ingen av de tre *S. vulgaris* hästarna utsöndrade parasitägg i träck men samtliga hade en hög antikroppstiter. Som nämnts ovan fann vi inga skillnader när samtliga fall jämfördes med sina kontroller, men när fallen analyserades utifrån kolikdiagnos fann vi att fallhästar med bukhinneinflammation hade signifikant högre antikroppstitrar än kontrollhästarna samt att de två hästar som avlivades och diagnostiserades med *S. vulgaris*-skador ingick i gruppen med bukhinneinflammation. Sammantaget visar den här delstudien inte på någon ökad förekomst av parasiter hos hästar med kolik, men våra resultat tyder på att det finns ett samband mellan *S. vulgaris* och bukhinneinflammation, vilket vi ska undersöka närmare inom ramen för pågående forskningsprojekt

Syftet med den sista delstudien var att undersöka om regelbunden manuell mockning av beteshage kan reducera parasitsmitta på betet och på så sätt utgöra ett effektivt komplement till avmaskning för hållbar parasitkontroll. De avmaskningsmedel som finns att tillgå till häst tillhör tre substansgrupper: 1) bensimidazoler (fenbendazol) 2) makrocykliska laktoner, (ivermektin och moxidektin) 3) tetrahydropyrimidiner (pyrantel). En kraftig överanvändning av avmaskningsmedel sedan 1970-talet har resulterat i att cyathostominer och spolmask har utvecklat resistens i Sverige. Bland cyathostominerna finns en utbredd resistens mot benzimidazoler och effekten av pyrantel är bristande. Därför är det viktigt att använda komplement till läkemedel för att bekämpa parasitsmitta. För att undersöka effekten av mockning delades en parasitfri hage in i två lika delar, A och B. Tre hästar som var infekterade med cyathostominer (300-500 EPG) betade varannan dag i A respektive B. Hage A mockades två gånger per vecka under betesperioden medan B utgjorde kontroll. För att undersöka effekten av mockning analyserades gräsprover från A och B varannan vecka och antalet infektiva cyathostominlarver per kg torrsubstans gräs fastställdes. I hage A hittades 55 larver/kg torrsubstans vid ett enda tillfälle i övrigt hittades inga larver. I B varierade antalet larver mellan 3271-12601/kg torrsubstans gräs. Resultaten visade att mockning två gånger per vecka är mycket effektivt. Detta projekt visar att förekomsten av *S. vulgaris* har ökat i Sverige.

Lösningen på problemet är dock inte att återgå till rutinmässiga avmaskningar på grund av tilltagande problem med resistens hos hästens små blodmaskar och spolmask. Det är avgörande för hästnäringens framtid att man tillämpar långsiktigt hållbara metoder för parasitkontroll. Till exempel är det viktigt att alltid inkludera analys för *S. vulgaris* på våren, speciellt hos hästar som utsöndrar inga eller få ägg eftersom den gruppen annars lämnas obehandlade. Vidare kommer det i framtiden att vara ännu viktigare att kombinera läkemedelsbehandling med alternativa metoder. Våra resultat visar att mockning av beteshage två gånger per vecka är mycket effektivt för att minska blodmasksmitta i hagarna. Det är sannolikt inga nya substanser för avmaskning av häst på väg ut på marknaden och därför måste de medel som finns att tillgå användas på bästa sätt så att resistensutveckling bromsas utan att det samtidigt leder till ökat lidande orsakat av parasiter.

Part 2: 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 cythostostomins, 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 cyathostmines and *S. vulgaris*. The L3 of cyathostomins 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 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 cythostomines, 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].

Anthelmintic drugs and drug resistance

It is necessary to limit the parasite burden especially in foals and young horses. For decades, the cornerstone for controlling parasites has been regular treatments with anthelmintic drugs (Figure 1). There are three major drug classes of broad-spectrum anthelmintics available for horses: i) macrocyclic lactones, ii) benzimidazoles, and iii) tetrahydropyrimidines. However, a frequent use of these drugs has resulted in widespread anthelmintic resistance among populations of small strongyles and roundworms in horses [4].

As only one new class of anthelmintic drugs for use in horses has been introduced on the market in the past 35 years, it is important to use available drugs in a sensible way. To slow down the development of resistance, selective treatment regimens were introduced in the beginning of the 2000s implying that all animals on a given farm must first be diagnosed with strongyle infection before treatment is initiated. Treatment is then applied to horses exceeding a chosen cut-off value, often 200 strongyle eggs per gram (EPG) faeces [5]. However, new data from Denmark indicate that the prevalence of *S. vulgaris* has increased possibly following the introduction of new prescription rules for anthelmintic drugs [6].

The aims of this project

The purpose of this project was to investigate whether selective worming is related to an increase of *S. vulgaris* in Sweden and/or and if there is an increase in parasite-related injuries. For the first time in Scandinavia, we will also measure the effect of pasture hygiene measures in addition to selective worming. We have performed the following sub-studies:

i. Investigated the presence of *S. vulgaris* in Swedish and Norwegian horse herds.
ii. Evaluated if *S. vulgaris* is more prevalent in horses with clinical signs of colic.
iii. Investigated whether regular removal of faeces from the pastures can significantly reduce parasite infestation and thus provide an effective complement to worming for sustainable parasite control.

Material and methods

Prevalence of S. vulgaris in Swedish horse herds (Study 1)

The study was performed during two consecutive years from March to June in 2016 and 2017. 20 horse farms were recruited each year from the three regions in Sweden: south, central and north. The inclusion criteria were: (i) a farm size of at least five horses; (ii) animal age minimum 2 years; (ii) no anthelmintic treatment performed within six months prior to sampling; and (iii) response to a web-based questionnaire about deworming routines (Table 1). After completing the questionnaire, faecal samples from five randomly selected horses older than two years on each farm were collected by the owners or staff working in the stable.

Table 1 Questionnaire data collected on the participating horse farms.

Information	**Descriptor**
Farm location	Zip code
Age of horses	Years
Time since last anthelmintic treatment	Months < 3; < 6; < 12; < 24; < 36; ≤ 48
Anthelmintic	Drug used at last treatment
Signs of colic[a] last 24 months	Yes/No
Tested positive for *Strongylus vulgaris* during the last 24 months	Yes/No
Deworming routines applied on the farm	i) only after FEC[b] ii) after FEC and cultivation for *S. vulgaris* iii) routine deworming 1-4 times/ year

[a] restlessness and pawing at the ground, irritated kicking to the stomach, rolling or attempting to roll [b] faecal egg count

Parasite analysis

Strongyle FECs were carried out for each horse using a modified McMaster technique with a theoretical sensitivity of 50 EPG (Coles et al., 1992). Nematode eggs in faecal samples (3 g) were flotated using a saturated NaCl solution (SG=1.18) (Coles et al., 1992). Irrespective of FEC, larval cultures for detection of *S. vulgaris* were performed on 50 g faeces from each horse according to Bellaw and Nielsen (2015). Pellet of harvested L3 was screened for *S. vulgaris* by real time PCR to amplify a 171 PCR product of the second internal transcribed spacer (ITS-2) of *S. vulgaris* (accession number in GenBank: X77863.1); forward primer 5`GTATACATTAAATTGTGTCCCCCATTCTAG3` and reverse primer 5`TTGCAAATATCATTAGATTTGATTCTTCCG3`. A non-template control and DNA extracted from an adult *S. vulgaris* were run for each reaction. Samples were considered as positive with a threshold value (Ct) <38 with a specific melt curve analyses. Samples with a Ct-value ≥38 were verified to be positive after Sanger sequencing.

Statistical analyses

A mixed logistic regression model was performed using the software R version 3.4.1 was used for all statistical calculations in study 1 and 2. All statistical analyses were interpreted as statistically significant up to p-value ≤ 0.05 and a confidence interval (CI) of 95%.

Prevalence of S. vulgaris in Norwegian horse herds (Study 1)

The study was conducted during two consecutive years from August 2017 to October 2018. Horses from 56 herds were included in the investigation. Horse owners from all of Norway were invited to participate in the study via direct mail. Additionally, the study was announced several times on the Facebook page of the Norwegian Veterinary Institute as well as on the webpages of Norwegian equine breed organisations. The inclusion criteria were: (i) a farm size of at least five horses; (ii) animal age minimum 2 years; (ii) no anthelmintic treatment performed within six months prior to sampling; and (iii) response to a questionnaire about deworming routines (Table 2). The faecal samples were collected by the owners or staff at the farms from at least five randomly selected horses.

Table 2 Questionnaire data collected from the Norwegian horse farms.

Information	**Descriptor**
Farm location	Zip code
Age	Years
Breed	Trotter/warmblood/pony/thorough/døla/fjordh/other
Sex	Stallion/mare/gelding
Time since last anthelmintic treatment	Months 0-3; 4-6; 7-12; >12
Anthelmintic	Drug used at last treatment
Clin. signs (diarrhoea, weight loss, colic) within 2 mths	Yes/No
Deworming routines applied on the farm:	
Grazing available	Yes/No
Separate outdoor winter paddocks	Yes/No
Regular removal of faeces from fields	Yes every week/Yes every month/No
Co-grazing with other animal species	Yes (species)/No
Duration of the grazing/day	1-6h/7-12h/24h/never
Type of grazing	Soil/grass/sand/forrest/other
Number of horses per field	1-3/4-10/>10
Parasites diagnosed within 24 mths	Small str/large str/tapeworms/roundworms/No/?
How do you chose anthelmintic treatment	Vet/Social media/Internet/Other
How often are anthelmintics used	Never/if pos test/once per year/2-4 per year
Do you treat all horses at the same time	Yes/No
How do you dose the drug/weigh the animal	Scale/equine weigh tape/estimate

Parasite analysis

Strongyle FECs were carried out for each horse using a modified McMaster technique with a theoretical sensitivity of 50 EPG (Coles et al., 1992). Nematode eggs in faecal samples (3 g) were flotated using a saturated NaCl solution (SG=1.18) (Coles et al., 1992). Irrespective of FEC, larval cultures for detection of *S. vulgaris* were performed on 50 g faeces from each horse according to Bellaw and Nielsen (2015). Pellets of harvested L3 were analysed with *S. vulgaris* real time PCR as described previously. In addition, 10 g samples from each horse were analysed for presence of the tapeworm *Anoplocephala perfoliata* by flotation and microscopy. Subsequently, presence of *S. vulgaris* eggs in the flotated material was analysed by real time PCR at NVI.

Strongylus vulgaris and colic in Swedish horses – a case-control study (Study 2)

This was investigated in a case-control study conducted from February 2017 to February 2018 at the equine clinic of SLU. A horse presenting to the clinic and diagnosed with disease related to the gastrointestinal canal was classified as a case. Each case was matched with a control horse presenting during the same week and, when possible, of same or similar age (+/- 1-2 years if older than five years of age), and diagnosed with either a disease unrelated to the gastrointestinal canal or presenting for prophylactic reasons (e.g. general health check, oral exam). Fecal and blood samples were collected by veterinarians from each case and control during the horse´s visit at the clinic. In addition, every owner was asked to respond to a questionnaire, with information regarding previous colic history, previous anthelmintic treatment, anthelmintic routines (Tabel 1). Sera were analysed by a *S. vulgaris* antigen ELISA and faecal samples for strongyle eggs with a modified McMaster method and for *S. vulgaris* with an ITS-2 specific PCR on larval cultures (see above for description). See figure 1 for outline of the study.

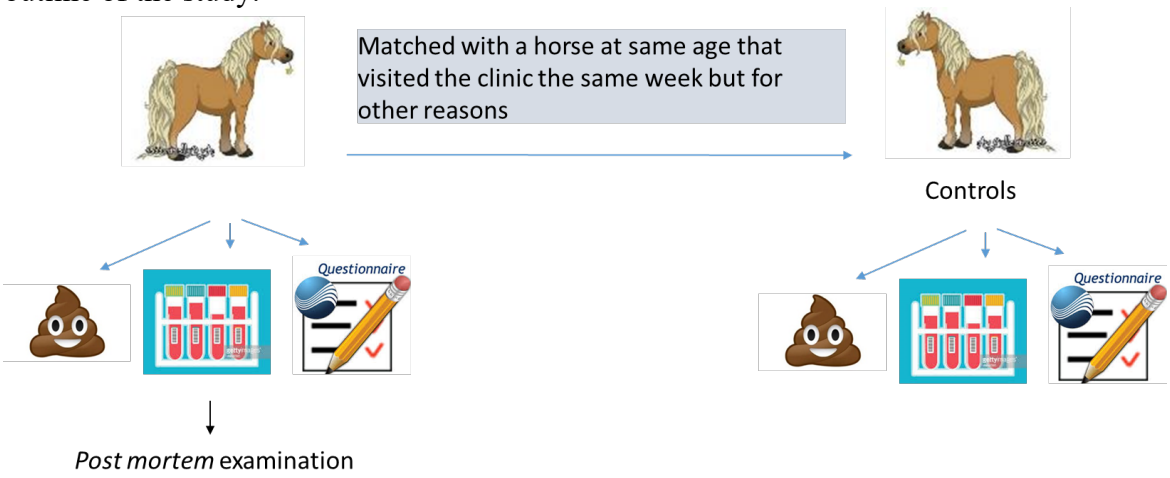

Figure 1. A schematic illustration of the sample collection in the case-control study.

Strongylus vulgaris and colic in Norweigan horses – a case-control study (Study 2)

A case-control study, identical to the Swedish study, was conducted in Norway. The samples (faeces and blood) was collected from November 2017 to March 2018.

The efficacy of faecal removal to reduce the number of cyathostomin larvae on pasture (Study 3)

A parasite free pasture, approximately 3 hectars, was divided into two equal halves, A and B (Fig x). Three horses shedding 300-750 EPG of strongyles were let to graze on the pasture,

every second day in A and every second day in B from 18th June until 24th July 2017. During this period faeces were removed manually twice per week from A but not from B. Faecal samples from the horses were analysed at the start and at the end of the grazing period by using a modified McMaster method. Grass samples were collected in the morning once every second week from 10th June until 24th October 2017 and once per month 17th June- until 2nd November 2018. The method used was as follows: while walking over the pasture in a zig-zag pattern (Fig 2), a pinch of grass, approximately 0.5 cm diameter, was cut close to the ground every seventh step. Approximately 2 x 250 g were collected from A and B, respectively. By using the Baermann funnel method, the larvae in the grass were collected and the number of third stage larvae per kilo dry grass was estimated.

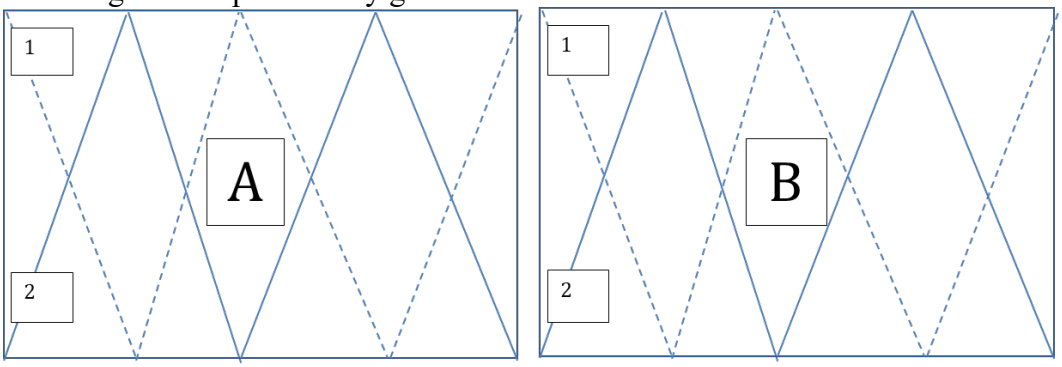

Figure 2. A schematic illustration of A and B and the zick-zack pattern for collection of grass samples.

Results and discussion

Prevalence of S. vulgaris in Swedish horse herds (Study 1)

In total, 529 horses from 106 farms participated in the study; 39 farms from the south, 40 farms from the central and 27 farms from the north of Sweden. Examination of 529 individual larval cultures by PCR showed an overall *S. vulgaris* prevalence of 28% (95% CI: 22–33%) with a mean of 1.4 positive horses out of five examined per farm. No significant differences were found between the years (p=0.15); 32% (95% CI: 24–39%) in 2016, and 24% (95% CI: 16-31%) in 2017. The overall prevalence of *S. vulgaris* at the farm level was 61% (95% CI: 51-70%, 65 of 106 farms), and no significant differences were found between the three regions; 64% (95% CI: 47-79%) in south, 60% (95% CI: 43-75%) in central, and 59% (95% CI: 39-78%) in northern Sweden.

Patent *S. vulgaris* infection was found in all age groups and was not significantly correlated to strongyle FEC levels (Fig. 3A, B). Prevalence of *S. vulgaris* in this study population in relation to latest deworming was based on questionnaire data provided by the horse owners. The prevalence tended to be positively correlated with time since the last deworming (Fig. 3 C), but this was not statistically significant. The only significant risk factor found was the deworming decision applied on the farms. Hence, no association was found between signs of colic within a period of 24 months prior to sampling or a positive *S. vulgaris* test outcome.

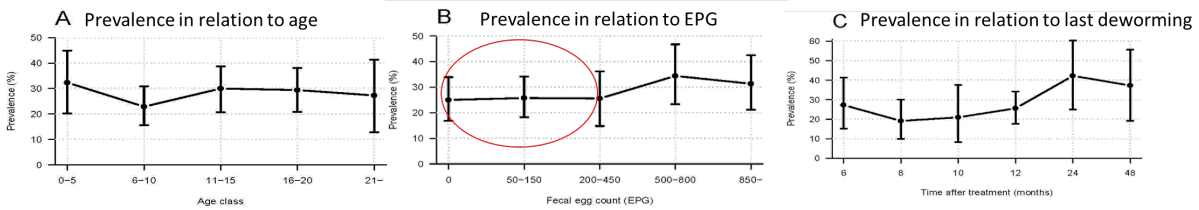

Figure 3. Prevalence of S. vulgaris A) in relation to the age of the horses. The horses were clustered in age groups 0-5; 6-10; 11-15; 16-20 and ≥ 21 years B) in relation to faecal egg counts clustered as follow: 0; 50-150; 200-450; 500-800; ≥850 eggs per g of faeces (EPG) C) in relation to last deworming.

Many horse owners and veterinarians in Sweden have adopted a selective deworming strategy, and since anthelmintic drugs to horses became available on prescription only, in 2008, markedly less anthelmintics have been sold. In parallel, the prevalence of *S. vulgaris* has increased approximately three times compared to the last survey 1999. In this study, we found no association between the prevalence of *S. vulgaris* and egg counts or horse age. The only risk factor for infection with *S. vulgaris* in our study was selective therapy based on strongyle FECs alone, which increased the odds risk of *S. vulgaris* by 2.9. It is important to point out that selective therapy based on a combination of strongyle FECs and larval cultures was not associated with an increased risk of *S. vulgaris* infection compared to regular blanket treatment 1-4 times per year. A key message to horse owners and veterinarians is the importance of including specific diagnostics for *S. vulgaris* even in situations when the excretion of strongyle eggs is low or below the detection limit in individual horses.

Prevalence of S. vulgaris in Norwegian horse herds (Study 1)

In total, 435 horses from 56 farms, representing all geographical regions, were included in the study. Based on examination of larval cultures from August 2017 to February 2018, we detected an overall individual *S. vulgaris* prevalence of 3.5% and a herd prevalence of 27.3%. Later, there was a very poor correlation between the number of eggs detected by faecal egg counts and the number of larvae harvested following larval culture. In many cases, no larvae were detected in the cultures despite high egg counts. Therefore, the results based on microscopy of the larval cultures could not be trusted, and all cultured larvae were submitted to SLU for real time PCR. However, SLU was also unable to detect any L3-larvae in our samples, and therefore eggs harvested following flotation are currently being analysed by real time PCR. The final prevalence results are expected in January 2020.

The potentially pathogenic tapeworm, *A. perfoliata*, was detected in 14 of 56 farms corresponding to a herd prevalence of 25% and an individual prevalence of 6.7% (29/435). This parasite is usually considered relatively non-pathogenic, but there is increasing evidence that heavy infections may be linked to intestinal obstruction and colic.

Long-lasting anthelmintic drugs belonging to the group of macrocyclic lactones were used in 84.7% of the Norwegian horse farms where the utilized drugs were known; in 10 farms, the drug was unknown. The regular and frequent use of macrocyclic lactones in Norwegian horse farms without any preceding parasitic diagnosis may explain the relatively low prevalence of *S. vulgaris* detected in Norway compared to Sweden.

Strongylus vulgaris and colic in Swedish horses – a case-control study (Study 2)

A total of 137 cases and 137 age-matched controls were included in the study. The final diagnoses of the case horses included impaction colic (large colon, small colon and cecal impactions) (38.0%), acute colitis (10.2%), large intestinal displacement (9.5%), peritonitis (8.0%), gas distention colic (5.8 %), eosinophilic enteritis/colitis (3.6 %), chronic colitis (2.2 %) and other (7.3 %). A nonspecific diagnosis was reached in 15.3 % of the cases. Of the 10 horses that had surgery performed, the final diagnoses were as follows: large colon displacement (4), ileocecal invagination (2), eosinophilic enteritis/typhlocolitis (2), non-strangulating intestinal infarction (2), gas distention colic (1) and undiagnosed (1). A total of four (2.9%) cases were euthanized, all of which had had abdominal surgery.

When analysing all cases compared to controls no significant associations were found between colic and positive *S. vulgaris* enzyme-linked immunosorbent assay (ELISA), level of faecal egg output or *S. vulgaris* positive larval cultures. Of the horses that had abdominal surgery and/or were euthanized, parasitic injuries were found in three cases, both of which were diagnosed

with *S. vulgaris* associated non-strangulating intestinal infarction of the colon. Interestingly, these three cases excreted 0 strongyle eggs and were negative on the ITS-2 PCR but positive in the *S. vulgaris* ELISA. There was no association of a positive ELISA titer and gastrointestinal disease in general (Table 5), with sixty-one percent (75/122) positive horses in the case group and 62.8% (86/137) positive horses in the control group. However, horses within the case group, peritonitis, was significantly associated with a positive ELISA, compared to the controls (Fig. 2).

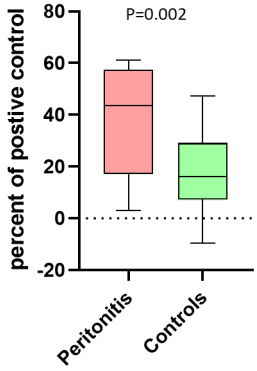

Figure 4. The antibody titre of S. vulgaris is significantly higher in the group of horses diagnosed with peritonitis compared to the matched control.

Form the questionnaire data no differences in parasite control strategies between cases and controls were found in the present study. Macrocyclic lacones was the most commonly used anthelmintic group overall, in accordance with several previous publications from various countries. Overall, this study we could not demonstrate a higher occurrence of *S. vulgaris* in horses with clinical signs of colic than in the control horses without colic. However, in the peritonitis group significant higher titre of the *S. vulgaris* specific antibody was observed compared to matched controls. Further on three horses in this group were confirmed with *S. vulgaris* after necropsy.

Strongylus vulgaris and colic in Norweigan horses – a case-control study (Study 2)

A total of 69 horses were included in this investigation i.e. far less than planned. However, the horse clinic at the Norwegian University of Life Sciences (NMBU), where the study took place, has been shut more or less continuously throughout the entire study period due to recurrent problems with salmonellosis. Therefore, we were unable to obtain results from which anything can be concluded regarding correlation between *S. vulgaris* infection and clinical symptoms/colic.

The efficacy of faecal removal to reduce the number of cyathostomin larvae on pasture (Study 3)

Faecal egg output

The three horses were shedding 300, 400 and 750 EPG, respectively, at the start of the study (18th June). Two individuals that grazed for five weeks were both shedding 400 EPG when they were removed from the pasture on the 24th July. The third horse with 750 EPG in June was removed from pasture after three weeks and was not sampled then. During the first half of the sampling period in 2017 the average temperature varied from 13.3°C and 20.1°C. From the end of August, the average temperature decreased and the highest temperature was 15.5°C. The weather was dry in June and in July 2017 but in August the rainfall increased (Fig 5).

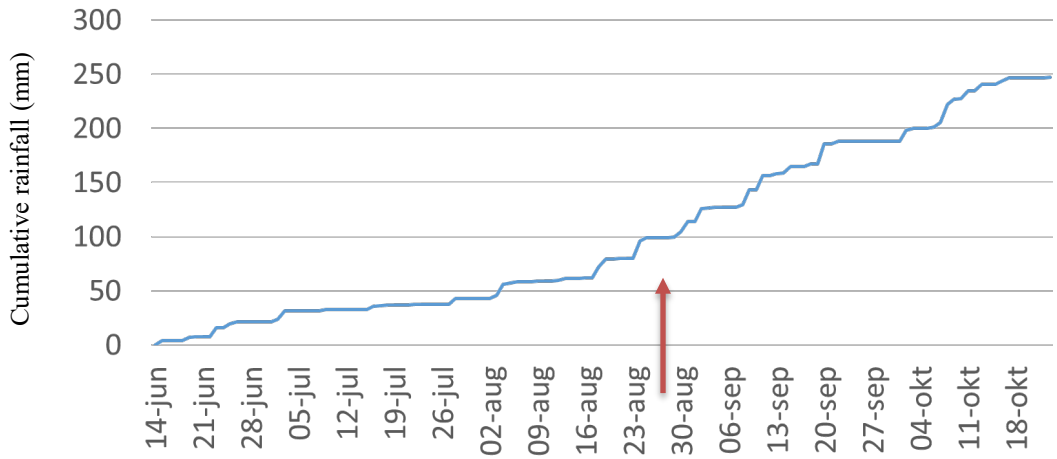

Figure 5. Cumulative rainfall during the study period in 2017. The arrow indicates the first sampling occasion with large amounts of larvae in the grass.

Except for a few larvae found in pasture B the 3rd July, no larvae were detected until the 27th August when suddenly high numbers were found in B (Table 2). The number of larvae remained high for the rest of the sampling period in 2017. In the samples from pasture A, only a few larvae were detected once in September.

Table 2 The number of infective strongyle larvae in the grass samples from A and B, respectively

Date for sampling	Larvae per kg A: Faeces removed	Larvae per kg B: Control
10 June	0	0
3 July	0	41
17 July	0	0
31 July	0	0
14 Aug	0	0
27 Aug	0	12601
10 Sept	0	3842
24 Sept	55	7219
8 Oct	0	3271
24 Oct	0	4350

In summary this study shows that the number of infective strongyle larvae were considerably lower in the samples that had been collected from the pasture where faeces had been removed manually twice weekly. Dung removal could thus be a very useful alternative or complement to anthelmintic treatments to reduce the contamination levels on pastures.

Conclusions

The overall prevalence of *S. vulgaris* at individual and farm levels were 28% and 61%, respectively, which indicates a threefold increase since 1999. In Norway, the corresponding prevalence were 3.5% and 27.3%, respectively. However, these results are preliminary, and we have to wait for the final real time PCR results, which are expected in January 2020, before firm conclusions can be made. We observed a 2.9 increased odds risk of *S. vulgaris* infection on Swedish farms that based their treatment on strongyle FEC only as compared to farms that complemented strongyle EPGs with larval cultures or dewormed regularly, 1-4 times per year, without prior diagnosis. We found no association between the prevalence of *S. vulgaris* and strongyle EPG level, but rather high prevalence (25%) of *S. vulgaris* in horses shedding ≤150

EPG. The horses with low strongyle EPGs that are left untreated could be an important source of *S. vulgaris* infection and might be an important reason for the increase in *S. vulgaris* prevalence. Although we notice a re-emerging of *S. vulgaris* in the Swedish horse population no difference overall could be found in positive *S. vulgaris* titers between controls and cases. Importantly, the percentage of horses positive for the ELISA test was higher (77.8%) than overall in the peritonitis group, with only two cases being negative, and may be supportive of a *S. vulgaris* etiology.

Faecal removal effectively reduce the number of small strongyle larvae on pasture and is a useful alternative or complement to anthelmintic treatments to reduce the contamination levels on the pastures.

Finally, *S. vulgaris* needs to be monitored continuously and should be taken into careful consideration when the treatment frequency is reduced. Thus, there is an urgent need for other diagnostic methods able to diagnose the larval stages of *S. vulgaris*.

Relevance for the practical horse sector incl. recommendations

Describe how the project results can be used in the practical horse sector, what is needed for the results to be implemented, and (if applicable) what needs further investigation after the project.

We report an increased prevalence of *S. vulgaris* in Swedish horses, with a potentially negative impact on equine health. In contrast, our preliminary results suggest a much lower prevalence of *S.vulgaris* in Norway, which is probably linked to the frequent deworming with macrocyclic lactones without previous coproscopic analysis. However, the solution is not to go back to routine anthelmintic treatments several times per year since that would likely accelerate the spread of multidrug resistance in small strongyles and ascarids. Instead we recommend horse farms to practice SAT and importantly, always include diagnostics of *S. vulgaris* in the spring. SAT should be complemented by pasture hygiene methods such as collection of faeces from the grazing areas twice weekly. In the beginning of 2020, we will invite equine practitioners from the University Animal Hospital, Evidensia and Mälaren Equine Clinic to a roundtable discussion at SLU where the aim is to reach consensus and formulate a common document with recommendations. The ambition is to provide veterinary surgeons and horse owners in Scandinavia with up to date information on worm control practices that will prevent clinical disease while minimising selection pressure for resistance. The new parasite recommendations will be disiminated by using the webpages and social media of the above mentioned bodies, the Swedish Congress of Veterinary Medicine and HästSverige. Moreover, they will be presented at lectures and courses.

Despite increasing awareness within the veterinary profession and the equine industry of the potential implications of anthelmintic resistance, there is a concern that insufficient measures are being taken to reduce its development and spread. Therefore, we are continuing this work with a newly founded application (H-18-47-389) with the aims (i) to investigate new approaches for control of equine strongyle nematodes based on biological, non-chemical principles, and ii) to validate means for early diagnosis of migrating *S. vulgaris* larvae.

References

References that are cited in the report including references to earlier projects finances by the Foundation.

Note that all references/publications that is a result of the current project needs to be given in the table in Part 3. Result dissemination.

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
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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)*
	Eva Tydén, Heidi Larsen Enemark, Mikael Andersson Franko, Johan Höglund, Eva Osterman-Lind, 2019, **Prevalence of Strongylus vulgaris in horses after ten years of prescription usage of anthelmintics in Sweden**, Veterinary Parasitology X, Volume 2, 100013 https://doi.org/10.1016/j.vpoa.2019.100013
Scientific publications, *submitted*	*Author(s), title*
	Ylva Hedberg-Alm, Miia Riihimäki, Johanna Penell, Eva Osterman-Lind, Eva Tydén (submit Dec 2019) ***Strongylus vulgaris* and colic in Swedish horses – a case-control study**, Animlas Special Issue "Equine Parasitology"
Scientific publications, *manuscript*	*Author(s), title*
	Eva Osterman- Lind, Eva Tydén, **The efficacy of faecal removal to reduce the number of cyathostomin larvae on pasture**
Conference publications/ presentations	*Author(s), year, title, conference name, location and date, (link if applicable)*
	E Tydén, Elin Werell, Heidi Enemark, Johan Höglund Eva Osterman Lind. *Prevalence of Strongylus vulgaris after 10 years of target selective treatment in Sweden* (2017) World Association for the Advancement of Veterinary Parasitology, Kuala Lumpur, Malaysia (Abstract, oral presentation)
	E Tydén, Ylva Hedberg Alm, Miia Riihimäki, Martin Nielsen, Eva Osterman Lind *S. vulgaris and intestinal diseases in Swedish horses – a case control study* (2019) World Association for the Advancement of Veterinary Parasitology, Madison, USA (Abstract, oral presentation)
	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)*
	Parasiter hos häst: avmaskning, resistens och diagnostik (Abstract) Hippokampus 2016
	Förekomst och kontroll av blodmask - hur har det gått? (Abstract) Hippokampus 2018
	Ökad förekomst av stor blodmask – vad är risken? (Popular science) Travsport 2019

	Effekt av mockning, nyhet på hästSverige – (Popular science) https://hastsverige.se/content/uploads/2019/07/examensarbete-mockningsstudie-helena-thorolfson-1.pdf
	Stor blodmask på frammarsch efter 10 år med selektiv behandling, (Popular science) Forskningsnyheter nr 1 2020
Oral communication, *to horse sector, students etc.*	*Title, year/date, group presented to (link if applicable)*
	Parasiter hos häst: avmaskning, resistens och diagnostik (Abstract) Hippokampus 2016
	UDS "open hus" 2017 Parasiter hos häst: avmaskning, resistens och diagnostik
	Förekomst och kontroll av blodmask - hur har det gått? (Abstract) Hippokampus 2018
	Eva Osterman-Lind (2016-2019) Kontroll av hästparasiter for Equine Practioners and horse owners (oral communications at Flyinge and Wången on a yearly basis)
	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
	Ylva Hedberg Alm, Miia Riihimäki, E Tydén, *S. vulgaris and intestinal diseases in Swedish horses – a case control study* Carenet 2019 (Oral presentation)
Student theses	*Author/Student, co-authors/supervisors, year, title, type of thesis (doi/link if applicable)*
	Werell, Elin, 2017. Prevalens av Strongylus vulgaris. (30 HP) https://stud.epsilon.slu.se/10013/
	Mörby, Camilla, 2017. Parasitförekomst hos häst – samband med kolik och avmaskningsrutiner. : en fall-kontrollstudie. https://stud.epsilon.slu.se/10041/
	Thorolfson Rainamo, Helena, 2018. Mockning som beteshygiensk åtgärd för parasitbekämpning hos häst. (15 HP) https://stud.epsilon.slu.se/13151/
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	Persson, My, 2019. Strongylus vulgaris : samband med äggurskiljning, ålder och bekämpningsåtgärder. https://stud.epsilon.slu.se/14645/
	Wilderoth, Hanna, 2019. Förebyggande åtgärder för bekämpning av blodmask. https://stud.epsilon.slu.se/14615/
Other	