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Detection of *Haemonchus contortus* on sheep farms increases using an enhanced sampling protocol combined with PCR based diagnostics

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ABSTRACT

An enhanced sampling strategy for detection of gastrointestinal parasites of sheep based on faecal sampling covering approximately 10% of the animals in the flock was evaluated with focus on the major sheep pathogen *Haemonchus contortus.* We also compared traditional diagnostics based on faecal eggs counts (FEC) by microscopy with DNA detection on frozen faeces samples using a droplet digital (dd)PCR assay. 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 a ddPCR assay. 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. 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 irrespective 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). No significant differences in infection levels were observed between the two production systems (conventional and organic) irrespective of the diagnostic method used. However, 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. In conclusion, we have validated a practical tool for sheep producers to assess *Haemonchus* infection with high precision.

1. Introduction

Infections with gastro-intestinal nematode (GIN) parasites is globally well-known as a major veterinary problem in the sheep industry and may contribute to low farming productivity (Sutherland and Scott, 2010). Especially the abomasal nematode *Haemonchus contortus* is a highly pathogenic parasite which sometimes causes anaemia, production loss and mortality particularly in lambs and pregnant ewes (Besier et al., 2016). Swedish lamb production must be characterized by high standards of animal health and welfare and low use of medication, but it has been recognized that it is problematic to carry on pasture-based lamb production without use of anthelmintic drugs both on conventional and organic sheep farms in Sweden. A major difference though is that national regulations stipulate that anthelmintics are only allowed on organic farms following a diagnostic test procedure, while the prophylactic use of anthelmintic drugs is still permitted on conventional farms if the veterinarian have good knowledge of the specific herd (www.krav.se/nationellariktlinjer).

The practice of anthelmintic treatments on sheep farms is currently threatened due to the increasing anthelmintic resistance (AR), which today is found to both drug classes registered on the Swedish market (i.e. benzimidazoles and macrocyclic lactones) (Höglund et al., 2016). Although AR in Sweden is limited from an international perspective, care must be taken to avoid a situation observed for example in the Netherlands, UK and Ireland were the animals can be treated up to eight times per year (Learmount et al., 2016; Patten et al., 2011; Ploeger et al., 2016). Thus, the way forward is to keep the use of anthelmintic to a minimum and to deworm only those flocks that require treatment according to the principles for targeted flock treatments (Kenyon and Jackson, 2012). This requires that advice is based on

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reliable diagnostic information.

The Farm and Animal Health is a stakeholder organisation which for several decades has offered an advisory service to Swedish sheep farmers. Over the years, this service has safeguarded the health and welfare in Swedish sheep flocks. In a nationwide survey on the efficacy of major anthelmintics in Sweden, conducted in 2006 and 2007 including 45 sheep farms, it was shown that only two flocks were infected with GIN that were resistant to the anthelmintic albendazole and in both instances *H. contortus* was the dominant species involved (Höglund et al., 2009). Ever since ivermectin (IVM) has often been the first choice in the treatment of flocks infected with *H. contortus*. However, recently ivermectin-resistant *H. contortus* was also documented. It was first discovered in 2015 in a flock on the island of Gotland which had suffered from recurrent problems with *H. contortus* since East Friesian Dairy sheep were imported from the Netherlands some years earlier (Höglund et al., 2016). Nevertheless, subsequent screening has shown that AR is probably today even more widespread because it is also more frequently observed in flocks in mainland Sweden (unpublished data).

Recently it has been realised that the procedures included in the Farm and Animal Health screening system needs to be adjusted to today's sheep flock types and management practices. This is not surprising as the sampling recommendations and diagnostic procedures dates back to at least the early 1990s. The sampling schedule, which was used until 2015 was based on the investigation of faeces collected from the same number of animals irrespective of flock size; first from six ewes before turnout in spring and then a second sample from six lambs after they have been on pasture for a minimum of three weeks. In the laboratory the faeces are pooled on each sampling occasion into two triplets before microscopical examination with focus on nematode faecal egg counts (FEC) and where the presence of *H. contortus* eggs are determined based on morphological criteria (Ljungström et al., 2018). Based on this diagnostic information in combination with other data, veterinary advice is then given based on the principles for targeted anthelmintic treatments. Although parasitological screening has contributed to limit the spread of AR in Sweden, the programme was launched when sheep flocks had an average of below 30 ewes. Today more flocks are larger, sometimes comprising several hundred ewes. Thus, the screening procedure must be improved so that it can be used with more confidence also in bigger flocks and especially for detection of *H. contortus*.

The aim of this study was to validate a screening strategy for larger commercial sheep flocks in Sweden specialised in pasture-based lamb production; i) by evaluation of extended faecal sampling protocol performed in relation to flock size, and ii) compare traditional diagnostics based on faecal eggs counts (FEC) with a novel diagnostic method based on detection based on droplet digital (dd)PCR. Focus was on the major sheep pathogen *H. contortus*. A questionnaire survey was also conducted to gather information about the management and deworming routines applied on the farms.

2. Materials and methods

2.1. Farms

The study was conducted in 39 commercial sheep flocks distributed throughout Sweden. An equal number of conventional (N = 20) and organic farms (N = 19) were randomly recruited with help of the Farm and Animal Health. The inclusion criteria were; i) farms with more than 70 pregnant ewes; ii) no anthelmintic treatment within six months prior to the first sampling, iii) answer a web-based questionnaire about farm management and deworming routines. Sheep farms were recruited from three regions of Sweden: Götaland (n = 27) in the southern part, Svealand (n = 7) in the central part, and Norrland (n = 5) in the northern part (Fig. 1).

2.2. Faecal sampling and egg counts

Samples were collected on each occasion from approximately 10% of the flock when the animals were handled in a sampling gate. The ewes were sampled once prior to turn-out, whereas the lambs were sampled after they had been out grazing for a minimum of six weeks. In flocks with 70–100 animals nine randomly selected sheep were sampled, in flocks with between 100 to 130 animals 12 sheep, and if there was > 130 animals 15 sheep. Samples from each animal were placed in individual airtight zipper bags where the air was squeezed out before being sealed and sent by regular post to the diagnostic laboratory (Vidilab AB). Upon arrival the following day, the samples were stored at 4 °C for a maximum of one day until analysis. In the laboratory the samples were pooled in triplets by careful mixing of 3 g of faeces from each of three animals. FEC were then carried out using a centrifugation enhanced McMaster technique based on detection of strongyle eggs in 3 g faeces dispersed in 42 mL saturated NaCl (density of 1.18 g/cm^3), providing a minimum diagnostic sensitivity of 50 eggs per gram faeces (epg). Nematode FEC were partitioned into trichostrongylids excluding *Nematodirus* spp and *Chabertia/Oesophagostomum*, which were separately counted. The proportions of *Trichostrongylus axei* and *H. contortus* were expressed as percentages of trichostrongylid. The eggs of *H. contortus* were identified as described earlier (Ljungström et al., 2018).

2.3. Detection of parasite DNA

DNA was extracted from faecal samples stored in a freezer at −4 °C. After thawing pooled samples were prepared by careful mixing of an equal amount of faeces from the same three sheep previously analysed by microscopy. From each triplet 1 g of faeces was put into 7 ml tissue homogenising tubes (CK28, Precellys©Lysing kit) with 4 mL InbititEx buffer (Qiagen) and 3.5 g (2.8 mm ceramic beads, Qiagen). The samples were homogenized on a Precellys© Evolution instrument (Bertin Technologies) for 10 cycles (30 s/30 s) at 500 g. Thereafter the tubes were centrifuged at 180 g for 5 min at room temperature on an IEC CL30 centrifuge (Thermo Scientific) from which 1.2 mL of the lysate was aspirated into a 1.5 mL microcentrifuge Eppendorf tube. Genomic DNA was extracted using the QIAamp fast DNA Stool Mini Kit (Qiagen) according to the instructions from the manufacturer for large faecal samples.

Samples were finally screened with three different primers probe sets targeting different positions in the internal transcribed spacer region 2 (ITS2) situated in the ribosomal RNA gene for DNA of; i) universal strongyle egg DNA, ii) *Haemonchus* specific DNA, and iii) *Teladorsagia* specific DNA as described previously by droplet digital PCR using the BioRad system (Elmahalawy et al., 2018). By using ddPCR two different duplex assays (universal / haemonchus and universal / teladorsagia) the total number of ITS2 gene copies of each genera were quantified in relation the total amount of strongyle DNA in the sample by dividing the gene copies of each genera with the total number of strongyle ITS2 copies detected with the universal probe

2.4. Questionnaire data

Each farm participating in the study completed a web-based questionnaire using the survey software Netigate I (www.netigate.net/sv/). The survey contained 19 obligatory and 7 open questions about farm management routines and the use of anthelmintics (Appendix 2).

2.5. Statistical analyses

Data summaries and descriptive statistical analyses were calculated using Microsoft Excel for Mac version 16.16.6 (Microsoft Corporation). Data was also imported into JMP® Pro ver. 12.0.1 where the distribution of the categorical response variables Y (with presence set to 1 and absence to 0) was conditioned by the values of a categorical X factors

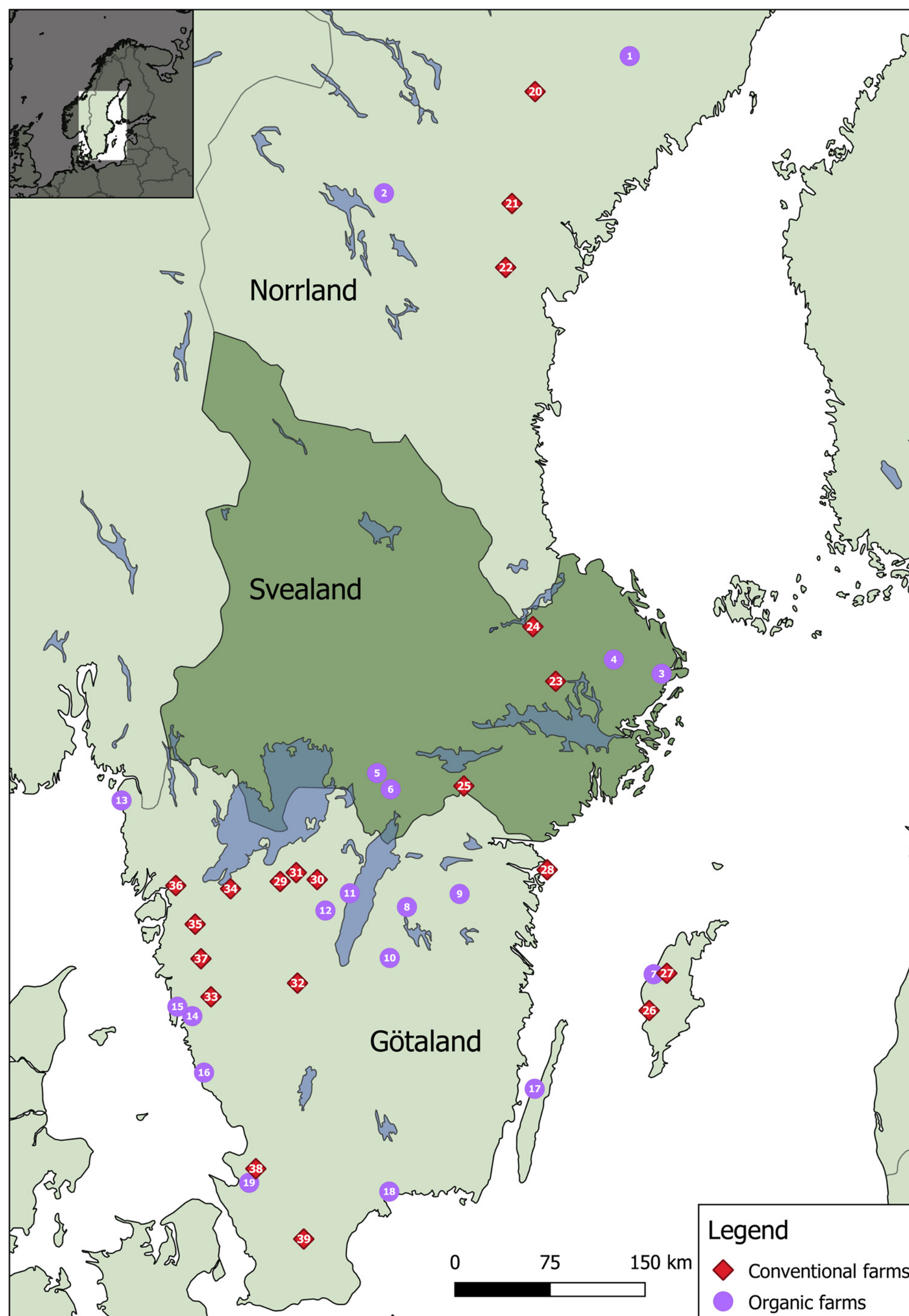

Fig. 1. Map of Sweden showing the location of the investigated farms.

(production [conventional or organic] and age group [ewe or lamb]) using contingency analysis in the fit Y by X platform. Data on FEC were analysed using the same platform by One-way Analysis using both Wilcoxon 2-sample non-parametric test and Student 2-sample *t*-test after logarithmic transformation of EPG + 1 to handle the skewed distribution. All tests were considered to be statistically significant at $P < 0.005$. Questionnaire data was summarized using the built-in application in Netigate.

3. Results

3.1. Farms

Sheep from 20 conventional farms (C) and 19 organic (O) farms participated in the study. From these farms 810 individual samples were collected and pooled into 270 triplets that were examined both by microscopy and ddPCR. The number of lactating ewes on the conventional farms varied between 70 to 210 with a mean of 111 ± SD 42 animals and on the organic farms the number of ewes varied between 72 to 250 animals with a mean of 115 ± SD 42. The geographical distribution of the sampled farms is shown in Fig. 1. The majority (74%) were located in south-central Sweden but with an almost equal regional

Table 1
Number and prevalence at the flock level of internal parasites identified in faecal samples by microscopy according to Ljungström et al. (2018).

Parasites	Conventional farms			Organic farms		
	Positive samples	Prevalence	95% ± CI	Positive samples	Prevalence	95% ± CI
Ewes	**N = 20**			**N = 19**		
Trichostrongylid eggs	18	90%	13%	18	95%	10%
Haemonchus	12	60%	21%	8	42%	22%
Trichostrongylus axei	4	20%	17%	2	11%	14%
Chabertia/Oesophagostomum	14	70%	20%	13	68%	21%
Nematodirus battus	0	0%		0	0%	
Nematodirus filicollis	1	5%	10%	2	11%	14%
Nematodirus spathiger	0	0%		0	0%	
Moniezia expansa	4	20%	17%	3	16%	16%
Coccidian oocysts	0	0%		1	5%	10%
Lambs	**N = 20**			**N = 17**		
Trichostrongylid eggs	17	85%	16%	16	94%	12%
Haemonchus	5	25%	19%	4	24%	20%
Trichostrongylus axei	1	5%	10%	3	18%	18%
Chabertia/Oesephagostomum	11	55%	22%	10	59%	23%
Nematodirus battus	6	30%	20%	2	12%	15%
Nematodirus filicollis	5	25%	19%	7	41%	23%
Nematodirus spathiger	3	15%	16%	1	6%	11%
Moniezia expansa	13	65%	21%	11	65%	23%
Coccidian oocysts	5	25%	19%	8	47%	24%

distribution of organic and conventional farms throughout the country; reflecting the flock sizes and geographical distribution of today's sheep flocks in Sweden. Most farms except two (O2 and O8) submitted samples both from their ewes and lambs. The ewes were sampled between mid-April to mid-May depending on the turn-out date, whereas the lamb samples were collected between early June to September.

3.2. Faecal egg counts

On the 76 sampling occasions 69 (91%) were positive for trichostrongyle eggs (Table 1). The mean FEC of the triplets from the ewes varied between < 50 to 1064 EPG and between < 50 to 1792 EPG in the lambs. There were no significant differences in FEC between the samples from the ewes (264 ± SD 273 EPG) and the lambs (271 ± SD 391 EPG), or between the samples from the conventional farms (286 ± SD 359 EPG) and the organic farms (223 ± SD192 EPG).

Eggs of *H. contortus* were identified on 29 (42%) occasions and more often conventional farms (43%) were infected than organic farms (33%), even if the difference was not significant. However, a gradient with an increased number of farms from north to south was observed with; i) one (O21) of five (20%) northern farm in Norrland, ii) three (O3, O6, C25) of seven (43%) central farms in Svealand, and iii) 16 (O8, O9, O10, O11, O12, O13, O14, C26, C28, C29, C31, C33, C35, C37, C38, C39) of 27 (59%) among the southern farms in Götaland (Figs. 1 and 2).

More samples from the ewes (N = 20) were *H. contortus* positive than from the lambs (N = 9). In the ewes *H. contortus* was found 51% out of the 39 farms. The proportion of eggs identified among trichostrongylid eggs counted, 3% to 84% with an average of 35 ± SD 26%. In the lambs *H. contortus* was found on 24% out of 37 farms investigated. Here the proportion of eggs counted, 5% to 33% with an average of 18 ± SD 10%. Seven samples out of the nine from the lambs came from farms where also the ewes were *H. contortus* positive. The remaining two samples were from farms (O13 and O17) where *H. contortus* eggs were absent in the ewes. On both of these farms was the mean FEC of the triplets from the ewes low; 50 EPG ± SD 40 and 138 EPG ± SD 118, respectively.

Other trichostrongyle eggs identified were *Trichostrongylus axei*, *Chabertia/Oesophagostomum* and *Nematodirus* spp., but also eggs of the tapeworm *Moniezia expansa* and coccidian oocysts were found (Table 1). There was in general no significant difference between conventional and organic farms. Significant differences were only observed for *Nematodirus spathiger, N. battus, Moniezia expansa*, and coccidian oocysts which were significantly more prevalent in the lambs than in the ewes.

3.3. Influence of sample size

On the 29 sampling occasions when *H. contortus* was identified by microscopy between one to all of the triplets were *H. contortus* positive (Appendix 1). However, only on 5 (17%) out the 29 occasions eggs were found in all triplets. Extra information about the *H. contortus* status was provided on 14 (48%) occasions, by including more than two triplets per sampling occasion as was the standard before 2016. The mean FEC on these 14 occasions varied between 33–733 EPG with an average of 265 ± SD 207 EPG. In contrast, on those sampling occasions when no extra information about *H. contortus* was obtained by including more than two triplets the mean FEC of was 601 ± SD 421 EPG and varied between 117–1792 EPG.

3.4. Detection of parasite DNA

DNA (ITS2) fragments of *Haemonchus* and/or *Teladorsagia* were amplified by ddPCR in 67 (88%) of 76 composite triplet samples. All of these had a FEC of > 50 EPG, whereas only two out of the nine ddPCR negative samples had a FEC of 100 EPG. DNA of *Haemonchus* was detected in 33 (43%) and DNA of *Teladorsagia* was found in 64 (84%) out of the 76 samples examined with ddPCR (Appendix 2). The proportion of ITS2 copies in the *Haemonchus* positive samples varied between 2% to 100% with a mean of 38 ± SD 31%, whereas the proportion of ITS2 copies in the *Teladorsagia* positive samples ranged between 1% to 100% with a mean of 47 ± SD 32%. In the ewes *Haemonchus* was dominating over *Teladorsagia* (i.e. with > 50% of the copies) in 9 (45%) out of the 21 *Haemonchus* positive samples and in 3 out of 12 (25%) positive samples from the lambs.

3.5. Comparison of diagnostic methods

There was substantial agreement between the diagnostic results obtained by microscopy and ddPCR (Cohen's kappa = 0.70 ± 0.087). *Haemonchus* was found by both diagnostic methods in 26 (39%) of the 67 samples where DNA was amplified (no amplification was observed

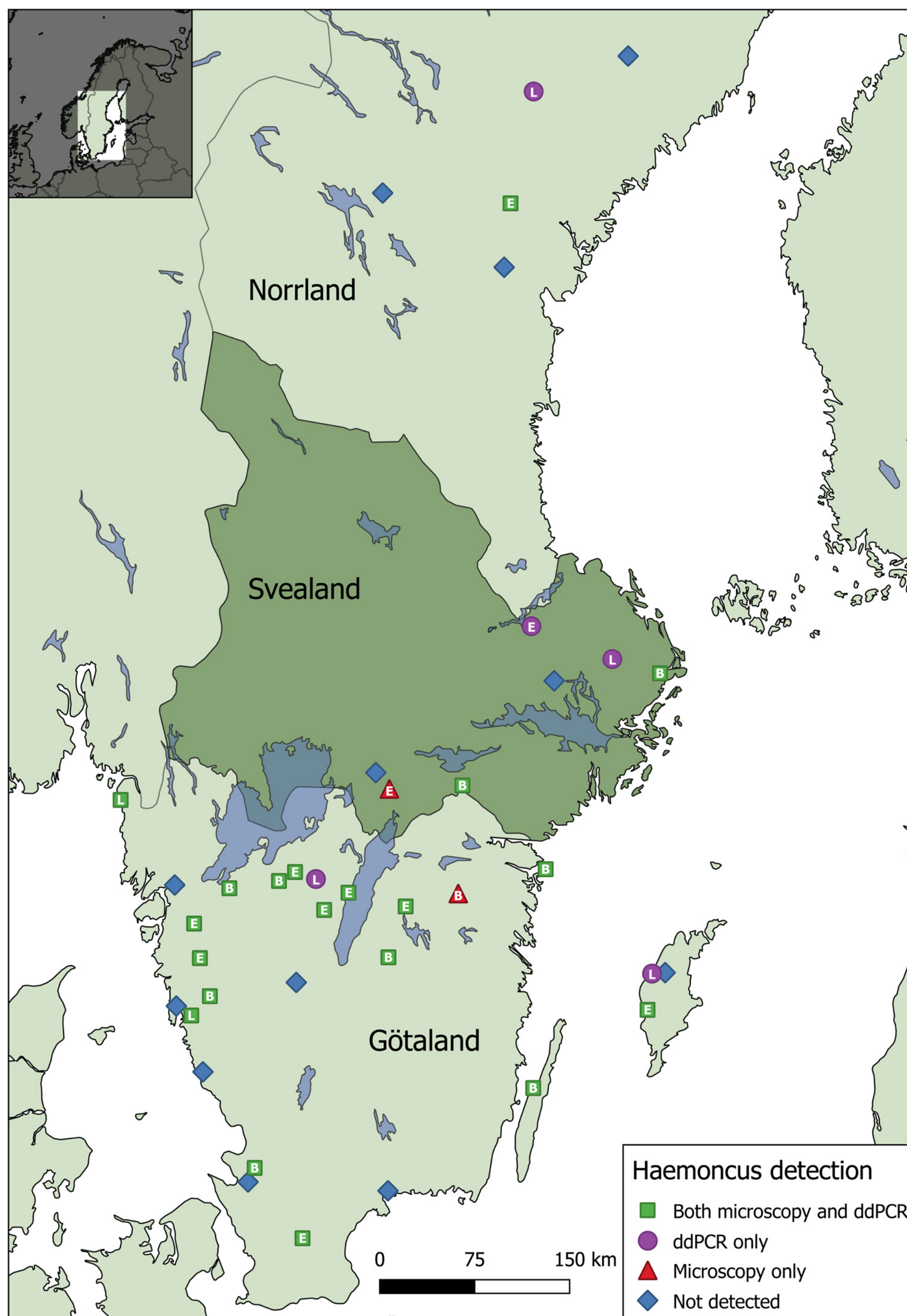

Fig. 2. Map of Sweden showing the location of *Haemonchus* infected farms; B = both in ewes and lambs, E = only in ewes, L = only in lambs.

in 9 samples), whereas it was not identified with any method in 31 (46%) samples. Furthermore, *Haemonchus* was detected in 6 (9%) of the samples with ddPCR only but that were negative according to microscopy. The parasite was also identified only by microscopy in 4 (6%) samples. On farm O9 it was found both in ewes and lambs, whereas on O6 it was only found in the ewes. On farm C29 it was found in the lambs only by microscopy but in ewes by both methods.

Like with microscopy a gradient with an increasing number of infected farms towards the south was observed by ddPCR; i) two out of five (40%) northern farms, ii) four out of seven (57%) central farms, and iii) 20 of 27 (74%) southern farms, although these differences were not significant (Fig. 2). On 22 (56%) farms *Haemonchus* was detected with both methods. Two farms (O6 and O9) were exclusively diagnosed as being infected by microscopy (Fig. 2). On five (13%) farms (O4, O7, C20, C24, C30) where the parasite was exclusively detected by ddPCR, the mean FEC were low both in the ewes (140 ± SD 104 EPG) and in the lambs (227 ± SD 212).

3.6. Farm management

All farms participating in the study completed the web-based questionnaire (Appendix 3). From this it appeared that there were only minor differences between the organic and conventional producers. Most farms (82%) buy in animals on a regular basis, but not from another country and only rarely (7.6%) from herds that in turn had imported animals from abroad. The lambing season was also similar in

organic and conventional flocks with the majority (64%) having lambing's during the same period in spring. Also, the grazing management routines did not differ with a majority of farms (59%) having a combination of natural pastures and arable land grazed by animal groups that were moved between different paddocks at least three times per season (67%).

3.7. Deworming routines

The parasite control methods were similar (Appendix 3). The great majority of the farms (97%) had used anthelmintics in the previous grazing seasons and both ewes and lambs were usually treated often at different occasions between one to two times per year. In the year of the study (2018) the ewes were dewormed on 74% of the farms and the lambs on 64% of the farms. The most commonly used anthelmintic was ivermectin, which was administered to 75% of the ewes and to 84% of the lambs on farms which dewormed their sheep. Other anthelmintics than ivermectin or albendazole was used only on three occasions on two of the farms (O9 for lambs and C35 both for ewes and lambs). Treatment decisions was in general based on the diagnostic information obtained from this study, apart on five occasions on two conventional farms (C23 and C26) that relied on blanket treatments. Several farms (64%) reported that they alternated between different substance classes at least once a year or at every deworming event. Most farms (59%) had tested at least on one occasion if the anthelmintics used had the expected efficacy. However, only one conventional farm (C31) stated that strongylid eggs had been observed in previous treatment control 7–10 days after deworming. Each of two conventional (C20 and C24) and organic farms (O5 and O19) reported that *H. contortus* is always diagnosed, whereas 54% stated that this parasite is occasionally found and 26% that it was found in the past but not during the last two years.

4. Discussion

Accurate diagnosis of GIN infections in sheep is central to their control. This study shows that the likelihood of finding *Haemonchus contortus* in sheep flocks increases when sampling size is extended from six animals (as was routinely examined per sampling occasions until 2015 in Sweden) to approximately 10% of the animals in the flock. With this extended sampling protocol, we found that *H. contortus* is widespread both on organic and conventional sheep farms in Sweden but with a somewhat increased prevalence according to a north to south gradient. It was also found that an adequate level of parasite control was in general achieved based on the collected diagnostic information. Although the diagnostics produced by microscopy and the molecular test basically produced congruent results the sensitivity was increased with 13% more farms identified using a ddPCR assay recently developed in our laboratory (Elmahalawy et al., 2018).

In sheep, the use of composite sampling methods to estimate the mean faecal egg count of a group of individuals has been evaluated before and the general conclusion is that a single count is highly correlated with the mean of the individual samples in the pooled faeces (Baldock et al., 1990; Nicholls and Obendorf, 1994; Morgan et al., 2005). There seems to be no world-wide consensus on the best sampling strategy and which diagnostic procedure to be used for screening of GIN parasites in sheep. This is not surprising simply because there is such a wide range of differing production systems working under diverse climatic conditions and with varying flock sizes and different management procedures providing different practical opportunities for parasite screening. Despite its northern location and likewise as in many other countries with commercial sheep production *H. contortus* has for decades been recognized as the major pathogen in Swedish flocks (Lindqvist et al., 2001). In Sweden it has been shown that *H. contortus* mainly overwinters as hypobiotic larvae inside the housed ewes and the parasite is thus relying on the lambing ewe to complete its life cycle (Waller et al., 2004), although it has also been demonstrated that a small proportion of larvae may also overwinter on pasture (Troell et al., 2005). Because of this above main attention in the screening for *H. contortus* is dedicated to examination of the housed ewes before they are turned out with their lambs on pasture, merely to avoid future massive pasture contamination and thereby protect the lambs from being exposed to numerous infective larvae. In order to control *Haemonchus* infection it is, therefore important to screen the ewes and if necessary apply targeted anthelmintic treatments before they are turned out on pasture with lambs. Similarly, grazing lambs need to checked in time but only treated if required.

It has been suggested that a composite sample from ten individual sheep is likely to give good results for routine examination of mixed trichostrongyle egg counts in grazing sheep in the UK (Morgan et al., 2005). Furthermore, a 94% correlation between composite FEC and mean FEC of individual samples was demonstrated in sheep regardless of pool size or analytical sensitivities (Rinaldi et al., 2014). In the present study, the sampling protocol was adjusted to approximately 10% of the animals in the flock. Composite faecal samples were then first examined by microscopy after careful mixing of 3 g samples from each of three animals mixed into between three to five composite samples depending on flock size. This way it was assumed that potential bias due to parasite egg aggregation between individual hosts could be avoided and that our results better reflected the true infection status in the investigated flocks than with only two triplets. We found that only on 17% of the sampling occasion when *H. contortus* was identified by microscopy it was present in all triplets, while extra information was provided on 48% of the occasions by including more than two triplets. According to our results the probability of finding *H. contortus* was thereby increased. This indicates that the protocol for the enhanced sampling strategy used in this study is superior to the one used in Sweden before 2015. This further demonstrates that the outcome of nematode faecal investigation from sheep is sensitive to which animals that are included in composite tests (Hood et al., 2006; Morgan et al., 2005).

Of importance is not only how many animals that are examined but also the time-point when sampling is conducted in the flock. As argued before, the screening of the ewes prior to turn-out is a time point of key importance during Swedish conditions. However, to avoid production losses it is equally important to determine the infection levels in the lambs before the clinical signs are observed, ideally by repeated sampling during the grazing season but also to evaluate the outcome of advocated control measures to the ewes (Waller et al., 2004). On the majority (70%) of the farms in the present study, most (73%) of the lamb flocks were sampled after they had been on grass for between five to 12 weeks. On a few farms it was for practical reasons difficult to sample the lambs within this time frame and it is likely that this affected the outcome on those farms. For example, one flock (O33) where the lambs were not sampled until in September had a mean FEC of 1792 EPG almost exclusively infected with *H. contortus*. This illustrates the importance of not delaying the sampling of lambs.

Our results revealed that the sensitivity of the *Haemonchus* investigation was improved by replacing microscopy with the use of a molecular diagnostic test based on a ddPCR assay (Elmahalawy et al., 2018). Today there are several PCR based methods described for the detection of GIN in sheep, however these have only rarely been compared with microscopy (Bott et al., 2009; Learmount et al., 2009; McNally et al., 2013; Roeber et al., 2012). Furthermore, to the best of our knowledge there is only one study comparing the results of microscopy with a PCR based assay for detection of strongyle nematode eggs directly without prior harvest of eggs from sheep faeces by a flotation method (Sweeny et al., 2011). Likewise, as in our study there was a substantial level of agreement between the two diagnostic techniques. A crucial difference between our studies, however is that we were searching specifically for *Haemonchus* DNA, which was found by ddPCR in 13% of the samples that were negative according to microscopy. On the other hand, the PCRs by Sweeny et al (2011) was comparing with

DNA from strongylid worms and in this case only an additional 2% of samples was detected. Nevertheless, in agreement with earlier results (Höglund et al., 2009; Ljungström et al., 2018), these observations confirm that PCR based detection of *Haemonchus* is more sensitive than by microscopy also when the test was based on presence of eggs in faecal samples directly. Interestingly, in the present study *Haemonchus* was also identified in a few samples only by microscopy. Likely explanations for this are that this was as a result of misidentification by microscopy, and/or that the sample examined with the molecular assay was not representative. As has been suggested before in earlier attempts to identify the eggs of GIN in livestock faeces directly, both the processing of the faecal samples and DNA extraction procedure used are central to the performance of PCR based assays (Harmon et al., 2006; McNally et al., 2013; Roeber et al., 2013). In the present study the molecular investigation was based on the detection of ITS2 nematode DNA by ddPCR in extractions based on 1 g of faeces and where the eggs prior to DNA extraction, were crushed by bead beating according to a modified protocol by Hamron et al. (2006). On a farm level 13% more samples were found *Haemonchus* positive by ddPCR. Thus, there is no doubt about that DNA investigation by ddPCR performed directly on faeces is a promising tool for screening of *Haemonchus* on commercial sheep farms. However, the protocol for sample processing can undergo further optimization to refine its precision and to determine its level of detection and it also needs to be automated. Not until then ddPCR can replace microscopy for the purpose of routine diagnosis

Overall, this study confirms that *H. contortus* is still common on Swedish sheep farms regardless if the production form is organic or conventional. It also indicates that the level of parasite control based on the new screening method was in general acceptable. We also confirmed that the parasite still has a widespread geographical distribution in Sweden approaching farms near the Arctic Circle even though there was a tendency of more infected farms in the southern part of the country. These results basically agree with the last survey carried out in Sweden between 1997–1999 where *H. contortus* was found by microscopy in 37% of the organic flocks (Lindqvist et al., 2001). This figure agrees with what was observed on the organic farms by microscopy in the present study (33%).

5. Conclusion

In conclusion, we show that the enhanced sampling protocol works better than the old protocol when combined with microscopy. We also found that the sensitivity of the screening can be further enhanced by replacing microscopy with a ddPCR based assay although the protocol for molecular testing is still open for further refinements. The described screening strategy should ideally be used in ewes prior to turn-out to plan effective control measures, based on targeted anthelmintic treatments and in combination with grazing strategies to avoid pasture contamination for the following grazing season.

Ethical approval

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

Author contributions

JH with help of KG devised the project, the main conceptual ideas and obtained the funding. KG with help of JH designed the questionnaire. KG contacted the farmers. SE with help of PH contributed to the molecular laboratory work. JH analysed the data and drafted the first version of the manuscript. All authors discussed the results and contributed to the final manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vpoa.2019.100018.

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