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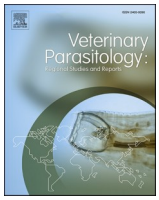

Original Article

Ramming the parasites: Evaluation of quarantine procedures against *Haemonchus contortus* at sheep markets in Sweden

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ABSTRACT

In Sweden, it is recommended to treat rams at sheep markets with ivermectin and then keep them in quarantine to minimise the risk of transmission of *Haemonchus contortus* between farms. The aim of this study was to evaluate the effectiveness of this practise. Accordingly the gastrointestinal parasite status of 50 rams purchased at two sheep markets in central Sweden in autumn 2019 was investigated using faecal samples collected on the day of sale (test 1), 12 ± 3 days after ivermectin injection (test 2, in quarantine) and a few months later before inclusion in the new flocks (test 3). We used both traditional diagnostic methods (i.e. identification of nematode eggs in faeces or larvae in cultures when *H. contortus* eggs could not be identified) and a molecular test based on the digital droplet PCR platform to further identify positive samples. In test 1, conducted in autumn, 40 % of rams were FEC-positive, but only 12 % (six rams) were infected with *H. contortus* according to conventional routine diagnostics. In test 2, 8 % (four rams) were FEC-positive, including one with 1050 eggs, 90 % of which were identified as *H. contortus*. This ram was therefore returned to the supplier. However, ivermectin was found to be effective when this animal and a group of lambs from the same farm were treated and tested again. Test 3 was performed on 44 of the same rams (in addition to the returned ram, two rams died in quarantine and the samples from three rams were never provided by the owners). The proportion of FEC-positive animals was 42 %, with an even higher proportion (27 %) of animals being *H. contortus*-positive than in test 1. The corresponding results for tests 1, 2 and 3 with the ddPCR assay were 18 %, 4 % and 76 %, respectively. This study demonstrates the superiority of DNA detection over microscopy, which is the mainstay in most diagnostic laboratories. Although the combined results confirm that *H. contortus* survived quarantine in two rams, in the other cases it is not clear whether the spring infection rates are due to re-emergence of persistent larvae from quarantine or reinfection on the new farm. These results suggest not only that we should recommend that sheep farmers use a more sensitive molecular test when purchasing and introducing new animals to their flock, but also that the reliability of injectable ivermectin as a quarantine treatment for removal of adult and larval stages needs further investigation.

1. Introduction

Ruminants are frequently exposed to mixed infections with gastrointestinal nematodes (GIN), which, if left uncontrolled, have a negative impact on animal welfare and health, thus reducing farmers' production and profitability (Charlier et al., 2020). This is a recognised problem worldwide, not least in pasture-based sheep production, where GIN infections are one of the main causes of diarrhoea, anaemia, poor digestion and sometimes even mortality, particularly in lambs, alongside subclinical weight loss (Mavrot et al., 2015). Since the 1960's use of anthelmintics has been the main approach to parasite control (Gilleard et al., 2021). However, the overuse of these drugs has led to the widespread development of drug resistance, which now threatens the sustainability of traditional worm control strategies (Rose Vineer et al., 2020). Particularly in countries with extensive sheep farming, anthelmintic resistance has been recognised as a threat that could ultimately affect the global food supply (Kaplan, 2020). In the last ten years, the occurrence of anthelmintic resistance (AR) has also increased in Sweden, especially in *Haemonchus contortus*, where multiple resistance has recently been detected on some farms (Höglund et al., 2022).

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In the present study, we investigated the status of *H. contortus* infection in rams purchased from two local sheep markets before and at two time points after treatment with injectable ivermectin in quarantine. We focussed on *H. contortus* as it is considered the most important pathogen as in many other parts of the world (Besier et al., 2016), and on ivermectin as it is the recommended drug of choice for quarantine in Sweden. In the Netherlands, for example, it has been shown that dangerous pasture infectivity with this highly prolific parasite can develop within a few weeks if it is not controlled (Eysker et al., 2005). Although the infective larvae of *H. contortus* can survive on pasture in winter in Sweden, unlike the other GINs they overwinter mainly in the abomasal mucosa of their host (Troell et al., 2005). In Sweden, the tendency of early stage 4 (EL4) larvae to arrest their development has been described as a unique epidemiological feature occurring from mid-July (Waller et al., 2004). It is also known that *H. contortus* lays fewer eggs at this time of year, which is due to an acquired immune response as the sheep get older (Stear et al., 1999). In recent decades, *H. contortus* has become more widespread. Today, almost three quarters of Swedish sheep farms are infected (Höglund et al., 2019), despite local attempts to eradicate the parasite (Waller et al., 2006).

According to statistics from the Swedish board of Agriculture, the number of sheep has increased to some extent since the 1970s. Today there are around 520,000 sheep of around 20 different breeds such as Gotland sheep, Texel, Finull and Dorset. They not only provide meat, milk, wool and pelt, but also contribute to ecological functions that maintain the biodiversity of the open landscape and the aesthetic value of the grassland (Benthien et al., 2018). Sheep flocks in Sweden are generally smaller than in many other countries, and most animals are kept indoors for half of the year in winter (Halvarsson et al., 2022). However, they must spend the entire summer on pasture, regardless of whether they are kept organically or conventionally. The use of anthelmintics is similar regardless of the production orientation, especially in flocks with more than 80 ewes (Halvarsson et al., 2022).

Most Swedish sheep flocks are concentrated in the south and centre of the country and are kept on a small scale for hobby purposes, as evidenced by the fact that less than 6 % of Swedish sheep flocks have 400 or more ewes (Kumm, 2009). Nevertheless, the demand for locally produced premium lambs is increasing in Sweden, so grazing sheep to produce premium lambs is currently a popular form of livestock farming. To increase the productivity and profitability of these flocks, crossbreeding programmes are essential. Although some of this can be achieved through artificial insemination, ewes are also mated with rams from other farms to introduce new genes. However, the livestock trade carries the obvious risk of contracting parasites (Leathwick et al., 2009). For example, there is evidence that resistant parasites can be transferred from one farm to another via the livestock trade in the Netherlands (Borgsteede et al., 2007). In addition, multiple AR first appeared in GIN in the former Czechoslovakia after goats were imported from New Zealand (Varady et al., 1993). Similarly, multiple AR was confirmed on a Swiss farm where South African Boer goats had previously been imported (Schnyder et al., 2005). As elsewhere in the world, importation from another country appears to have been the trigger for the spread of ivermectin-resistant *H. contortus* in Swedish sheep flocks (Höglund et al., 2015). Less is known about whether this is also the case within the country. All in all, well-designed quarantine procedures appear to be an essential part of minimising the spread of new and/or resistant parasites, regardless of the origin of the animals purchased.

A working group (Sampar) in Sweden with representatives from the Swedish University of Agricultural Sciences, the Swedish Veterinary Agency, Växa Sweden and Farm and Animal Health (Gård & Djurhälsan, G&D) is sharing experiences and working on developing recommendations for effective parasite control in ruminants. Sampar has produced the document "How to prevent the spread of resistant GIN in sheep", which contains recommendations for sampling, treatment and other handling of purchased animals. In this study, we evaluated the effectiveness of the quarantine recommendations currently in place in Sweden. To this end, we analysed 50 breeding rams sold at two markets in the country.

2. Material and methods

2.1. Quarantine practice, animals and sampling

The quarantine procedures for sheep that we have evaluated are available online (https://www.gardochdjurhalsan.se/karantansrekommendationer/). They state that new animals should be kept isolated in quarantine, dewormed with ivermectin and not exposed to parasites in the interim. Finally, it is recommended that a coprodiagnostic test is carried out before the new animal is released onto pasture with the recipient herd. A total of 45 buyers of 50 rams had the opportunity to participate in the project at two ram markets. These took place between September and October 2019 in Linköping and Tenhult in south-central Sweden, and 25 rams were selected at each market. All rams had previously been on pasture and some may have been dewormed by the seller before arriving at the market. Regardless, all rams were dewormed on site at the market by the local veterinarian with an injectable formulation of ivermectin (Noromectin®vet, N-vet) at a dose of 0.2 mg kg^{-1}. At the same time, rectal faecal samples (test 1) were collected in zip-lock plastic bags and sealed immediately after expulsion of air. Buyers were also given written instructions on how to quarantine the animals on arrival at their farm (as described above) and gave their consent for the study. These instructions specified when and how to collect samples for tests 2 and 3 (see below) and that the rams should be kept separate until integration into the recipient flock (test 3). Farmers were also provided with sample material for two further tests, i) a treatment control (test 2) 12 ± 3 days after treatment at market and ii) an additional sample (test 3) the following spring prior to release in the 2020 grazing season.

2.2. Parasitology

On the day of sampling, collected faeces were sent overnight by post to the diagnostic laboratory (Vidilab AB, Enköping) for parasitological examination. On arrival, the nematode eggs were counted using a modified McMaster method with a minimum diagnostic sensitivity of 50 eggs per gramme (EPG) to determine the number of GIN eggs in 3 g of faeces (EPG). For faecal egg counts (FEC), the results were expressed as the total number of trichostrongylids (i.e. including *Haemonchus, Trichostrongylus* and *Teladorsagia* eggs), with the proportion of *H. contortus* eggs determined based on the appearance and morphology of the eggs. Similarly, the FECs of *Chabertia/Oesophagostomum* and *Nematodirus* spp. were counted separately according to the criteria described in Ljungström et al. (2018).

If no *H. contortus* eggs were found at FEC in test 1, larval cultures (LC) were prepared by mixing the remaining faeces with vermiculite and then incubating at room temperature (i.e. at 20 to 25 °C) in a moist state for 7–10 days. The infective larvae (L3) were then harvested by adding water using the Petri dish method previously described by Elmahalawy et al. (2018), after which the presence of *H. contortus* larvae was determined by microscopic examination according to van Wyk and Mayhew (2013). In tests 2 and 3, a culture was established regardless of the FEC result if sufficient faeces was present, and kept for ddPCR testing (see next paragraph). In reporting, we created a third category based on FEC or LC results: Findings of *H. contortus* based on conventional routine diagnostics.

After concentration and removal of excess water, the resulting larvae were stored in Eppendorf tubes in the freezer at approximately −18 °C. After thawing, genomic DNA was extracted using the Nucleospin® tissue kit (Macherey-Nagel) according to the manufacturer's instructions. The absolute number of Haemonchus-DNA copies was quantified in a duplex reaction using a combination of two different primer-probe sets targeting different positions in the internal transcribed spacer region 2 (ITS2)

in the ribosomal RNA gene array: (i) for the detection of the universal strongylid DNA (serving as an internal control) and (ii) for the detection of the specific Haemonchus-DNA as described in (Baltrušis and Höglund, 2023). For this, we used the BioRad® Droplet Digital (dd)PCR assay platform as previously described (Elmahalawy et al., 2018).

2.3. Faecal egg count reduction assesment

A ram (#14) had 1050 EPG, 90 % of which were identified as *H. contortus* after arrival at the receiving farm. The purchase was therefore cancelled and the animal was returned to the supplier. To further investigate the resistance status in the supplier flock, lambs from the same farm were tested using the faecal egg count reduction test (FECRT). In brief, 15 randomly selected, ear-tagged lambs were treated orally with 1.2 of the recommended dose (200 µg per kg body weight) of ivermectin (Noromectin®vet, N-vet), after being weighed on a calibrated scale, with the dosing equipment calibrated to avoid underdosing. In this context, individual faecal samples were also taken from each animal as previously described. Subsequently, 10 days after treatment, further samples were taken from 12 animals with the highest pre-treatment FEC levels. All samples, both before and after treatment, were individually packed in airtight plastic zip-lock bags and labelled with the animal's unique identity number and sent to the diagnostic laboratory where they were analysed as described above. The effect of the treatments (level of clinical resistance) was finally evaluated essentially according to the recommendations of the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines Kaplan et al. (2023).

2.4. Additional information

Of the 45 buyers, the majority, 82 % (n = 37), were customers in connection with the services offered by G&D. For these farms, previous records of *H. contortus* detection following faecal testing in samples from the farms in question for 2018, 2019 and 2020 could be partially verified. In addition, data was collected from 18 purchasing farms (43 %) that participated in a nationwide questionnaire use of anthelmintics and farm management, which was distributed by e-mail in October 2019.

2.5. Statistics

Data summaries and descriptive statistical analyses were performed in Microsoft® Excel version 16.81, while statistical comparisons and graphical representations were performed using Prism version 10.1.1. for macOS X (GraphPadSoftware LLC). Comparisons of FEC values were performed non-parametrically as the normality hypothesis was rejected (Shapiro-Wilk test). The Kruskal–Wallis test was then used to assess the effect of test occasion and, if significant, followed by a pairwise comparison with a Mann–Whitney test. Results of ddPCR amplifications were generated using the Droplet Digital PCR XQ200 System (Bio-Rad Laboratories Inc.) and data were analysed using QuantaSoft™ Analysis Pro (version 1.7.4.0917). Thresholds were set by the software and fine-tuned manually. More than 10*H. contortus* ITS-2 copies were required for a sample to be categorized as positive.

3. Results

3.1. Parasitology

3.1.1. Faecal egg counts

The FEC-values differed significantly between the three time points for both the trichostrongylids (p = 0.0012) and *Chabertia/Oesophagostomum* (p > 0.0001), but not for *Nematodirus* (see Appendix 1).

In test 1, the majority, 60 % (n = 30) of rams were FEC negative on arrival at the market. As shown in Fig. 1A, only 28 % (n = 14) contained trichostrongylid eggs. The FEC of these eggs was higher than the FEC of the other two categories (i.e. *Chabertia/Oesophagostomum* and *Nematodirus* spp.) (Fig. 2A). Although FEC values varied considerably (Fig. 2B), *H. contortus* was only detected in four samples (#14, 20, 21, 22). In addition, two samples contained only *Chabertia/Oesophagostomum* (#19, 38), and two (#17, 40) contained only *Nematodirus* spp. Eggs of all taxa (including *H. contortus*) were found only in one ram (#21).

In test 2, which was collected after an average of 12 ± 3 days post treatment (i.e. test 1) and while the animals were in quarantine, both the number of FEC-positive rams (Fig. 1) and EPG values had fallen in all categories (Fig. 2). At this point, one ram (#14) still had a FEC of 1050 eggs per gramme (EPG) with the majority (90 %) identified as *H. contortus*. As already mentioned, this ram was returned to the supplier. Otherwise, 50 *Nematodirus* EPG were diagnosed in one animal (#6) and 100 *Chabertia/Oesophagostomum* eggs in another ram (#19).

The samples in test 3, which was carried out in 2020, arrived at the laboratory after on average of 227 ± 25 days after test 2. We then received faeces from 45 rams, including two samples in March, 22 in April, 16 in May, 3 in June and 2 in July. Among the missing samples one ram (#20) died in quarantine from a clostridial infection and another (#6) was euthanised due to respiratory problems that started in quarantine and worsened over a month. In addition, samples from three rams (#10, 16, 41) were never received despite several reminders. As shown

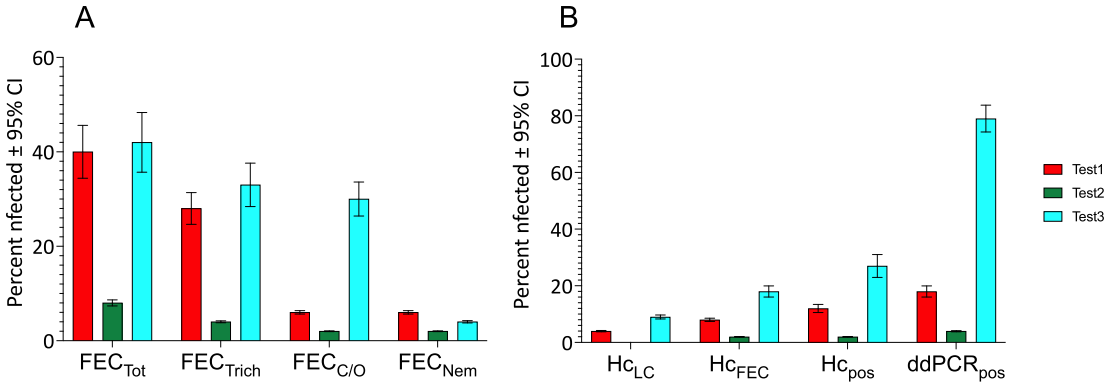

Fig. 1. (A) Percentage of rams infected with gastrointestinal strongylid nematodes on 3 test dates, based on faecal egg count (FEC) examination. FEC_{Tot} = all strongylid eggs, FEC_{Trich} = trichostrongylids, $FE_{C/O}$ = *Oesophagostomum/Chabertia*, FEC_{Nem} = *Nematodirus* spp. (B) Proportion of *H. contortus*-positive rams based on different diagnostic tools. Hc_{LC} = larvae detected in cultures (n = 46 $_{T1}$, 49 $_{T2}$, 45 $_{T3}$), Hc_{FEC} = eggs detected in faecal egg counts (n = 50$_{T1}$, 49$_{T2}$, 45$_{T3}$), Hc_{pos} = detected by conventional diagnostic methods (i.e. LC and/or FEC, n = 50 $_{T1}$, 49 $_{T2}$, 45 $_{T3}$), $ddPCR_{pos}$ = detected by digital droplet PCR (n = 45 $_{T1}$, 48 $_{T2}$, 42 $_{T3}$). Test 1 (T1) = upon arrival at the sheep market, Test 2 (T2) = 12 ± 3 days after ivermectin injection (T1), Test 3 (T3) = the following spring before being pastured. Note that the LC of four rams recognised as *H. contortus*-positive by their eggs were missing in T1 and some were not available in T2 and T3.

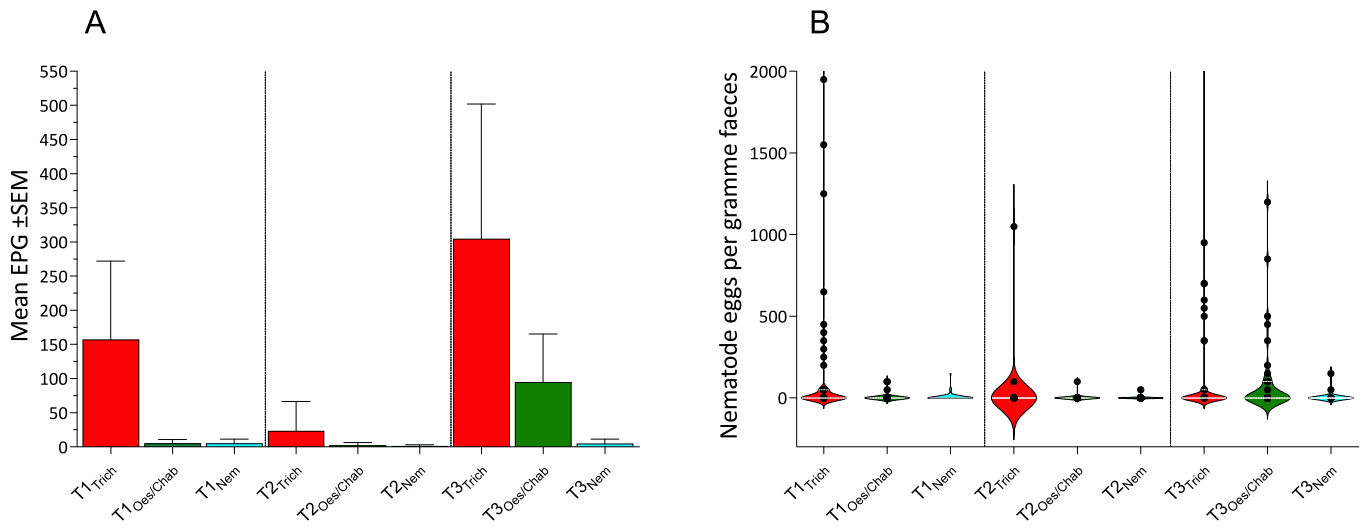

Fig. 2. (A) Nematode eggs per gramme of faeces (EPG) in rams (n = 50–45) purchased at Swedish sheep markets on 3 test occasions. Test 1 (T1) = on arrival at the sheep market, Test 2 (T2) = 12 ± 3 days after ivermectin treatment (T1), while Test 3 (T3) = the following spring before being pastured. Trich = Trichostrongylid eggs, Oes/Chab = *Chabertia* or *Oesophagostomum*, Nem = *Nematodirus* spp. (B) Truncated violin plots showing the number of nematode eggs in faeces of rams tested on the same occasions. Note that one sample with 8950 EPG is outside the Y-axis in $T3_{Trich}$ in B.

in Fig. 1, the number of FEC-positive samples had risen to approximately the same level as in test 1. One third (33 %) contained trichostrongylid eggs, with *H. contortus* detected in eight samples (#5, 8, 19, 30, 38, 45, 49, 51). Although the average here was higher than in test 1, this difference was not significant. Interestingly, no less than 13 samples (#5, 9, 13, 19, 21, 22, 23, 24, 27, 38, 39, 40, 51), contained between 50 and 1200 *Chabertia/Oesophagostomum* EPG, usually in combination with other eggs (exceptions were #22, 24, 39). When compared to test 1 the observed increase was significant ($p = 0.0039$). In addition, two rams (#40, 44) had between 50 and 150 *Nematodirus* EPG (Fig. 2).

3.2. ddPCR and comparison with routine diagnostics

The conventional larval culture (LC) analysis focused exclusively on *H. contortus*, and larvae were detected in 4 %, 0 % and 9 % of rams in tests 1, 2 and 3, respectively (Fig. 1B). Of note, in test 1, cultures were not available in four rams (#14, 20, 21, 22) as they were identified as positive by FEC alone. Molecular results were also missing from two rams (#14, 43) in test 2, and from eight rams (# 6, 10, 15, 16, 20, 25, 41) in test 3. Based on the routinely used combined parasitological tests (i.e. FEC and/or LC), 12 %, 2 % and 27 % of rams were diagnosed as *H. contortus*-positive in tests 1, 2 and 3, respectively, while the corresponding figures for the ddPCR test were 18 %, 4 % and 76 % (Fig. 1B). Interestingly, 54 % of samples that were negative by routine diagnostics were identified as positive by ddPCR. In contrast, all samples that were diagnosed as *H. contortus*-positive were also positive by ddPCR.

Firstly, of the eight rams (#12, 13, 16, 22, 23, 27, 44, 46) that tested positive for *H. contortus* by ddPCR in test 1, only 2 (#12, 22) were also identified by routine diagnostics. Notably, four rams (#14, 20, 21, 22) diagnosed by FEC in test 1 LC for ddPCR were not available. Secondly, of the two rams (#15, 21) identified by ddPCR in test 2, both were negative after routine diagnostics. In addition, the only ram (#14) that tested positive with FEC in test 2 was never tested by ddPCR as it was returned to the supplier. Third, of the 33 of the 45 rams identified as positive for *H. contortus* by ddPCR in test 3, only 12 (#5, 9, 19, 21, 22, 28, 30, 32, 38, 45, 49, 51) were also identified as positive by the combined routine diagnostic.

3.3. Historical data and owners compliance with quarantine practises

According to the historical data, *H. contortus* was identified in 12 of the farms that received rams that tested positive with ddPCR in the spring (#2, 5, 7, 22, 23, 28, 29, 30, 39, 47, 49, 50), five of which (#5, 22, 28, 30, 49) had rams that were also positive after routine diagnostics. In 11 cases (i.e. farms that received rams #9, 16, 18, 21, 32, 34, 35, 38, 40, 44, 45) the historical status of these farms was unknown, while of the historically negative recipient farms, 7 (#8, 12, 13, 26, 27, 42, 46) had rams that tested positive for Hc-ITS2 by ddPCR. In total, only two rams (#12, 40) were confirmed not to have been outdoors in autumn and/or spring and not to have received fresh grass from the buyer's pastures. Both rams tested positive by ddPCR in both test 1 and test 3. In test 1, ram #12 had 0 EPG but with *H. contortus* larvae according to the LC results, while ram #40 had 50 EPG without *H. contortus*. In test 2, both rams were negative in all tests. In test 3, ram #12 was negative after routine diagnostics but had 191*H. contortus* ITS-2 copies, while ram #40 had 550 trichostrongylid eggs (but no *H. contortus*) and 1200 eggs of *Chabertia/Oesphagostomum* with 1006 copies of Hc-ITS2. This indicates that this animal was misdiagnosed during routine diagnostics.

3.4. Faecal egg count reduction

The tested lambs included were all *H. contortus*-positive and had an average of 395 ± 206 EPG, which varied between 100 and 750 EPG when the lambs were treated orally. No eggs were found in the same animals after treatment. Ram #14 was also successfully treated in this way after being returned.

4. Discussion

In this study, the quarantine recommendations for rams transferred between Swedish sheep farms were evaluated. Interestingly, most rams were FEC-negative at the first inspection at the sheep market and only a few of them were infected with *H. contortus*. This indicates that many rams are adequately dewormed by the seller before they are brought to market to be sold. In the few cases where eggs were present, monitoring of treatment shortly after ivermectin injection also showed that most rams responded as expected. Overall, therefore, there is little evidence that the adult parasites tested here were resistant to ivermectin. Nevertheless, infection rates returned. in test 3 and were relatively high in several rams, even when samples were taken in April and May, i.e. at a time before the grazing period. This pattern was further emphasised by the results of the molecular test. In cases where procedures were

followed, and perhaps also in the recipient farms where *H. contortus* had not previously been detected, this suggests that quarantine procedures need to be improved. In light of these findings, the efficacy of ivermectin needs to be continuously monitored and investigated. In the meantime, there is a risk of further spread of *H. contortus* throughout the country, regardless of whether the parasites are resistant to ivermectin or not.

It is known that trade in livestock can contribute to the spread of resistant parasites both within and between countries (Borgsteede et al., 2007; Schnyder et al., 2005). It is therefore important to encourage farmers to take advantage of quarantine treatment before adding new animals to their farms, but also to evaluate existing practises. All buyers in the present study were advised to keep the recruited rams indoors or separate from contaminated pastures after their arrival at the new farm until test 3 was performed. However, we cannot guarantee that this practise was always followed. This complicates the interpretation, as it is not always possible to say with 100 % certainty whether the apparently high infection rates observed in test 3 are due to surviving worms or to an infection picked up on the new farm. On the other hand, two cases it was confirmed that the procedures were strictly followed, even when *H. contortus* was present, suggesting that the parasite can survive the current quarantine treatment, even if this does not happen as often. It is known that handling animals in the trade can be stressful despite high welfare standards, making them more susceptible to disease (Gregory et al., 2009). Quarantine is often used to determine whether purchased animals can become ill and/or infect other animals in their new environment, even though they showed no signs of disease on arrival. The fate of the two rams that died in this study shows that this can be the case and illustrates that even animals with valuable genetic traits in good condition can suddenly become seriously ill. However, it is important to emphasise that in neither of the two fatal cases observed here was a parasitic infection the main cause of death.

For the interpretation of the data, it is important to know that all diagnostic tests used in this study (i.e. FEC, LC and ddPCR) only detect animals shedding eggs produced by adult females. Therefore, there is no information on the presence of preadult and male nematodes present in the animal at the time of sampling. Although our results clearly show that the routine and molecular parasitological tests differ in their diagnostic ability, information on the arrested larval stages is missing in each case. At the same time, the manufacturer's instructions for use postulate, in agreement with Yazwinski et al. (1983) and Benz et al. (1989), that ivermectin kills almost all (99.9 %) stages if the strain in question is susceptible to the drug. To a certain extent, this view was confirmed by our data, as most samples in test 2 were FEC-negative shortly after treatment. However, there were a few exceptions. For example, one ram (#14) was returned to the supplier. In this case, it was later determined that this was not due to anthelmintic resistance, as this ram and all lambs tested from the same farm were FEC-negative after oral ivermectin treatment. It is likely that this ram was not treated appropriately at the market.

In addition to this ram, two other rams (#15, 21) were identified by ddPCR in test 2, although no *H. contortus* larvae were found in the cultures or by FEC. These observations are in line with previous studies demonstrating the higher sensitivity and accuracy of the molecular tests (Ljungström et al., 2018; Höglund et al., 2019). Similar results were also observed for test 3: according routine diagnostics, *H. contortus* was found in 12 rams (27 %). In contrast, 33 rams (79 %) were ddPCR positive, although only 42 samples were tested (3 cultures were missing, 2 rams had died, and no third sample had been obtained from 3 rams). In addition, the sample from one ram (#40) was probably misdiagnosed by microscopy. This shows firstly that a molecular test is more sensitive and accurate in identifying animals with patent infection. Secondly, our results emphasise the importance of testing and treating purchased rams in the spring before they are brought into the new flock for mating. Otherwise, it may be difficult to prevent the build-up of larval parasite populations in the coming grazing season. This applies regardless of where the infection originated (i.e. whether it was introduced with the ram or picked up on the receiving farm).

Two factors (arrestment/larval inhibition and acquired immunity) may have contributed to the suboptimal timing of the samples collected in tests 1 and 2. A third test was therefore carried out the following spring before the rams were put on pasture. This was to further verify the effect of the deworming in quarantine. It was assumed (based on the instructions to the farmers) that the rams had not previously been on a contaminated pasture or paddock and had therefore not come into contact with infective larvae in the meantime. However, as already mentioned, it was not always possible to determine this. At the same time, we have shown in a previous study that arrested *H. contortus* re-emerge under the influence of increasing light in spring and soon develop into mature worms, whereupon oviposition begins again (Höglund et al., 2021). This is an additional factor that may have contributed to the fact that the samples from the same rams in test 3 tended to have higher values than in the tests carried out in autumn. If this is the case, it suggests that the efficacy of ivermectin against the inhibited larval stages was reduced. In any case, this observation is consistent with previous observations showing that the efficacy of ivermectin against EL4 in naturally infected sheep varies between 46 % and 100 % (Wescott and LeaMaster, 1982). However, this aspect certainly requires further confirmation. As resistance genes to multiple substances are still rare in the strains found on most Swedish sheep farms, the use of combination anthelmintics with a similar spectrum of activity needs to be investigated as an alternative to ivermectin alone, for reasons explained by e.g. Leathwick et al. (2009), although this is considered controversial by local veterinarians.

5. Conclusions

The current Swedish quarantine recommendation for livestock appears to reduce the risk of introducing *H. contortus* to the buyer's farm. In the treatment control (test 2), only three rams were infected. One was detected by FEC, while the other two rams could only be detected by the molecular method (ddPCR). It can be concluded that the molecular method allows a more sensitive diagnosis. Thus, although FEC remains a valuable tool, it should preferably be used in conjunction with DNA detection. Furthermore, we have shown in one case that a positive sample in quarantine does not necessarily mean that treatment failure is due to anthelmintic resistance, but that other factors such as inadequate deworming may also play a role. Despite all this, infection rates were relatively high in the rams in spring. To summarise, this study shows that when buying rams, it is important to examine them in the spring before they are put out to pasture with the new flock and to treat them if necessary. This applies regardless of whether they were infected at the time of purchase and/or whether they appear to have responded to quarantine treatment.

Ethics statement

This study was conducted with animals and with anthelmintic drugs in compliance with the current laws of the country in which they were performed.

CRediT authorship contribution statement

Johan Höglund: Writing – review & editing, Writing – original draft, Visualization, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Giulio Grandi:** Writing – review & editing, Conceptualization. **Nizar Enweji:** Methodology, Investigation. **Katarina Gustafsson:** Writing – review & editing, Methodology, Conceptualization.

Declaration of competing interest

I hereby declare that the information we have provided is true and

that we are not aware of any other situation of actual, potential, or apparent conflict of interest. I undertake to inform you of any change in these circumstances, even if a problem arises in the course of the work itself.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2024.101125.

References

Baltrušis, P., Höglund, J., 2023. Digital PCR: modern solution to parasite diagnostics and population trait genetics. Parasit. Vectors 16, 143. https://doi.org/10.1186/s13071-023-05756-7.

Benthien, O., Braun, M., Riemann, J.C., Stolter, C., 2018. Long-term effect of sheep and goat grazing on plant diversity in a semi-natural dry grassland habitat. Heliyon 4, e00556. https://doi.org/10.1016/j.heliyon.2018.e00556.

Benz, G.W., Roncalli, R.A., Gross, S.J., 1989. Use of Ivermectin in cattle, sheep, goats, and swine. In: Campbell, W.C. (Ed.), Ivermectinn and Abamectin. Springer-Verlag, New York, pp. 215–229.

Besier, B., Aucamp, J., Wyk, V., Besier, R.B., Kahn, L.P., Sargison, N.D., Van Wyk, J.A., Van Wyk, J.A., Besier, B., Aucamp, J., Wyk, V., Besier, R.B., Kahn, L.P., Sargison, N. D., Van Wyk, J.A., 2016. The pathophysiology, ecology and epidemiology of Haemonchus contortus infection in small ruminants. Adv. Parasitol. 93, 95–143. https://doi.org/10.1016/bs.apar.2016.02.022.

Borgsteede, Dercksen, D.D., Huijbers, R., 2007. Doramectin and albendazole resistance in sheep in the Netherlands. Vet. Parasitol. 144, 180–183. https://doi.org/10.1016/j.vetpar.2006.09.031.

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. Prev. Vet. Med. 182, 105103. https://doi.org/10.1016/j.prevetmed.2020.105103.

Elmahalawy, S.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. Vet. Parasitol. 261, 1–8. https://doi.org/10.1016/j.vetpar.2018.07.008.

Eysker, M., Bakker, N., Kooyman, F.N.J., Van Der Linden, D., Schrama, C., Ploeger, H.W., 2005. Consequences of the unusually warm and dry summer of 2003 in The Netherlands: poor development of free living stages, normal survival of infective larvae and long survival of adult gastrointestinal nematodes of sheep. Vet. Parasitol. 133, 313–321. https://doi.org/10.1016/j.vetpar.2005.05.058.

Gilleard, J.S., Kotze, A.C., Leathwick, D., Nisbet, A.J., McNeilly, T.N., Besier, B., 2021. A journey through 50 years of research relevant to the control of gastrointestinal nematodes in ruminant livestock and thoughts on future directions. Int. J. Parasitol. 51, 1133–1151. https://doi.org/10.1016/j.ijpara.2021.10.007.

Gregory, N.G., Benson, T., Smith, N., Mason, C.W., 2009. Sheep handling and welfare standards in livestock markets in the UK. J. Agric. Sci. 147, 333–344. https://doi.org/10.1017/S002185960800837X.

Halvarsson, P., Gustafsson, K., Höglund, J., 2022. Farmers' perception on the control of gastrointestinal parasites in organic and conventional sheep production in Sweden. Vet. Parasitol. Reg. Stud. Rep. 30, 100713. https://doi.org/10.1016/j.vprsr.2022.100713.

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. Vet. Parasitol. Reg. Stud. Rep. 1–2, 10–15. https://doi.org/10.1016/j.vprsr.2016.02.001.

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. Vet. Parasitol. X 2, 100018. https://doi.org/10.1016/j.vpoa.2019.100018.

Höglund, J., Carlsson, A., Gustafsson, K., 2021. Effects of lambing season on nematode faecal egg output in ewes. Vet. Parasitol. Reg. Stud. Rep. 26, 100633. https://doi.org/10.1016/j.vprsr.2021.100633.

Höglund, J., Baltrušis, P., Enweji, N., Gustafsson, K., 2022. Signs of multiple anthelmintic resistance in sheep gastrointestinal nematodes in Sweden. Vet. Parasitol. Reg. Stud. Rep. 36, 100789. https://doi.org/10.1016/j.vprsr.2022.100789.

Kaplan, R.M., 2020. Biology, epidemiology, diagnosis, and management of anthelmintic resistance in gastrointestinal nematodes of livestock. Vet. Clin. North Am. Food Anim. Pract. 36, 17–30. https://doi.org/10.1016/j.cvfa.2019.12.001.

Kaplan, R.M., Denwood, M.J., Nielsen, M.K., Thamsborg, S.M., Torgerson, P.R., Gilleard, J.S., Dobson, R.J., Vercruysse, J., Levecke, B., 2023. World Association for the Advancement of veterinary parasitology (W.A.A.V.P.) guideline for diagnosing anthelmintic resistance using the faecal egg count reduction test in ruminants, horses and swine. Vet. Parasitol. 318, 109936. https://doi.org/10.1016/j.vetpar.

Kumm, K.I., 2009. Profitable Swedish lamb production by economies of scale. Small Rumin. Res. 81, 63–69. https://doi.org/10.1016/j.smallrumres.2008.11.006.

Leathwick, D.M., Hosking, B.C., Bisset, S.A., McKay, C.H., 2009. Managing anthelmintic resistance: is it feasible in New Zealand to delay the emergence of resistance to a new anthelmintic class? N. Z. Vet. J. 57, 181–192. https://doi.org/10.1080/00480169.2009.36900.

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

Mavrot, F., Hertzberg, H., Torgerson, P., 2015. Effect of gastro-intestinal nematode infection on sheep performance: a systematic review and meta-analysis. Parasit. Vectors 8, 1–11. https://doi.org/10.1186/s13071-015-1164-z.

Rose Vineer, H., Morgan, E.R., Hertzberg, H., Bartley, D.J., Bosco, A., Charlier, J., Chartier, C., Claerebout, E., de Waal, T., Hendrickx, G., Hinney, B., Höglund, J., Ježek, J., Kašný, M., Keane, O.M., Martínez-Valladares, M., Mateus, T.L., McIntyre, J., Mickiewicz, M., Munoz, A.M., Phythian, C.J., Ploeger, H.W., Rataj, A. V., Skuce, P.J., Simin, S., Sotiraki, S., Spinu, M., Stuen, S., Thamsborg, S.M., Vadlejch, J., Varady, M., von Samson-Himmelstjerna, G., Rinaldi, L., 2020. Increasing importance of anthelmintic resistance in European livestock: creation and meta-analysis of an open database. Parasite 27, 69. https://doi.org/10.1051/parasite/2020062.

Schnyder, M., Torgerson, P.R., Schönmann, M., Kohler, L., Hertzberg, H., 2005. Multiple anthelmintic resistance in Haemonchus contortus isolated from south African Boer goats in Switzerland. Vet. Parasitol. 128, 285–290. https://doi.org/10.1016/j.vetpar.2004.12.010.

Stear, M.J., Strain, S., Bishop, S.C., 1999. Mechanisms underlying resistance to nematode infection. Int. J. Parasitol. 29, 51–56. https://doi.org/10.1016/S0020-7519(98)00179-9.

Troell, K., Waller, P.J., Höglund, J., 2005. The development and overwintering survival of free-living larvae of Haemonchus contortus in Sweden. J. Helminthol. 79. https://doi.org/10.1079/JOH2005286.

van Wyk, J.A., Mayhew, E., 2013. Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: a practical lab guide. Onderstepoort J. Vet. Res. 80. https://doi.org/10.4102/ojvr.v80i1.539.

Varady, M., Praslicka, J., Corba, L., Vesely, L., 1993. Multiple anthelmintic resistance of nematode in imported goats. Vet. Rec. 132, 387–388.

Waller, P.J., Rudby-Martin, L., Ljungström, B.L., Rydzik, A., 2004. The epidemiology of abomasal nematodes of sheep in Sweden, with particular reference to over-winter survival strategies. Vet. Parasitol. 122, 207–220. https://doi.org/10.1016/j.vetpar.2004.04.007.

Waller, P.J., Rydzik, A., Ljungström, B.L., Törnquist, M., 2006. Towards the eradication of Haemonchus contortus from sheep flocks in Sweden. Vet. Parasitol. 136, 367–372. https://doi.org/10.1016/j.vetpar.2005.11.017.

Wescott, R.B., LeaMaster, B.R., 1982. Efficacy of ivermectin against naturally acquired and experimentally induced nematode infections in sheep. Am. J. Vet. Res. 43, 531–533.

Yazwinski, T.A., Greenway, T., Presson, B.L., Pote, L.M., Featherstone, H., Williams, M., 1983. Antiparasitic efficacy of ivermectin in naturally parasitized sheep. Am. J. Vet. Res. 44, 2186–2187 (PMID: 6689112).