Slutrapport SLF 2021

Molekylära tester för detektion av mag- och tarmparasiter hos får och för mätning om de är resistenta mot avmaskningsmedel

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Utförlig sammanfattning

Parasiter är en av de största och viktigaste hälsostörningarna i fårbesättningar runt om i världen. En fungerande strategi för parasitkontroll är därför en förutsättning för att kunna bedriva betesbaserad lammuppfödning med god djurvälfärd. Tillgång till effektiva avmaskningsmedel är en av hörnstenarna för framgångsrik parasitbekämpning. Pågående resistensutveckling hos parasiterna mot dessa läkemedel hotar dock denna resurs och kan på sikt äventyra en långsiktigt hållbar produktion.

Syftet med projektet var att utveckla molekylära verktyg särskilt för diagnos av stor löpmagsmask, *Haemonchus contortus*, som är den viktigaste patogenen hos svenska får. Men även att ta fram förbättrade metoder för provtagning samt identifiera genetiska markörer för detektion av läkemedelsresistens.

Genom en serie försök utvecklades och validerades olika molekylära tester dels för att kunna ta reda på hur de viktigaste parasiterna hos får påverkades vid avmaskning, dels för att på ett effektivare vis kunna påvisa om de bär på genetiska anlag för resistens. När testerna sedan användes för att studera effekten av antingen albendazol, ivermektin eller levamisol, visade det sig att framför allt *H. contortus* i flera fall överlevde, särskilt när djuren behandlades med ivermektin men i ibland även med albendazol. Inom ramen för projektet upptäckte vi även att användning av monepantel snabbt kan leda till resistens. Däremot förefaller det som om levamisol fortfarande fungerar tillfredsställande. Resultaten är oroande eftersom de är i skarp kontrast till de som erhölls i en svensk studie av ett 40-tal slumpvis utvalda fårbesättningar för cirka 15 år sedan. Det förefaller alltså som om resistensläget har förvärrats under senare år. Samtidigt måste det poängteras att chansen för att upptäcka stor löpmagsmask ökar vid användning av molekylära diagnostik samt med de metoder för provtagning som utvecklades i projektet. Dessutom var de undersökta gårdarna i det aktuella projektet inte slumpmässigt utvalda utan fångades upp inom ramen för Gård & Djurhälsans fortlöpande verksamhet.

Med avseende på molekylära tester för detektion av resistens fann vi dessvärre att endast analysen för att påvisa bensimidazol-resistenta *Haemonchus* gav tillförlitliga resultat. Följaktligen behövs ytterligare forskningsinsatser för att utveckla liknande tester även för de andra substanserna och parasiterna. För att kunna konstatera om resistensläget förändrats krävs även en förnyad kartläggning som genomförs i enlighet med den tidigare svenska studien.

Projekt har fått finansiering genom:
Stiftelsen Lantbruksforskning

Genom projektet har vi idag tillgång till olika molekylära metoder såväl för att påvisa de viktigaste parasiterna som förekomst av bensimidazol-resistenta *H. contortus* i fårflockar. Däremot visade det sig att de genetiska markörer som utvecklades för att påvisa resistens mot ivermektin eller levamisol inte var korrelerade med effektivitet hos dessa avmaskningsmedel. Däremot kan *H. contortus* och förekomst av resistens mot bensimidazoler nu övervakas med högre precision än tidigare.

1. Introduction

The total number of sheep in Sweden (i.e., ewes, rams and lambs) was around 550,000 to 600,000 in June 2020[1]. The production is mainly pasture-based, which besides from providing meat and pelts, is a sustainable way to maintain biodiversity of open landscape and contributes to the aesthetic values of grassland (Metera et al., 2010). There is an ever-increasing demand for grass-fed Swedish-produced lamb. Production must therefore escalate, but according to the action plan for increased domestic lamb production[2], this must not be done at the expense of the animal's health and well-being.

Infections with GINs is globally well-known as a major problem, which contributes to reduced productivity in the sheep industry (Sutherland and Scott, 2010). Strategic use of use of anthelmintic drugs remains a cornerstone of most parasite control programmes due to its ease of implementation and low cost of therapy compared to other methods (Waller, 2006). Therefore, consequences can be fatal in herds where the parasites have developed anthelmintic resistance (AR).

Grazing sheep are hosts for more than 20 of parasitic worms, but some species are more harmful than others. This is especially true for the barber's pole worm (*Haemonchus contortus*), which is a blood-sucking nematode in the abomasum. This parasite is very pathogenic and can cause major problems at high burdens, both in pregnant ewes and lambs (Besier et al., 2016). It is therefore important to be aware of this parasite which can require immediate control measures.

The Farm and Animal Health (G&D) in Sweden has therefore for decades offered evidence-based advice for the use of anthelmintics to sheep farmers[3]. This is based in part on diagnostic information from composite nematode eggs counts. Over the years, this service has safeguarded sheep health and welfare in Swedish flocks. It has also prevented problems with AR, as the use of these chemicals has been restricted.

For example, in a randomized nationwide survey on the efficacy of major anthelmintics, conducted from 2006 and 2007, it was shown that only two herds of 45 were infected with GIN resistant to one of the most widely used anthelmintic; at the time the benzimidazole (BZ) albendazole (Valbazen®). In both instances, *H. contortus* dominated (Höglund et al., 2009). However, recently, *H. contortus* which were also resistant to ivermectin (IVM) was documented on a farm on Gotland (Höglund et al., 2015). This flock had suffered from recurrent *H. contortus* problems for some years, most likely since they imported resistant parasites with East Friesian

[1] https://jordbruketisiffror.wordpress.com/
[2] http://www.kottforetagen.se/handlingsplan-lamm.html
[3] http://www.gardochdjurhalsan.se/sv/far/

Dairy sheep from the Netherlands via Finland. Subsequent monitoring of the sheep on the same farm, and in neighbouring plus contact farms, showed that the resistance was more widespread also on mainland Sweden (Höglund et al., 2015). Furthermore, ongoing investigations have demonstrated that in pafrticular IVM-resistance is also present in several other flocks throughout the country (unpublished).

Through the mentioned activities, it was realized that both the diagnostics and sampling methods used on sheep farms in Sweden must be updated. The sampling recommendations and diagnostic procedures currently in use dated back to the early 1990s. The sampling schedule, used until recently, was based on the investigation of faeces collected from six animals per flock on two different occasions per year: first from six ewes before turnout and then during summer most often from six grazing lambs around weaning. On each sampling occasion, faeces were pooled from each of three animals and analysed for strongyle nematode faecal egg counts (FEC). Although this sampling strategy has been useful, it was launched when sheep flocks were smaller, with an average of 30 ewes (Lindqvist et al., 2001). Today, sheep flocks in Sweden are larger, sometimes with up to 1,000 ewes and sheep farmers are therefore sometimes unable to adjust to the old protocol and thus forced to depart from the recommended sampling schedule. Of major concern is if the older protocol affected the outcome of diagnostic test results and, thereby, the advice given to the farmers.

It was also realized that there was a need to develop genetic markers for the detection of AR as well as genetic assays to support the diagnosis. Today AR is diagnosed based on microscopical examination and by calculating the reduction in the nematodes' egg output after treatment using the Faecal Egg Count Reduction Test (FECRT) (Coles et al., 2006). Although this is a simple method, FECRT requires access to at least ten high egg shedding animals per anthelmintic substance tested and that must be sampled both pre- and post-treatment. Thus, FECRT cannot be used on single animals. Other disadvantages are that FECRT requires trained expertise and that it is time-consuming and costly to perform. It also only allows the detection of resistance when at least 25% of the population is resistant (Martin et al., 1989). Despite these shortcomings, FECRT is still regarded as the gold standard for AR testing on farms.

Aims

The overall purpose of the project was to develop novel diagnostic tools and sampling routine in particular for the detection of *Haemonchus contortus*, which is the most important pathogen in Swedish sheep.

Specific research objectives were to:

- Develop molecular methods for the detection of the most important gastrointestinal nematode parasites and their resistance status
- Validate a new method for parasite research used on sheep farms with grazing-based lamb production
- Examine the parasitic dynamics of ewes and lambs in relation to deworming

2. Material and methods

2.1 Diagnostic markers

First, we compared four methods for the identification of *H. contortus* eggs. To validate the outcome of diagnostic tests, faeces were collected from naturally infected sheep (n = 38). Subsamples from each animal were then analysed by two coproscopy methods (a) McMaster egg counting (MM); and (b) differential counting of eggs after staining with peanut agglutinin (PNA); as well as by (c) detection of parasite DNA following amplification by real-time quantitative polymerase chain reaction (qPCR); and (d) loop-mediated isothermal amplification (LAMP). Differences between similar tests (coproscopy and molecular) were analysed with Bland–Altman plots and Spearman rank correlation.

Second, we presented a new tool, based on Droplet Digital™ Polymerase Chain Reaction (ddPCR), for absolute quantification of key genera of gastrointestinal (GI) nematode parasites of grazing livestock. Four combinations of primers/probe sets targeting the internal transcribed spacer region 2 (ITS2) of the ribosomal RNA gene array were designed using the Primer3 software, following *in silico* analysis of nucleotide sequences from nematodes of interest downloaded from the NCBI database. The amplified regions include both a universal region for detection of any strongylid gastrointestinal parasite and three different genus specific regions, making it possible to differentiate between the most important GI nematode genera of sheep in Sweden: *Haemonchus, Teladorsagia* and *Trichostrongylus*.

2.2. Effects of treatment

To assess the effects of anthelmintic treatment larval culture samples collected in relation to treatment were analysed for their nemabiome using the PacBio platform followed by bioinformatic sequence analysis with SCATA. Species richness and diversity were calculated and analysed in R. A total of 158 samples were collected (n = 35 in 2007 and n = 123 in 2013–2016) and cultured from groups of sheep on 61 commercial farms in the south-central part of the country where most animals are grazed. Among the samples, 2 × 44 (56%) were paired collections from the same groups pre- and post-treatment with anthelmintics such as macrocyclic lactones (i.e. ivermectin), benzimidazoles (i.e. albendazole) or levamisole.

2.3 Sampling methods

To improve the sampling methods, we conducted a pilot study to determine optimal time-points and number of sheep required for parasite surveillance in sheep flocks. We then validated an enhanced sampling strategy for detection of gastrointestinal parasites of sheep based on faecal sampling covering approximately 10% of the animals in the flock with focus on *H. contortus*. In this study we also compared traditional diagnostics based on faecal eggs counts (FEC) by microscopy (coproscopy) with DNA detection on frozen faeces samples using the droplet digital (dd)PCR assay developed earlier. The investigation was carried out in 2018 in 20 conventional and 19 organic sheep flocks in Sweden with between 70 and 250 production ewes. On 76 different sampling occasions a total of 810 individual faecal samples were collected. Samples were pooled in the laboratory into 270 triplets which were examined both by microscopy and the univ-Hc ddPCR assay.

2.4 Molecular markers for AR

BZ-resistance First, we proposed a novel droplet ddPCR assay protocol for rapid and precise identification of *H. contortus* strains as being resistant or susceptible to benzimidazole drugs based on the presence or absence of the most common resistance-conferring mutation F200Y (TAC) in the β tubulin isotype 1 gene. The assay was optimized and validated utilizing DNA templates from single-worm samples, which were previously sequenced using the next

generation PacBio RSII Sequencing (NGS) platform. Subsequent NGS results for faecal larval cultures were then used as a reference to compare the obtained values for fractional abundances of the resistance-determining mutant allele between ddPCR and NGS techniques in each sample. Secondly, the utility of the assay was validated in two *H. contortus* laboratory strains (MHco5 and MHco4), characterized by different BZ resistance levels, by investigating changes in allele frequencies with respect to this mutation, when subjected to increasing concentrations of thiabendazole. Additionally, we investigate whether exposure to a discriminating dose of thiabendazole in the egg hatch test resulted in the death of all *H. contortus* eggs with a susceptible genotype. Thirdly, we screened 174 pooled larval culture samples on 67 farms around Sweden, collected either pre- or post-treatment, for the frequencies of the mutations F167Y and F200Y.

IVM-resistance Moreover, we also investigated the suitability of A141G and G153T single nucleotide polymorphisms (SNP) of *dyf-7* as a marker for IVM resistance. Initially, we designed dyf-7 primers from a worldwide collection of adult *H. contortus* DNA. With the sequence data, we created a haplotype network. We then optimised and used the same sets of primers and probes in a ddPCR assay for quantification of *dyf-7* allele frequencies in seven faecal larval cultures collected pre- and post-IVM treatment.

LEV-resistance Based on the sequencing data of different isolates of *H. contortus*, primers and probes were designed and validated with a novel droplet digital PCR assay for the quantification of the *acr8* deletion reported to contain the "resistant" allele. Single adult worms from six phenotypically described isolates (n = 60) and from two Swedish sheep farms (n = 30) where levamisole was effective were tested. Furthermore, field larval culture samples, collected pre- (n = 7) and post- (n = 6) levamisole treatment on seven Swedish sheep farms where levamisole was fully efficacious according to Faecal Egg Count Reduction Test results, were tested to evaluate the frequency of the "resistant" allele in each.

3. Results and discussion

3.1 Diagnostic markers

The observed ranking in terms of test sensitivity was MM < PNA < LAMP < qPCR (Ljungström et al., 2018). Although we found that *H. contortus* can be identified by McMaster counting, without major mistakes regarding false positive results, molecular methods provide the capacity to diagnose *H. contortus* eggs with increased accuracy compared to coproscopy. This can be essential when animals are investigated for routine diagnostic purposes as well as in studies evaluating anthelmintic treatment efficacy.

The suitability of ddPCR for detection and absolute quantification of three major sheep pathogens when tested on larval cultures from pooled ovine faeces was confirmed (Elmahalawy et al., 2018). Taken together, our data confirm the suitability of ddPCR for detection and absolute quantification of three major sheep pathogens when tested on larval cultures from pooled ovine faeces. The results also indicate that ddPCR can be a useful complement to applications based on conventional egg counting methods such as the faecal egg reduction test (FECRT).

3.2 Effects of treatment

In the analysis of the nemabiom of sheep ITS2 sequences were found in all samples except two, even though the faecal egg counts were below the McMaster threshold in 20 samples (Halvarsson and Höglund, 2021). Sequencing yielded, on average, 1008 sequences per sample.

In total, 16 operational taxonomical units (OTU), all with ≥ 98 % identity to sequences in the NCBI database, were recognized. The OTUs found represented nematode species of which ten are commonly associated with sheep. Multiple species were identified in all pre-anthelmintic treatment larval culture samples. No effects on nematode diversity were found in relation to host age. On the other hand, recent anthelmintic treatment lowered species richness, especially after use of IVM and albendazole where *Haemonchus* in particular survived the treatment. Interestingly, despite zero egg counts after use of levamisole, these samples still contained nematode DNA and especially *H. contortus*. Taken together, our findings provide evidence that nemabiome analysis combined with diversity index analysis provides an objective methodology in the study of the efficacy of anthelmintic treatment as both high and low abundant species were detected.

3.3 Sampling methods

On most farms (95%) a minimum of three triplets were investigated, first from the ewes prior to turn-out and later from the lambs after they had been grazing for at least six weeks (Höglund et al., 2019). Extra information about the *Haemonchus* status was provided on 48% of the 76 sampling occasions by including more triplets compared with the old sampling strategy applied in Sweden before 2015 based on two triplets per sampling occasion irrspective of flock size. At a farm level *H. contortus* was identified by microscopy in 22 (56%) of the 39 flocks and by ddPCR it was found in 28 (72%) flocks with the enhanced protocol. There was a substantial agreement between the two diagnostic tests (Cohens kappa = 0.70 ± 0.087). In this study, samples from the ewes were more often *Haemonchus* positive than those from the lambs indicating that the level of parasite control was in general acceptable. Combined, our results show that *Haemonchus* infection is widespread throughout Sweden.

4. Molecular markers for AR

BZ-resistance. Both molecular methods (ddPCR and NGS) produced highly similar results (Baltrušis et al., 2018). Thus, the ddPCR assay proved to be a reliable tool which can be used to create a powerful mutation detection and quantification assay. In the validation study we found the MHco5 strain to maintain an overall higher frequency of the F200Y mutation (80–100%) over all drug concentrations, whilst a steady, gradual increase from around 30%–60% was observed in the case of the MHco4 strain (Baltrušis et al., 2020b). This was further supported by the dose-response curves, displaying a much higher tolerance of the MHco5 strain (LD50 ¼ 0.38 μg/ml) in comparison to the MHco4 strain (LD50 ¼ 0.07 μg/ml) to the effects of thiabendazole. In the subsequent field study, we did only find the F200Y to be present at much higher frequencies than F167Y, but the overall levels of this mutation have stayed stable throughout the years 2014-2019 at an average value of 88.5 ± 20.3% in the pre-treatment samples across the tested farms ($p = 0.61$) (Baltrušis et al., 2020a). Furthermore, after establishing a mixed model and fitting our data, we found a significant ($p < 0.01$) difference in the average frequency of the mutation F200Y between paired, pre- and post-treatment with albendazole, samples. Although the frequency difference in samples treated with albendazole was relatively minor (88.5% in pre- and 95.6% in post-treatment), no significant ($p = 0.15$) change in F200Y mutation frequency was observed between the samples from the flocks treated with IVM (90.8% and 92.6 %, respectively). These findings conform with several other studies.

ML-resistance. The fractional abundance (FA) of SNP in *dyf-7* previously associated with anthelmintic resistance (Urdaneta-Marquez et al., 2014), was within the range 7.8 and 31%. However, in our case the fractional abundance (FA) was generally stable in samples collected from the same farms, even though they were obtained on different occasions up to 25 months

apart. There was also no indication that the level of IVM resistance as measured by the faecal egg count reduction test, was higher on farms with high FA. Furthermore, by comparing FA in samples from the same farms pre- and post-IVM treatment, we found no evidence of a correlation between dyf-7 and level of resistance. Based on these results, *dyf-7* is not a suitable marker for field testing of IVM resistance in *H. contortus* (Elmahalawy et al., 2018).

LEV-resistance. We also reported the development of a digital PCR assay as a molecular tool to detect a 63 bp deletion in the *hco-acr-8* that has been previously associated with LEV resistance (Neveu et al., 2007). In our study Sanger sequencing of single adult *H. contortus* yielded 56 high-quality consensus sequences surrounding the region containing the deletion (Baltrušis et al., 2021). However, even though a significant difference in genotype frequencies between the resistant and susceptible reference isolates was found (p = 0.01), the homozygous "resistant" genotype was observed to be abundantly present in both the susceptible isolates as well as in some Swedish samples. Also, the frequencies of the deletion in the pre-treatment larval cultures ranged from 35 to 80%, whereas no amplifiable *H. contortus* genomic DNA was detected in the post-treatment samples. Taken together, these data reveal relatively high frequencies of the 63 bp deletion in the *hco-acr-8* both on individual *H. contortus* and field larval culture scales. This cast doubt on the utility of this deletion as a molecular marker for levamisole resistance detection on sheep farms.

4. Conclusions

In this project we showed that it is possible to determine the species identity of *H. contortus* eggs in faecal samples by coproscopy. Still, in certain situations there is a need for molecular diagnostics. For example, in order to detect the parasite to prevent it from spreading with animal movements as well as to investigate with increased certainty how the parasite responds to anthelmintic treatment. Likewise, the great need for cost-effectiveness and reliable diagnostics that can be used even in smaller groups of animals remains, where genetic markers continue to be seen as a viable path. We have also validated a practical tool for sheep producers to assess *Haemonchus* infection with higher precision than according to previous practice. Thus, through the developments in this project, informed interventions against parasitic diseases can now be implemented with enlarged precision.

Although we show that the F200Y mutation is a viable and reliable marker for the detection and surveillance of BZ resistance in *H. contortus*, no similar tests are as yet available neither for IVM nor LEV as we failed to correlate the genetic markers studied herein (*dyf7* for IVM and *acr8* for LEV) with faecal egg count reduction results. Thus, the technique used for AR detection for both of these drugs must undergo further investigations in order to be modernized. To achieve this the key genes/loci conferring resistance must be identified. Not until reliable diagnostic AR tests are available, factors involved in the emergence and spread of AR as well as AR in smaller groups or in individuals cannot be studied effectively.

5. Benefit for the industry and recommendations

The findings in this project are useful because they contribute to informed use of anthelmintic drugs in sheep flocks. This will in the long run reduce the risk for the selection and spread of anthelmintic resistant parasites. From a veterinary medicine standpoint, gastrointestinal nematode infections are common in grazing livestock. Here they occur at the expense of the animal's health and welfare, but they are also responsible for costly production-limiting diseases (Charlier et al., 2020). Overreliance of anthelmintics, however has resulted in the development of drug-resistant nematode populations which is a growing problem around the

world (Wolstenholme et al., 2004). AR is now reported in 21 European countries (Vineer et al., 2020), including emerging levels that recently have been described in sheep in Sweden (Höglund et al., 2020, 2015). Concurrently, it has been recognised that it is challenging to breed sheep without access to efficient anthelmintics both on conventional and organic farms (Höglund et al., 2019). Access to up-to-date and sensitive diagnostic markers for early detection of AR is therefore of vital importance for the maintenance of healthy and productive sheep flocks.

To avoid an increased spread of *H. contortus*, which according to recent estimates occur in approximately 40% of the flocks in Sweden, it is essential to avoid its introduction. Thus, in case animals are recruited, these needs be separated and carefully dewormed as well as checked (diagnosed) after treatment before they are released into the existing flock. Especially when the animals come from abroad but also when they are recruited from other national herds. From the results in this project, it is evident that the chance for detection is increased with the use of the novel molecular diagnostic tools developed herein, and particularly those based on digital-PCR and/or nemabiom sequencing.

Unfortunately, with respect to the molecular tests for detection of resistance, we found that only the digital-PCR assay that was developed to detect BZ-resistant *Haemonchus* produced reliable results. Consequently, further research efforts are needed to develop similar tests for the other parasites and anthelmintic substances used in Sweden. Furthermore, before any molecular assays can be used on a larger scale it is also essential to establish them in laboratories working with routine diagnostics.

References

Baltrušis, P., Charvet, C.L., Halvarsson, P., Mikko, S., Höglund, J., 2021. Using droplet digital PCR for the detection of hco-acr-8b levamisole resistance marker in H. contortus. International Journal for Parasitology: Drugs and Drug Resistance 15, 168–176. https://doi.org/10.1016/j.ijpddr.2021.03.002*

Baltrušis, P., Halvarsson, P., Höglund, J., 2020a. Utilization of droplet digital PCR to survey resistance associated polymorphisms in the β tubulin gene of Haemonchus contortus in sheep flocks in Sweden. Veterinary Parasitology 288, 109278. https://doi.org/10.1016/j.vetpar.2020.109278*

Baltrušis, P., Halvarsson, P., Höglund, J., 2018. Exploring benzimidazole resistance in Haemonchus contortus by next generation sequencing and droplet digital PCR. International Journal for Parasitology: Drugs and Drug Resistance 8, 411–419. https://doi.org/10.1016/j.ijpddr.2018.09.003*

Baltrušis, P., Komáromyová, M., Várady, M., von Samson-Himmelstjerna, G., Höglund, J., 2020b. Assessment of the F200Y mutation frequency in the β tubulin gene of Haemonchus contortus following the exposure to a discriminating concentration of thiabendazole in the egg hatch test. Experimental Parasitology 217. https://doi.org/10.1016/j.exppara.2020.107957*

Besier, B., Aucamp, J., Wyk, V., Besier, R.B., Kahn, L.P., Sargison, N.D., Wyk, J.A. van, van Wyk, J.A., Besier, B., Aucamp, J., Wyk, V., Besier, R.B., Kahn, L.P., Sargison, N.D., Wyk, J.A. van, 2016. The Pathophysiology, Ecology and Epidemiology of Haemonchus contortus Infection in Small Ruminants. Advances in Parasitology 93, 95–143. https://doi.org/10.1016/bs.apar.2016.02.022

Charlier, J., Rinaldi, L., Musella, V., Ploeger, H.W., Chartier, C., Vineer, H.R., Hinney, B., von Samson-Himmelstjerna, G., Băcescu, B., Mickiewicz, M., Mateus, T.L., Martinez-Valladares, M., Quealy, S., Azaizeh, H., Sekovska, B., Akkari, H., Petkevicius, S., Hektoen, L., Höglund, J., Morgan, E.R., Bartley, D.J., Claerebout, E., 2020. Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe. Preventive Veterinary Medicine 182, 105103. https://doi.org/10.1016/j.prevetmed.2020.105103

Coles, G.C.C., Jackson, F., Pomroy, W.E.E., Prichard, R.K.K., von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M.A.A., Vercruysse, J., 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology 136, 167–185. https://doi.org/10.1016/j.vetpar.2005.11.019

Elmahalawy, Safaa T., Halvarsson, P., Skarin, M., Höglund, J., 2018. Droplet digital polymerase chain reaction (ddPCR) as a novel method for absolute quantification of major gastrointestinal nematodes in sheep. Veterinary Parasitology 261, 1–8. https://doi.org/10.1016/j.vetpar.2018.07.008*

Elmahalawy, S. T., Halvarsson, P., Skarin, M., Höglund, J., 2018. Genetic variants in dyf-7 validated by droplet digital PCR are not drivers for ivermectin resistance in Haemonchus contortus. International Journal for Parasitology: Drugs and Drug Resistance 8. https://doi.org/10.1016/j.ijpddr.2018.04.005*

Halvarsson, P., Höglund, J., 2021. Sheep nemabiome diversity and its response to anthelmintic treatment in Swedish sheep herds. Parasites & Vectors 14, 114. https://doi.org/10.1186/s13071-021-04602-y

Höglund, J., Elmahalawy, S.T., Halvarsson, P., Gustafsson, K., 2019. Detection of Haemonchus contortus on sheep farms increases using an enhanced sampling protocol combined with PCR based diagnostics. Veterinary Parasitology: X 2, 100018. https://doi.org/10.1016/j.vpoa.2019.100018*

Höglund, J., Enweji, N., Gustafsson, K., 2020. First case of monepantel resistant nematodes of sheep in Sweden. Veterinary Parasitology: Regional Studies and Reports 22, 100479. https://doi.org/10.1016/j.vprsr.2020.100479*

Höglund, J., Gustafsson, K., Ljungström, B.-L., Engström, A., Donnan, A., Skuce, P., 2009. Anthelmintic resistance in Swedish sheep flocks based on a comparison of the results from the faecal egg count reduction test and resistant allele frequencies of the beta-tubulin gene. Veterinary Parasitology 161. https://doi.org/10.1016/j.vetpar.2008.12.001*

Höglund, J., Gustafsson, K., Ljungström, B.L., Skarin, M., Varady, M., Engström, F., 2015. Failure of ivermectin treatment in Haemonchus contortus infected-Swedish sheep flocks. Veterinary Parasitology: Regional Studies and Reports 1–2, 10–15. https://doi.org/10.1016/j.vprsr.2016.02.001

Lindqvist, Å., Ljungström, B.L., Nilsson, O., Waller, P.J., 2001. The Dynamics, Prevalence and Impact of Nematode Infections in Organically Raised Sheep in Sweden. Acta Veterinaria Scandinavica 42, 377–389.

Ljungström, S., Melville, L., Skuce, P.J.P.J., Höglund, J., 2018. Comparison of four diagnostic methods for detection and relative quantification of Haemonchus contortus eggs in feces samples. Frontiers in Veterinary Science 4. https://doi.org/10.3389/fvets.2017.00239

Martin, P.J., Anderson, N., Jarrett, R.G., 1989. Detecting benzimidazole resistance with faecal egg count reduction tests and in vitro assays. Australian veterinary journal 66, 236–240. https://doi.org/10.1111/j.1751-0813.1989.tb13578.x

Metera, E., Sakowski, T., Słoniewski, K., Romanowicz, B., 2010. Grazing as a tool to maintain biodiversity of grassland - a review. Animal Science Papers and Reports.

Neveu, C., Charvet, C., Fauvin, A., Cortet, J., Castagnone-Sereno, P., Cabaret, J., 2007. Identification of levamisole resistance markers in the parasitic nematode Haemonchus contortus using a cDNA-AFLP approach. Parasitology 134, 1105–1110. https://doi.org/10.1017/S0031182007000030

Sutherland, I., Scott, I., 2010. Gastrointestinal nematodes of sheep and cattle : biology and control, 1st ed. Wiley-Blackwell, Oxford, United Kingdom.

Urdaneta-Marquez, L., Bae, S.H., Janukavicius, P., Beech, R., Dent, J., Prichard, R., 2014. A dyf-7 haplotype causes sensory neuron defects and is associated with macrocyclic lactone resistance worldwide in the nematode parasite Haemonchus contortus. International Journal for Parasitology 44, 1063–1071. https://doi.org/10.1016/j.ijpara.2014.08.005

Vineer, H.R., Morgan, E.R., Hertzberg, H., Bartley, D.J., Bosco, A., Charlier, J., Chartier, C., Claerebout, E., Waal, T. de, Hendrickx, G., Hinney, B., Johan, H., Keane, O.M., 2020. Increasing importance of anthelmintic resistance in European livestock : creation and meta-analysis of an open database. Parasite 27. https://doi.org/10.1051/parasite/2020062

Waller, P.J., 2006. Sustainable nematode parasite control strategies for ruminant livestock by grazing management and biological control. Animal Feed Science and Technology 126, 277–289. https://doi.org/10.1016/j.anifeedsci.2005.08.007

Wolstenholme, A.J., Fairweather, I., Prichard, R., von Samson-Himmelstjerna, G., Sangster, N.C., 2004. Drug resistance in veterinary helminths. Trends in Parasitology 20, 469–476. https://doi.org/10.1016/j.pt.2004.07.010

Del 3: Resultatförmedling

Vetenskapliga publiceringar	• In total ten scientific publications - for details see the marked with an asterix* in the refernce list above
Övriga publiceringar	• Höglund, J 2018. Ny metod för artbestämning av parasiter hos får. Forskningsnytt från SLU, https://www.slu.se/. • Gustafsson, K. Projekt med SLU. Fårskötsel nr 3 2018. • Gustafsson, K. Betessäsong betyder också masksäsong. Fårskötsel nr 3 2019. • Gustafsson, K., Höglund, J., 2020. Resistenta parasiter – nytt läkemedel hjälpte inte. Fårskötsel nr 4 2020. • Höglund, J & Gustafsson, HÅLL KOLL PÅ MASKEN 3. Parasiter hos får på bete, Fårskötsel nr 4 2019.
Muntlig kommunikation	• Höglund, J. Feb 2018, 2nd COMBAR Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland. • Höglund, J. Sep 2018, 2nd Meeting of Sheep and Goat Researchers within SLU, Uppsala, Sweden. • Höglund 8/3 2019, Ongoing research regarding sampling of parasites from sheep herds. 3rd Meeting of Sheep and Goat Researchers within SLU, Uppsala, Sweden. • Höglund, J. 17/5 2019. Forskning får, BVF-dag, SLU, Uppsala, Sverige. • Höglund, J July 2019, Assessment of an enhanced sampling protocol and use of a ddPCR assay for detection of *Haemonchus contortus* on commercial sheep farms, 27th WAAVP conference, Madison, USA. • Halvarsson, P. July 2019, Nemabiome analysis reveal change in parasite fauna after years of anthelmintic treatment. 27th WAAVP conference, Madison, USA. • Baltrušis, P. July 2019. Exploring benzimidazole resistance in *Haemonchus contortus* by Next Generation Sequencing and Droplet Digital PCR. 27th WAAVP conference, Madison, USA. • Höglund, J. Sep 2020, Anthelmintic resistance in Swedish sheep flocks. 4th LiHRA and 2nd COMBAR meetings, León, Spain. • Höglund, J & Gustafsson, K. Dec 2020. Hur får svenska får resistenta parasiter. Fårpodden (organisatör Titti Strömne,) Webinar.
Studentarbeten	• Elmahalawy, S. Single Nucleotide Polymorphism (SNP) in *dyf7- 141* of *Haemonchus contortus* as potential marker for ivermectin resistance. Master Degree Project in Infection Biology, 30 credits., SLU och Uppsala university. Spring 2017. • Baltrušis, P. Exploring Benzimidazole resistance in *Haemonchus contortus* by Next Generation Sequencing and Droplet Digital PCR. Degree Project in Infection Biology, 30 credits., SLU och Uppsala university. Spring 2018. • Hammarsten, E. Metoder för parasitundersökning i fårbesättningar, Examensarbete 30 hp inom veterinärprogrammet, ISSN 1652-8697, 2018:77 • Ameen, V. Comparison of Droplet Digital PCR and Pyrosequencing Assay for Detection of Benzimidazole Resistance in *Haemonchus contortus*. short term scientific mission (STSM) report, April 2018, COST CA16230. • Komáromyová, M. Genotyping of *Haemonchus contortus* L1 larvae in the Egg Hatch Test via droplet digital PCR. STSM report, Feb 2019. COST CA16230. • Gravdal, M. Introduction to molecular techniques for detection of gastrointestinal helminths and possible anthelmintic resistance, STM report, Jan 2020, COST CA16230.
Övrigt	• The results have been discussed regularly at SAMPAR meeting and with the reference groups • Intervju, 2021 februari, Svensk veterinärtidning / Mats Jansson • Intervju, 2021. DNA-forskning ger hopp om mer effektiv avmaskning. Fårskötsel 2, 21 / Kristina Räf