Occurrence, epidemiology, and control strategies of *Strongylus vulgaris* in horses in Denmark

Background

The equine bloodworm, *Strongylus vulgaris* is the most pathogenic equine helminth parasite, causing severe colic and death in horses of all age groups. The parasite is characterized by a long migratory lifecycle, with larval stages migrating into the arterial bloodstream and undergoing two molts before returning to the lumen of the large intestine about six months post infection. The migrating larvae induce arteritis and thrombosis in the mesenteric arteries leading to intestinal ischemia and potential rupture, which is fatal for the horse (Drudge, 1979).

Strongylus vulgaris is enzootic in Denmark and Sweden with up to 83% of equine operations infected (Nielsen et al., 2012; Tydén et al., 2019). Intestinal ischemia associated with *S. vulgaris* larval migration has been diagnosed with a 10-fold increased frequency (Pihl et al., 2015; Thoefner et al., 2003) after the availability of anthelmintic products was restricted to prescription-only in 1999 in Denmark (Pihl et al., 2018). A similar trend has been observed in Sweden in recent years (Hedberg Alm et al., 2022, 2020).

A previous study has suggested that one of *S. vulgaris'* migrating stages, the fifth stage larva (L5), is not susceptible to anthelmintic treatment, whereas all other stages appear to be fully susceptible (Nielsen et al., 2015). The L5 stage appears about three months post infection and exists for about one month in the mesenteric artery, before returning to the intestine and maturing into adult worms. This means that a single anthelmintic treatment may not eliminate all pathogenic stages of *S. vulgaris*, and these observations raise questions about how to design an effective protocol for anthelmintic treatment, once *S. vulgaris* has been diagnosed in a given equine operation.

Evidence-based treatment guidelines for equine operations diagnosed with *S. vulgaris* are frequently requested by equine veterinary practitioners, but studies evaluating the efficacy of different treatment strategies have never been performed, and no useful evidence exist to address these questions.

In the past year we conducted a cross-sectional study with support from Sveland Stiftelsen, with the aim of estimating the prevalence of *S. vulgaris* in Danish horses in Zealand, Denmark and to evaluate the outcome of two different treatment protocols for *S. vulgaris* (a single ivermectin treatment compared to two ivermectin treatments administered with a 6-week interval). The study estimated the *S. vulgaris* prevalence to be 5.74% (17/296 horses) on the horse level and 33.33 % (10/30) on the operation level. This is remarkably lower than previously reported prevalences in both Denmark and Sweden and could possibly be due to a raised awareness among horse owners and veterinarians of the risks of bloodworm infection and, thus, a changed treatment approach.

The study also demonstrated that a fecal PCR test significantly outperformed the more traditional larval culture method for diagnosing *S. vulgaris* infection. The PCR detected approximately twice as many positive cases as the larval cultures.

All horses enrolled in the treatment study became PCR and larval culture negative for *S. vulgaris* regardless of treatment protocol. Although this result suggested that a single ivermectin treatment could be sufficient for eliminating all stages of *S. vulgaris*, the number of positive horses (n=17) in this study did not allow any conclusions regarding the possible difference between the two treatment protocols. To increase the power, the study needs to be expanded and include more horses testing positive for *S. vulgaris*. For this purpose, we have established a collaboration with two

laboratories performing a high volume of equine fecal larval cultures every year. The two laboratories are located in Jutland (Hestelaboratoriet) and in Zealand (Hesteklinik Vestsjælland).

Aim

The aims of this study are 1) to investigate possible risk factors for occurrence of *S. vulgaris* in Danish horses. And 2) to evaluate the effect of two anthelmintic treatment protocols for reducing the occurrence of *S. vulgaris* in equine operations.

Materials and Methods

Timeline

Enrollment of equine operations, collection of first fecal samples and administration of anthelmintic treatments will start in April 2024. The last samples will be collected in November 2024 and all laboratory analyses completed by the end of 2024. Data analysis and report writing will start at the end of 2024 and be finalized during 2025.

Operation enrollment

Equine operations will be identified through collaboration with local equine veterinary practitioners and a specialized laboratory (Hestelaboratoriet). The aim is to enroll approximately 100 *S. vulgaris* positive horses.

The inclusion criteria will be as follows: 1) The horse has tested positive for *S. vulgaris* by larval culture performed by one of two collaborating laboratories in the spring of 2024 (April 1 to May 27th), 2) No anthelmintic treatment administered after the diagnosis, 3) Owner willingness to answer a questionnaire, treat according to the assigned protocol and send two control samples to the laboratories.

Positive larval culture is used as inclusion criteria since this is the standard test offered by equine practitioners and laboratories in Denmark. Even though the sensitivity is low, the specificity of this test is very high (Nielsen et al. 2010), decreasing the risk of including false positives in the study. Exclusion criteria will be if horses are not confirmed to be positive on our PCR analyses for *S. vulgaris* before treatment.

Laboratory techniques

McMaster and larval cultures will be performed by the collaborating laboratories. Larvae from positive samples will be frozen in alcohol and shipped to Taastrup, where a validated PCR test will be performed on extracted larvae from larval cultures (Nielsen et al., 2008).

Treatment protocols

Horses will be allocated randomly to two treatment groups, using a random number generator (50 horses in each treatment group). Follow-up fecal egg counts, larval culture and PCR testing will be

performed at 8- and 24-weeks post treatment to evaluate the responses of the two treatment protocols.

- Treatment group 1 (n=50 horses) will receive a single ivermectin (200 µg/kg) treatment immediately after initial diagnosis.
- Treatment group 2 (n=50 horses) will receive one ivermectin treatment (200 µg/kg) directly after first diagnosis and a second treatment (200 µg/kg) 6 weeks after the first treatment.

These protocols are designed to test the hypothesis that two treatments administered 6 weeks apart will effectively eliminate all stages of the parasite, as this time interval will allow migrating L5 stages to progress to the adult stage and thus become susceptible to treatment.

The time intervals for follow-up testing are chosen to accommodate the six-month life cycle of the parasite. If any stage of the parasite survives anthelmintic treatment, this should become detectable by fecal PCR within this time frame, as demonstrated in a previous study (Nielsen et al., 2015).

Power calculation:

Based on the aim of detecting at least a 15 % difference in incidence between the two treatment protocols, with a power of 80% (1-beta) and confidence level of 95%, we will need 47 horses in each treatment group (calculated with Sample Size Calculator (Sample Size Calculator (clincalc.com)).

Risk factor analysis

Electronic questionnaires will be sent out to owners of included horses to collect information about possible risk factors for *S. vulgaris*. Information about horses that tested negative in the same herd will also be collected.

Research group

The research will be conducted at the University of Copenhagen by a group of leading equine parasite researchers (see attached CVs). The group includes Professor Stig Milan Thamsborg parasitologist at University of Copenhagen, Professor Martin Krarup Nielsen, equine parasitologist at the University of Kentucky, USA, PhD student Katrine Toft, and Associate professor Tina Holberg Pihl, both equine veterinary clinicians at the University of Copenhagen Large Animal Hospital.

Profs. Nielsen and Thamsborg will be involved in the scientific planning of the study and supervision of the veterinary students. Drs. Toft and Pihl are responsible for the practical and logistical parts of the study as well as contact to equine practitioners and horse owners. All four researchers will participate in the statistical analyses, interpretation, writing and presentation of results.

The study will include 2 veterinary students working on their veterinary theses and two experienced laboratory technicians. Salaries to the researchers are covered by their employers

The study will be conducted with collaboration from Hestelaboratoriet and a large Danish equine veterinary practitioner group Hesteklinikken Vestsjælland, who will help identify *S. vulgaris* positive horses and collect, store, and ship samples. Anthelmintic treatments will be distributed through the

participating veterinary practitioners. The study is financially supported by Boehringer-Ingelheim and Sveland Stiftelsen. None of them have influence on the study protocol or results.

Specific outputs

This study will provide important and relevant knowledge to all equine practitioners and horse owners regarding risk factors and treatment of *S. vulgaris*. This will help veterinarians and horse owners make informed and evidence-based decisions about parasite control, mitigating risks of parasite associated disease and further development of anthelmintic resistance.

The project will specifically test the hypothesis that two ivermectin treatments are more effective than a single treatment and explore the potential risk factors for *S. vulgaris* infection in horses.

Results of the study will be published as a veterinary student final research project and in a peer-reviewed veterinary scientific journal. Furthermore, results will be disseminated to equine practitioners and horse owners by relevant equine, veterinary, and social media outlets.

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