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A follow-up on the Swedish roundworm control program: strengths and weaknesses

B. Tarbiat [*,1] **N. Enweji,*** **D. S. Jansson** [†,‡] **E. Wallström,**[§] **E. Osterman-Lind,**[#] **and J. Höglund** [*]

[*]*Department of Biomedical Sciences and Veterinary Public Health, Section for Parasitology, Swedish University of Agricultural Sciences (SLU), SE-750 07 Uppsala, Sweden;* [†]*Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU), SE-750 07 Uppsala, Sweden;* [‡]*Department of Animal Health and Antimicrobial Strategies, National Veterinary Institute (SVA), SE751 89 Uppsala, Sweden;* [§]*The Swedish Egg Association, Vreka kloster, Sweden; and* [#]*Department of Microbiology, Section for Parasitological Diagnostics, National Veterinary Institute (SVA), SE-751 89 Uppsala, Sweden*

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SUMMARY

Poultry roundworms have re-emerged in laying hens in many European countries due to the increase in noncaged housing. This is worrying because, at high parasite loads, *Ascaridia galli* can impact birds' welfare, health, and productivity. Worm control is therefore an important aspect of the successful management of the egg production industry. In 2009, the Swedish Egg Association initiated a voluntary control program to tackle the problem and reduce the appearance of worms in table eggs by encouraging producers to submit fecal samples for analysis. Since the start of the program, its data have never been thoroughly explored. Moreover, after more than a decade of challenges, our understanding of how egg producers perceive worm infection is still inadequate. This study was therefore designed to address these issues. The research data in the present study are drawn from 2 sources. First, through the control program and second, through an online survey. We have summarized the control program's achievements and discussed its findings and limitations. Although this work contributes to existing knowledge of roundworm control in laying hens in general, it also identifies gaps in knowledge. In conclusion, the control program can be improved by incorporating more strategic sampling and utilizing well-suited diagnostic tools for better assessment of infection status. It is equally important to educate producers on anthelmintics (**AH**) use and the development of resistance.

Key words: *Ascaridia galli*, questionnaire, treatment, monitoring, anthelmintic resistance

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[1]Corresponding author: behdad.tarbiat@slu.se

DESCRIPTION OF PROBLEM

During the 2000s, an increase in the occurrence of *Ascaridia galli* took place among

noncaged laying hens in Sweden. Both conventional flocks indoors and free-range flocks were affected (Jansson et al., 2010). The transition from conventional battery cages to cage-free housing systems has been the main reason for the increased occurrence of roundworms. Unlike cages, where hens are separated from their droppings, in litter-based production hens come in contact with feces, which may contain infective eggs of several roundworm species (Thapa et al., 2015). This facilitated the nematode lifecycle and resulted in the re-emergence of these infections (Jansson et al., 2010, 2011). A mild infection often passes unnoticed unless expelled worms are seen or fecal analysis reveals parasite eggs. However, the magnitude of the parasite's impact on the host, such as reduced growth, increased feed consumption, decreased egg production, anemia, and diarrhea, grows with an increased worm burden (Hinrichsen et al., 2016). In addition, concurrent bacterial infection can have an even greater adverse impact on the host in contrast to solitary bacterial infections (Dahl et al., 2002; Permin et al., 2006).

In the late 2000s, there was an increase in Swedish consumer complaints about the presence of ascarids in table eggs. Therefore, the Swedish Egg Association initiated a voluntary roundworm control program in 2009, when a benzimidazole drug was registered for laying hens in Sweden. Another reason was to encourage farmers to diagnose their flocks for parasite infection before anthelmintic treatment. Guidelines on the sampling of parents, pullets and laying hens, deworming, disinfection of barns, and follow-up measures were drafted in cooperation with the National Veterinary Institute (**SVA**) that performs the diagnostics. The program was later integrated as a requirement for table eggs to be certified by the Swedish Egg Association (www.svenskaagg.se). A certified egg can be traced through the entire production chain and guarantees that the product meets Sweden's strict requirements in animal welfare, food safety, infection control, and product quality. Since the launch of the control program, no compiled results have been presented publicly.

A systematic understanding of how egg producers perceive worm infection is still lacking. A search of the literature revealed few studies aimed at demonstrating producers' understanding of parasite worm infections and how they affect the welfare, health, and productivity of laying hens (Feyera et al., 2022). Despite the importance of worm infection in laying hens, there remains a paucity of data on existing sustainable parasite control programs worldwide.

There were 2 primary aims of this study: i) to analyze data generated within the Swedish roundworm control program aiming at summarizing its achievements and limitations; ii) to provide an overview of the Swedish producers' perception of the impact of parasite worms as well as on worm control practices. This paper also compared more modern molecular diagnostic tools such as ddPCR with its conventional counterpart, the flotation technique as used in the roundworm control program. Understanding the link between scientific evidence of the extent of worm infection problems and producers' awareness of these problems will help to provide better consultations and guidelines when it concerns the cost-benefit of roundworm control.

MATERIALS AND METHODS

All materials collected for this study were part of the roundworm control program conducted by the Swedish Egg Association. No extra animal discomfort was caused for sample collection for the purpose of this study. An ethical review by the Swedish Ethical Committee was therefore not required.

Roundworm Control Program

Fecal samples submitted to SVA during 2019 and 2020 from pullets and laying hens were included in this study. On each sampling occasion, the producer collected 4 samples of feces (0.5−1 dL each) from different locations in the barn (slats, litter belts, etc.). The following data were included on the referral form: farm and flock identity, production type/housing, age at sampling, sampling date, and time/date of the most recent deworming. At the laboratory, the 4 samples were analyzed individually from pullets while for layers, they were pooled before analysis. A modified flotation

method with centrifugation was used to analyze the samples at a routine diagnostic parasitology laboratory at SVA. The fecal samples were first carefully homogenized and ≈5 g of feces was mixed with 30 mL saturated sugar-salt solution (density: 1.280 g/mL at 25˚C) and poured through gauze with a 17 × 17 mm aperture. The sieved solution was then transferred to a Clayton Lane test tube and centrifuged for 5 min at 214 × *g* with a coverslip, 18 × 18 mm, on the top. The coverslip was transferred to a slide and microscopically examined at 100× magnification. The number of eggs was categorized as: i) negative (no eggs), ii) low (fewer than 10 eggs under the whole coverslip), iii) moderate (between 3 and 5 eggs in a microscope field of view), and iv) high (more than 5 eggs in a microscope field of view). The 4-graded scale was used to ensure a semiquantitative fecal egg count (**FEC**) assessment in a standardized way since it would be unrealistic to count the total number of eggs under the coverslip at high parasite egg numbers.

ddPCR Analysis

The laboratory at SVA was asked to freeze all samples (between August 2019 and November 2020) from laying hens after routine analysis, regardless of the results. These samples were reanalyzed at SLU using a duplex droplet digital (dd)PCR assay able to differentiate between *Ascaridia galli* and *Heterakis gallinarum* as described earlier (Tarbiat et al., 2021). Briefly, the fecal slurry was obtained by mixing feces and water with 1:2 ratio. DNA extraction was then performed in accordance with the protocol of the NucleoSpin DNA Stool kit using 220 μL of the slurries. We used a single bead beating step using a universal tissue homogenizer (Precellys Evolution, Bertin Technologies-le BretonneuxFRANCE) to facilitate egg disruption. The ddPCR assay was performed (Applied Biosystems 2720 Thermal Cycler) under the following conditions: a single reaction (22 μL reaction volume) containing 11 μL of 2× ddPCR Supermix for Probes (no dUTP, Bio-Rad, California, USA), 1.1 μL of each 20× stock solution for *H. gallinarum* primers/probe (FAM) and *A. galli* primers/probe (HEX), 1 μL DNA and Nuclease-Free water. Approximately 20,000 uniform nanoliter-sized droplets were generated using the automatic droplet generator (AutoDG Instrument) prior to the amplification. The parameters for the amplification steps consisted of 95˚C for 10 min followed by 40 cycles of 94˚C for 30 s, 62˚C for 1 min (annealing step), followed by an additional 10 min at 98˚C. QuantaSoft software (version 1.7.4.0917) was used to assign positive/negative droplets. Thresholds were manually adjusted in order to separate droplet clusters (channel 1 (FAM dye)—4,000 AU and channel 2 (HEX dye)—1,500 AU).

Before the molecular analysis, the samples were kept frozen at SVA for up to one and a half years. The results of the ddPCR presented in the present study were regarded as positive if more than one ITS2-copy were observed above the 1,500 and 2,400 thresholds for *A. galli* and *H. gallinarum*, respectively.

Survey of Worm Control Practices

An online survey was conducted with descriptive data being gathered using the Netigate platform (Netigate AB, Stockholm, Sweden, www.netigate.net). Invitations to participate were: i) sent by email to egg producers affiliated with The Swedish Egg Association; ii) announced in a national poultry magazine; and iii) promoted during an annual meeting for egg producers in October 2021. The survey was adapted to be used on personal computers, tablets, or smartphones, with the respondent remaining anonymous. A structured questionnaire in Swedish (based on 38 questions) was designed to gather information on farm and flock characteristics, perceived risks of worm infections, monitoring practices, as well as deworming, and other management strategies related to worm control (Supplement 1, translated questionnaire). Before distribution, the questionnaire was pilot tested by 6 poultry veterinarians or other egg industry stakeholders and 2 egg producers and was revised according to their suggestions. The questionnaire was open for 334 d. The original survey and several reminders were sent by the Swedish Egg Association. Data collection, processing, and distribution are done per GDPR requirements.

Statistical Analysis

The SVA FEC result together with the data on housing and management were summarized in Microsoft excel where most of the descriptive statistics were performed. The data were then transferred to GraphPad Prism 8 (GraphPad Softwar, Boston, USA) where graphs were created. For the online survey, responses were downloaded to Microsoft excel and checked for meaningful content. For each variable, a descriptive analysis was carried out, producing frequencies for categorical variables and means and medians for continuous variables, by respondent characteristics of interest. To measure the agreement between the results obtained with the flotation technique and the ddPCR assay, we calculated the kappa coefficient using JMP Pro version 16 (SAS Institute Inc., Cary, NC). To interpret the result, we referred to the guidelines provided by Landis and Koch (1977).

RESULTS AND DISCUSSION

We have analyzed data gathered between 2019 and 2020 through the roundworm control program initiated by the Swedish Egg Association in 2009. Three major findings were obtained. First, a majority of the flocks were sampled too late during the flock cycle, and they were sampled only once. Moreover, some flocks were treated before diagnosis (sample submission). Second, the results suggest a lack of efficacy in some flocks. Whether this is due to anthelmintic resistance warrants further investigation. Third, the questionnaire data showed that the number of treatments (without checking for treatment efficacy) varied between respondents.

Control Program 2019 and 2020

Pullets. In total, 1,310 samples from 344 flocks from 25 companies were analyzed individually. Out of these, 28 (8%) samples were submitted from organic flocks, 30 (9%) from enriched cages, and 281 (81%) from indoor barns/aviaries (missing data, $n = 5$). The samples were submitted from pullets between 12 and 18 wk of age with a mean (SD) of 14 ± 0.9. None of the flocks had been dewormed before sampling (missing data $n = 19$ flocks). All fecal samples were negative for roundworms.

Laying Hens. In total, 765 pooled samples from 620 flocks and 206 companies collected across all 21 counties in Sweden were analyzed. Of the 206 companies, 67 submitted samples from a single flock. Of the remaining 139 companies, 100 submitted samples from multiple flocks, one sampling occasion (four ≈0.5−1 dL of feces) per flock. The rest ($n = 39$) submitted samples from multiple flocks, on several occasions from each flock. At flock level, 525 out of 620 (85%) were sampled once. From the remaining 95 flocks, samples were submitted on 2 to 9 occasions (2.5 ± 1). Sample submission intervals ranged between 3 and 58 wk (median: 12) with a mean (SD) of 15 ± 11.

More than half of the samples (56%) came from farms with multitier systems (**M**) followed by organic production (**Or**, 24%), single-tier (**S**, 13%), 4% free-rage (**F**, nonorganic), and 3% from enriched cages (**En**). Of all samples, 2% were collected from flocks at an age of ≤30 wk. In floor-based housing systems (M, S, and Or) with or without outdoor access, the majority (71%) of the first samples were collected from hens between 30 and 50 wk of age. In contrast, 63% of the samples from En and F were collected at an age of 50 to 70 wk of age (Figure 1).

The results of the SVA analysis (flotation method) of the first submitted sample from each flock (multiple samples from the same flocks were excluded) showed that 281 (45%) were negative and 339 were positive for ascarid eggs. Figure 2A presents the breakdown of these results according to the FEC levels and housing/production systems. Except for the En system, there were no significant differences ($P > 0.05$) between housing/production systems. The distribution of these samples based on the age ranges and the FEC levels are shown in Figure 2B (missing data $n = 12$). From the graph, it is apparent that the lowest infection pressure, at the time of sampling, was obtained among those flocks that were sampled at an age of <30 wk. The infection status of those flocks ($n = 95$) that were sampled multiple times during the same production cycle is shown in

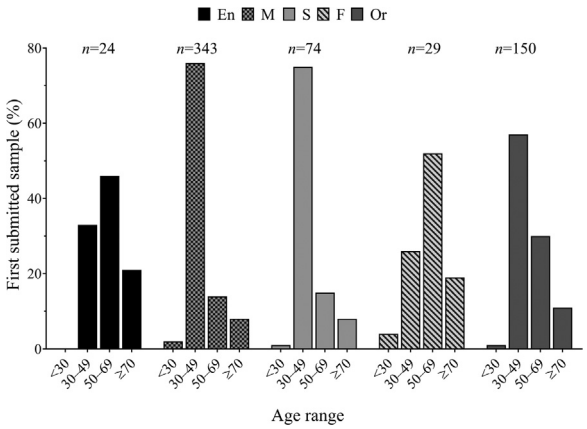

Figure 1. Breakdown of the first submitted sample (in %) according to age range and housing/production system. En: enriched cage; M: multitier; S: single-tier; F: free-range; and Or: organic. n: number of flocks.

Figure 3A. Overall, the infection status was consistent (negative/negative: NN or positive/positive: PP) in 67% of the flocks whereas 31% turned positive from negative and 2% from positive to negative. The infection status between consecutive flocks kept in the same barn (n = 173) is shown in Figure 3B. In this case, it was consistent between flocks (NN or PP) in 78% of the cases. On the other hand, the infection status changed from positive to negative in 7% and from negative to positive in 15% of the cases when flocks were resampled.

Most flocks (79%) had not been treated against roundworms before submission of the first sample (Figure 4A) (missing data n = 1). In total, 56% of these flocks were sampled when hens were older than 35 wk. Figure 4B shows the FEC levels in the samples that were sent from the treated flocks. Among the treated flocks, 66 pooled samples were taken within 6-wk post-treatment (**WPT**) (4.6 ± 2.1 SD) of which 41 (62%) were still positive for roundworms and they were classified as low (61%), medium (36%), and high FEC (3%).

ddPCR. In total 323 samples were analyzed with ddPCR of which, 222 (69%) were positive for roundworms; either *A. galli* or *H. gallinarum* or both. The proportion of FEC-positive samples reported by flotation in the same group was 64%. With ddPCR, 14% were positive for both species while 50 and 5% were monoinfected with *A. galli* and *H. gallinarum*, respectively. Figure 5A shows the proportion of the positive samples for the 2 species in relation to the different housing systems. As can be seen from the graph, roundworms were detected by ddPCR assay in all the housing/production systems. Test results from SVA (flotation test) from the same samples showed a lack of parasite eggs detection in the En housing system (Figure 5B). The result of Cohen's kappa indicated an overall moderate agreement of 82% between the 2 tests (Cohen's k: 0.59). The number of samples that were classified as positive by the flotation method was 22 out of which 59% were low, 36% were medium, and 5% were high. In contrast, the number of samples classified as positive only with ddPCR was 37 with ITS2 copy numbers/μL ranging between 1 and 2,876.

Questionnaire

Respondents' Information. A total of 54 out of ∼300 laying hen companies responded to our survey of which 48 answered all the questions (response rate 30%). The responses came from 17 counties (out of 21) with the highest representation from Östergötland (20%), Västra Götaland (13%), and Örebro (9%) counties, which are all situated in south-central Sweden. Most of the egg producers (96%) were members of the Swedish Egg Association of which 87% had more than 10 yr of poultry farming experience. A lower proportion (13%) had less than 10 yr of experience. Most respondents (78%) identified themselves as the owner of the company, 35% were managers with leadership responsibilities and 8% were administrators or

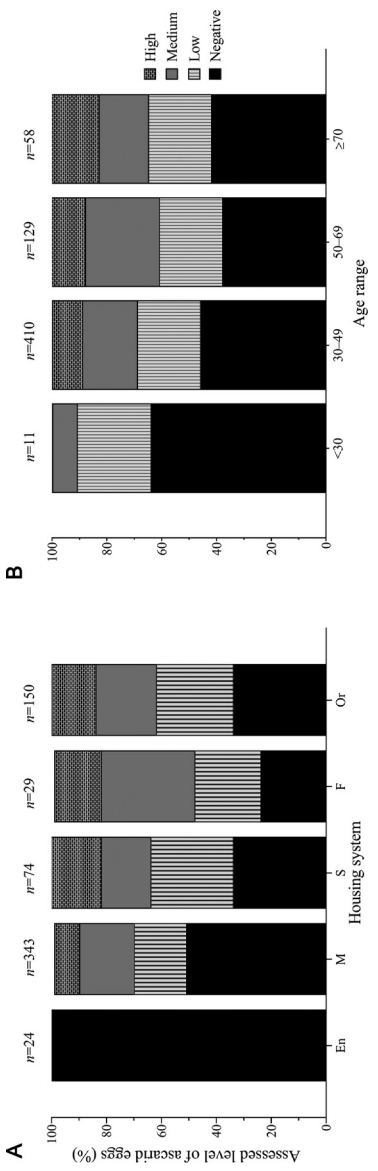

Figure 2. Semiquantitative assessment of the number of parasite eggs in the first submitted sample (in %) according to (A) housing/production systems and (B) age range (missing data $n = 12$). En: enriched cage; M: multitier; S: single-tier; F: free-range; and Or: organic. The number of parasite eggs detected by the flotation method is presented as negative, low, medium, and high. n: number of flocks.

economists who did not work directly with the birds.

Farm and Flock Characteristics. According to the respondents, 79% had conventional egg production whereas 21% had organic. Most respondents (85%) selected the multitier aviary system followed by 14% single-tier, 12% free-range, and 5% enriched cage operations in their farm. Most farms (64%) had more than one flock and 81% housed flocks at different ages. Forty-eight respondents (96%) stated that their farm was affiliated with the voluntary roundworm control program run by the Swedish Egg Association.

Perceived Intestinal Worm Importance. When asked if adult worms or parasite eggs had ever been found in one or more of their flocks, 75% answered "Yes," 22% said "No," and 2% were uncertain about the existence of roundworms in their flocks. On a scale of 1 to 10 where 1 was the least effect and 10 was the most, respondents' responses were on average 6.3 (±2.6 SD), 6 (±2.3 SD), and 7 (±2.7 SD) for the effect of worm infection on birds' health, farm workload, and profitability, respectively.

Among the effects on productivity, reduced egg production was the most common (50%) effect followed by increased feed consumption (23%) followed by cracked eggs (16%). Diarrhea and soiled bedding material each received 21% of the votes. In the free-text option, respondents also stated reduced egg weight, poor general condition, and mortality. However, 24% of respondents stated that worm infection had no effect.

About 90% of respondents monitored their flocks for worm infection using laboratory diagnosis (e.g., flotation). The monitoring frequency ranged from analysis of a single fecal sampling occasion (60%) to several (30%) per production cycle. Only a single respondent (2%) indicated that they never monitored for worm infection. Likewise, 4% stated that they had stopped sending samples. Both groups stated the reason for this was because they already knew the results. When asked if respondents were willing to be engaged in more frequent sampling (every 8 wk), 53% were positive, 22% disagreed, and 24% were uncertain.

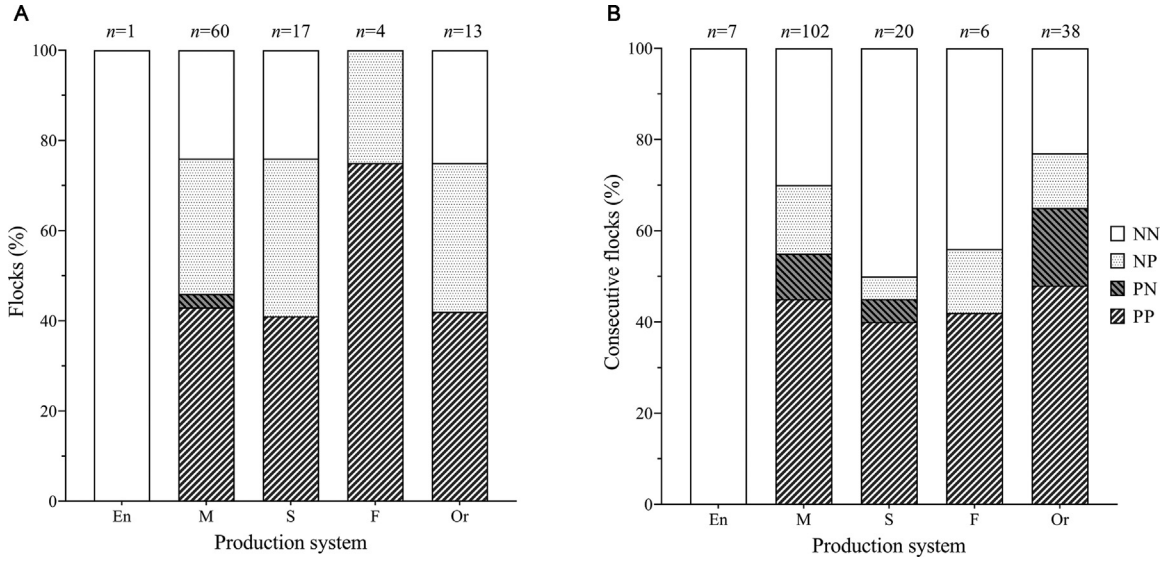

Figure 3. Infection status (flotation test ascarid eggs) in (A) flocks with multiple sampling occasions during the production cycle. (B) Between 2 consecutive flocks in the same barn. PP: the first sample was positive, and flock/consecutive flock remained positive. PN: the first sample was positive, and flock/consecutive flock became negative. NP: the first sample was negative, flock/consecutive flock became positive. NN: first sample was negative, flock remained negative. En: enriched cage; M: multitier; S: single-tier; F: free-range; and Or: organic. n: number of flocks.

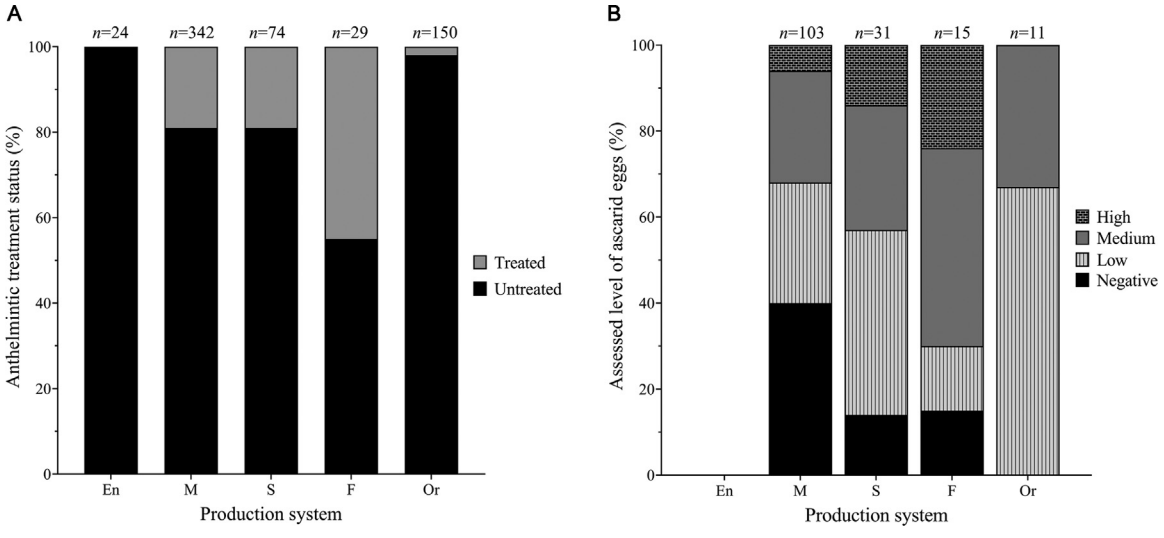

Figure 4. (A) Anthelmintic treatment status (in %) prior to the submission of the first sample. (B) Flotation results of the samples collected from flocks that were treated prior to the submission of the first sample. The number of parasite eggs is presented as negative, low, medium, and high. En: enriched cage; M: multitier; S: single-tier; F: free-range; and Or: organic. n: number of flocks.

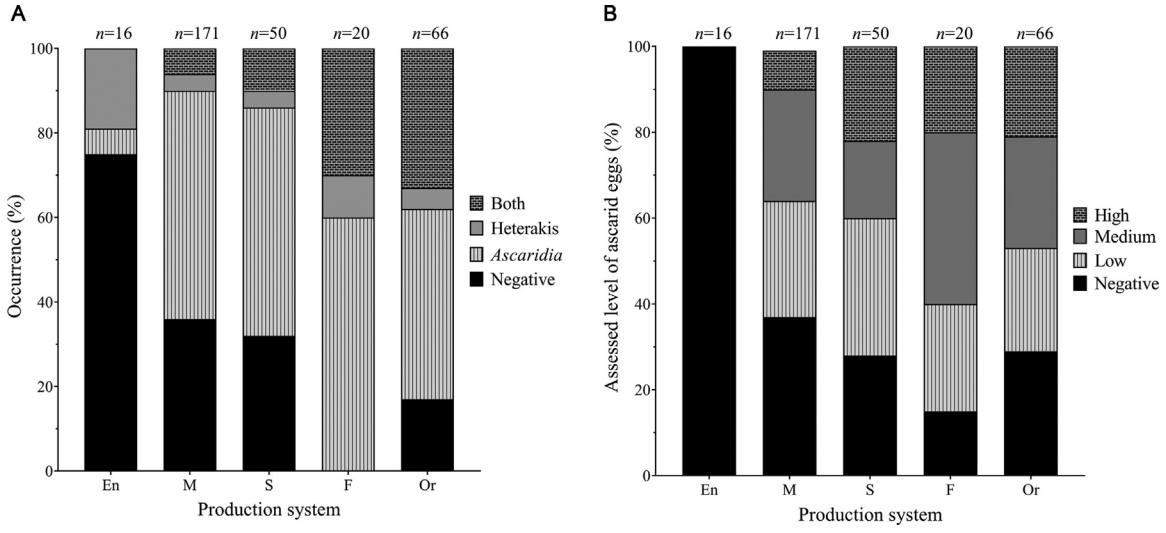

Figure 5. (A) Occurrence (in %) of *Ascaridia galli* and *Heterakis gallinarum* in 323 samples according to the ITS-2 copy numbers measured by ddPCR. (B) Results of the flotation method for the same samples that were tested with ddPCR. The number of parasite eggs is presented as negative, low, medium, and high. En: enriched cage; M: multitier; S: single-tier; F: free-range; and Or: organic. n: number of samples.

Deworming Practices and Anthelmintic Use. During the past 2 yr, more than half of the respondents (53%) had dewormed all their flocks while 18% treated only some. The main reasons for the treatment were: i) to reduce the risk of worm appearance in table eggs (74%), ii) to increase egg production (68%), and iii) to reduce feed consumption (40%). Many respondents also stated that they used anthelmintics (**AH**) in response to the recommendations given by their veterinarians (37%) and egg packers (31%). Twenty-seven percent (of which two-thirds were nonorganic producers) had never used AH during the past 2 yr. The stated motives were: i) absence of worms (71%), ii) withdrawal period (14%), and iii) other reasons (21%) including being organic. The majority of the respondents who used AH (46%) treated their flocks for the first time when the hens were between 36- and 45-wk old while 20% did the treatment between 26 and 35 wk of age. A single respondent reported that they treated their flock at around 25 wk of age. In total, 26% stated that the age of treatment varied between different flocks. When asked about the number of treatments per flock, 9% answered once, 20% 2 to 3 times, 20% 4 to 5 times, and 20% more than 6 times. Around 31% stated that the number of treatments varied between different flocks. Nearly half of the respondents (46%) stated that they were uncertain about the effect of treatments on the health and productivity of the hens. About 38% had a positive opinion about the treatment effect and 17% had not seen any effect. Positive effects observed were i) increased egg production (69%), ii) decreased morbidity (62%), and iii) decreased feed consumption (54%). While 20% said that they always checked the success of treatment through post-treatment fecal analysis, most respondents (57%) did not monitor the success of the treatments.

Husbandry Practices. When asked if the empty barns were cleaned between 2 consecutive flocks, 92% responded "after each flock" and 8% stated that they only cleaned between certain flocks. Less than 60% stated that they use wet or dry cleaning followed by manure removal (63%), high-pressure cleaning (86%), and using disinfectants (84%). Some of these measures were applied in combination. Regarding the disinfectants, 53% claimed always using products with known effects against roundworms whereas, 24% did not use such products. The most used disinfectant contained the organic compound chlorocresol as the active ingredient. As for litter management during an ongoing production cycle, 51% removed soiled/wet litter, 49% added fresh litter to the existing litter bed, and 37% had scrapers under the slats in the multitier aviary system. Around 20% replaced the entire litter bed at least once during a production cycle and 8% stated that they undertook no particular action. Most respondents (78%) had a downtime period between different consecutive flocks of 3 to 6 wk. However, 16% of responders stated that this period was sometimes shorter than 3 wk and 6% stated a period longer than 6 wk.

More than two-thirds of the investigated flocks had only submitted samples on one occasion from each flock. Thus, the overall sampling intensity was low. This agrees with the monitoring frequency reported in the questionnaire survey by more than two-thirds of the respondents. There are some issues regarding infrequent monitoring that can hinder proper assessment of the infection level/intensity at the flock level. First, it is well established that parasite eggs can survive harsh environmental conditions (Tarbiat et al., 2015, 2018; Maurer et al., 2021). Thus, ascarid eggs can be transmitted between different houses on the same farm or they can be introduced to other farms or flocks by contaminated equipment, vehicles, or people at any time during a production cycle. This is supported by the control program showing that the infection status changed from negative to positive in 30% of the flocks that were sampled on more than one occasion (Figure 3A). Second, these authors stated that the samples collected during the daytime have a higher FEC and therefore higher diagnostic value (Wongrak et al., 2015). Thus, samples collected strictly at night might (as is sometimes done in Sweden when samples are obtained from litter belts), therefore result in low FEC or perhaps false negatives. Third, in the absence of a treatment history, a negative result can be misleading. Altogether, it seems that 1 sample per entire production cycle does not fulfill its purpose. It must be realized that a negative result of

a single sample just provides a snapshot of the infection status. Furthermore, the sampling strategy has never been scientifically investigated. Thus, exploring how well the diagnostic sample reflects the infection level is of the highest priority.

The average sampling interval for those flocks that submitted more than 2 samples was around 4 mo. However, looking at the questionnaire result, more than half of the respondents stated that they are willing to send samples more frequently (every 8 wk). There is an unambiguous relationship between the early detection of parasite infection and the ability to slow down the accumulation of ascarid eggs in a barn through a more proper treatment regime and suitable management practices. Although this will increase the workload, it has been shown that frequent monitoring combined with targeted treatment ideally early during the production cycle, can significantly reduce the worm burden and thus is likely to contribute to improved bird health and production economy (Tarbiat et al., 2016b, 2022). The high percentage of positive samples with medium-high FEC levels that were taken after 30 wk of age in this study indirectly confirms the accumulation of parasite eggs in the environment. From the productivity and welfare standpoints, the extra workload is also worthwhile (Sharma et al., 2019; Tarbiat et al., 2022). It is important to highlight that together with worm infections other complications such as secondary bacterial infections can drastically affect both welfare and productivity (Dahl et al., 2002; Permin et al., 2006). Clearly, a better picture of the flock's infection status would be gained by more frequent sampling.

In general, AH is used in livestock strategically to control the infection pressure in the environment. This often means repeated treatments to reduce the adult worm population whose eggs are passed and accumulate in the environment. In this study, more than 40% of the samples were submitted from flocks that were not treated and were older than 45 wk. Moreover, responses to our questionnaire revealed that the use of drugs in treated flocks varied from a single treatment up to 6 times per flock. However, of those flocks with more than 3 treatments per flock cycle, only 20% had sent in follow-up samples to monitor the infection status or efficacy of the treatment. Hence, it could conceivably be inferred that AH was mainly used to briefly reduce the adverse effects of worms on health and productivity rather than to strategically reduce the infection pressure in the barn environment. This indicates that there is room for improvement in how anthelmintics are used.

Based on the control program it appears that there is a difference between AH use in different housing and production systems (Figure 4A). As expected, the use of AH was most intense in the conventional aviary systems according to both the control program and the questionnaire data. As for organic production, the use of anthelmintics is allowed if needed, but the withdrawal time set by EU legislation will most likely restrict their use. On the other hand, restrictions on AH use in enriched cage systems do not exist. Lack of treatment particularly in the enriched cage systems may partly be explained by the general consensus on the low occurrence of roundworms in this type of housing.

What is somewhat alarming is the number of positive samples taken within 6 WPT. Several factors may have contributed to this observation. First, fecal samples collected post-treatment may come in contact with residual parasite eggs deposited pretreatment. Second, the role of intestinal passants caused by coprophagia of feces containing nonviable parasite eggs cannot be ruled out (Boes et al., 1997; Roepstorff, 1997; Tarbiat et al., 2016a). Third, shortcomings concerning medication could potentially impair the treatment effects. Those include incorrect drug preparation, inaccurate dosing/administration, and the formation of biofilm in water pipes. Despite these confounders, having such a high number of positive samples post-treatment raises a critical concern about the development of anthelmintic resistance (**AR**). It is therefore important to view this phenomenon in relation to the recent publications by Collins et al. (2019, 2022) who reported AR in *A. dissimilis* in turkey and *H. gallinarum* in the broiler industry in the United States. Both species are closely related to *A. galli*. It is also worth mentioning that positive samples post-treatment (2 WPT) has been reported earlier from several Swedish laying

farms (Tarbiat et al., 2022). A thorough investigation into the underlying factors behind such an observation is urgent. In the absence of scientifically approved methods, it is important to develop assays and sampling protocols to assess AR at the farm level.

As regards molecular diagnostic tools, ddPCR-based duplex assays have proven to be a reliable tool for the quantification of parasites of veterinary interest (Elmahalawy et al., 2018; Baltrušis et al., 2019). Likewise, Tarbiat et al. (2021) demonstrated that the relative abundance of *A. galli* and *H. gallinarum* in fecal samples can be quantified in laying hens using this tool. Substantial agreement was found between the ddPCR and the flotation method. However, in the present study, a poorer agreement was achieved. The ddPCR failed to detect roundworms in 22 samples even though these samples were categorized as positive by the flotation method. The most likely cause of this incidence is damage to the DNA material. Samples used in our ddPCR were first examined at a routine laboratory at SVA and after which they were subjected to storage and freeze-thaw conditions under which DNA is prone to degradation as previously shown (Högberg et al., 2022). Still, 37 samples were labeled positive exclusively by ddPCR demonstrating its high sensitivity. More importantly, according to the ddPCR results, only 5% of the samples were monoinfected with *H. gallinarum*, whereas 14% carried mixed infections. This result is in line with previous studies in Sweden and suggests that the occurrence of *H. gallinarum* is low compared to other countries (Permin et al., 1999; Phiri et al., 2007; Jansson et al., 2011; Shifaw et al., 2021). Interestingly, our results showed that not only both *A. galli* and *H. gallinarum* were present in cage systems but also in a higher percentage (25%) than previously (4.3%) reported (Jansson et al., 2010). The reasons for this remain unclear and warrant further investigation. In addition to ddPCR, 2 other promising noninvasive diagnostic methods are available for the early detection of ascarid infections in laying hens, such as the detection of antibodies in egg yolk and worm copro-antigens in feces (Daş et al., 2017; Oladosu et al., 2022). Comparing the performance and costs of all 3 diagnostic tools and how each of these can assist in targeted treatment is another practical issue of top priority

The questionnaire revealed that the egg producers perceive worm infection as of moderate concern. This together with survey fatigue may explain the low survey response rate in the current study. A similar observation has been made on Australian laying hen farms (Feyera et al., 2022). Even though economic reasons, including product quality assurance (absence of worm in table eggs), improve egg production and, lower feed consumption were the main motives to use anthelmintics, almost half of the respondents were uncertain about the effect of the treatment. Interestingly, well over two-thirds of those who responded indicated that they didn't check the success of any treatments. Although treatment failure against *A. galli* as a result of the development of anthelmintic resistance is yet to be reported in laying hens in Europe, this claim has been strongly contested in other poultry parasite species in recent years by several authors (Collins et al., 2019, 2021; Saemi Soudkolaei et al., 2021). This concern was further raised in the present study.

In terms of flock management, the respondents, overall, demonstrated that they clean the barns properly during the downtime period. Moreover, over half of respondents reported that they used disinfectants with known effects against parasite eggs. The active substance in the most used disinfectants was chlorocresol which has proved to be effective against *A. galli* eggs under laboratory (Tarbiat et al., 2015) and field conditions (Höglund and Jansson, 2011). Only one-fourth of the respondents practiced complete removal of the litter during ongoing production. The remaining respondents replied that they had either partial removal or added material to the existing litter bed. A search of the literature revealed a single study on the role of the litter as a possible source of ascarid infection in poultry houses (Maurer et al., 2009). The authors claimed that litter might not be a potential source of infection because the litter negatively affects the viability and infectiousness of parasite eggs. A comparison of the control program and questionnaire revealed that despite proper sanitation, most farmers experienced moderate to high levels of parasite infection in their flocks. According to the control program, nearly half of the barns stayed positive or become positive between consecutive flocks

(Figure 3B). This suggests that sanitation is not a stand-alone activity and will be effective only when combined with other measures. Having said that, all evidence points to the complexity of eradicating ascarid infection once it is established (Heckendorn et al., 2009). In accordance with the present results confirming the lack of infection among pullets, previous studies have demonstrated that new flocks are exposed upon arrival (Höglund et al., 2012).

CONCLUSIONS AND APPLICATIONS

1. The program, in its current state, is limited in what it can deliver, that is to say, detecting and predicting roundworm infection levels which is essential to formulate recommendations intended for egg producers to control ascarid infections.
2. It is essential to encourage more frequent monitoring, starting earlier in the production cycle.
3. More reliable sampling techniques and novel diagnostic tools should be evaluated to better reflect the parasite species present and the level of infection. This will help us establish a greater degree of accuracy in monitoring ascarid infections in laying hens.
4. It is important to educate producers on the benefits of optimal treatment strategies, the importance of monitoring treatment efficacy, and the consequences of the development of drug resistance.

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DISCLOSURES

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.japr.2023.100356.

REFERENCES

Baltrušis, P., P. Halvarsson, and J. Höglund. 2019. Molecular detection of two major gastrointestinal parasite genera in cattle using a novel droplet digital PCR approach. Parasitol. Res. 118:2901–2907.

Boes, J., P. Nansen, and L. S. Stephenson. 1997. False-positive Ascaris suum egg counts in pigs. Int. J. Parasitol. 27:833–838.

Collins, J. B., B. Jordan, L. Baldwin, C. Hebron, K. Paras, A. N. Vidyashankar, and R. M. Kaplan. 2019. Resistance to fenbendazole in *Ascaridia dissimilis*, an important nematode parasite of turkeys. Poult. Sci. 98:5412–5415.

Collins, J. B., B. Jordan, A. Vidyashankar, A. Bishop, and R. M. Kaplan. 2022. Fenbendazole resistance in *Heterakis gallinarum*, the vector of Histomonas meleagridis, on a broiler breeder farm in South Carolina. Vet. Parasitol. Reg. Stud. Rep. 36:100785.

Collins, J. B., B. Jordan, A. N. Vidyashankar, P. J. Castro, J. Fowler, and R. M. Kaplan. 2021. Impact of fenbendazole resistance in *Ascaridia dissimilis* on the economics of production in turkeys. Poult. Sci. 100:101435.

Dahl, C., A. Permin, J. P. Christensen, M. Bisgaard, A. P. Muhairwa, K. M. D. Petersen, J. S. D. Poulsen, and A. L. Jensen. 2002. The effect of concurrent infections with Pasteurella multocida and Ascaridia galli on free range chickens. Vet. Microbiol. 86:313–324.

Daş, G., M. Hennies, B. Sohnrey, S. Rahimian, K. Wongrak, M. Stehr, and M. Gauly. 2017. A comprehensive evaluation of an ELISA for the diagnosis of the two most common ascarids in chickens using plasma or egg yolks. Parasit. Vectors 10:187.

Elmahalawy, S. T., P. Halvarsson, M. Skarin, and J. Höglund. 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.

Feyera, T., A. Shifaw, B. Sharpe, T. Elliott, I. Ruhnke, and S. W. Walkden-Brown. 2022. Worm control practices on free-range egg farms in Australia and anthelmintic

efficacy against nematodes in naturally infected layer chickens. Vet. Parasitol. Reg. Stud. Rep. 30:100723.

Heckendorn, F., D. A. Häring, Z. Amsler, and V. Maurer. 2009. Do stocking rate and a simple run management practice influence the infection of laying hens with gastrointestinal helminths? Vet. Parasitol. 159:60–68.

Hinrichsen, L. K., R. Labouriau, R. M. Engberg, U. Knierim, and J. T. Sørensen. 2016. Helminth infection is associated with hen mortality in Danish organic egg production. Vet. Rec. 179:196 -196.

Högberg, N., P. Baltrušis, N. Enweji, and J. Höglund. 2022. Assessment of three DNA extraction kits for the absolute quantification of strongyle nematode eggs in faecal samples. Acta Vet. Scand. 64:5.

Höglund, J., and D. S. Jansson. 2011. Infection dynamics of *Ascaridia galli* in non-caged laying hens. Vet. Parasitol. 180:267–273.

Höglund, J., D. A. Morrison, A. Engström, P. Nejsum, and D. S. Jansson. 2012. Population genetic structure of *Ascaridia galli* re-emerging in non-caged laying hens. Parasit. Vectors 5:97.

Jansson, D. S., A. Nyman, M. Göransson, J. Frössling, and J. Höglund. 2011. Increase of *Ascaridia galli* infections in Swedish laying hens. Svensk Vet. 63:11–16.

Jansson, D. S., A. Nyman, I. Vågsholm, D. Christensson, M. Göransson, O. Fossum, and J. Höglund. 2010. Ascarid infections in laying hens kept in different housing systems. Avian Pathol. 39:525–532.

Landis, J. R., and G. G. Koch. 1977. The measurement of observer agreement for categorical data. Biometrics 33:159–174.

Maurer, V., Z. Amsler, E. Perler, and F. Heckendorn. 2009. Poultry litter as a source of gastrointestinal helminth infections. Vet. Parasitol. 161:255–260.

Maurer, V., E. Perler, Z. Amsler-Kepalaite, and A. Bieber. 2021. Ascarid eggs disappear faster from gravel and wood chips than from soil. Br. Poult. Sci. 62:1–7.

Oladosu, O. J., M. Hennies, M. Gauly, and G. Daş. 2022. A copro-antigen ELISA for the detection of ascarid infections in chickens. Vet. Parasitol. 311:109795.

Permin, A., M. Bisgaard, F. Frandsen, M. Pearman, J. Kold, and P. Nansen. 1999. Prevalence of gastrointestinal helminths in different poultry production systems. Br. Poult. Sci. 40:439–443.

Permin, A., J. P. Christensen, and M. Bisgaard. 2006. Consequences of concurrent *Ascaridia galli* and *Escherichia coli* infections in chickens. Acta Vet. Scand. 47:43.

Phiri, I., A. Phiri, M. Ziela, A. Chota, M. Masuku, and J. Monrad. 2007. Prevalence and distribution of gastrointestinal helminths and their effects on weight gain in free-range chickens in Central Zambia. Trop. Anim. Health Prod. 39:309–315.

Roepstorff, A. 1997. Helminth surveillance as a prerequisite for anthelmintic treatment in intensive sow herds. Vet. Parasitol. 73:139–151.

Saemi Soudkolaei, A., G. A. Kalidari, and H. Borji. 2021. Anthelmintic efficacy of fenbendazole and levamisole in native fowl in northern Iran. Parasit. Vectors 14:104.

Sharma, N., P. W. Hunt, B. C. Hine, and I. Ruhnke. 2019. The impacts of *Ascaridia galli* on performance, health, and immune responses of laying hens: new insights into an old problem. Poult. Sci. 98:6517–6526.

Shifaw, A., T. Feyera, S. W. Walkden-Brown, B. Sharpe, T. Elliott, and I. Ruhnke. 2021. Global and regional prevalence of helminth infection in chickens over time: a systematic review and meta-analysis. Poult. Sci. 100:101082.

Tarbiat, B., N. Enweji, P. Baltrusis, P. Halvarsson, E. Osterman-Lind, D. S. Jansson, and J. Höglund. 2021. A novel duplex ddPCR assay for detection and differential diagnosis of *Ascaridia galli* and *Heterakis gallinarum* eggs from chickens feces. Vet. Parasitol. 296:109499.

Tarbiat, B., D. S. Jansson, and J. Höglund. 2015. Environmental tolerance of free-living stages of the poultry roundworm *Ascaridia galli*. Vet. Parasitol. 209:101–107.

Tarbiat, B., D. S. Jansson, and J. Höglund. 2022. Implementation of a targeted treatment strategy for the sustainable control of *Ascaridia galli* infections in laying hens. Vet. Rec. Open 9:e37.

Tarbiat, B., D. S. Jansson, L. Moreno, C. Lanusse, M. Nylund, E. Tydén, and J. Höglund. 2016a. The efficacy of flubendazole against different developmental stages of the poultry roundworm *Ascaridia galli* in laying hens. Vet. Parasitol. 218:66–72.

Tarbiat, B., D. S. Jansson, E. Tydén, and J. Höglund. 2016b. Comparison between anthelmintic treatment strategies against *Ascaridia galli* in commercial laying hens. Vet. Parasitol. 226:109–115.

Tarbiat, B., S. Rahimian, D. S. Jansson, P. Halvarsson, and J. Höglund. 2018. Developmental capacity of *Ascaridia galli* eggs is preserved after anaerobic storage in faeces. Vet. Parasitol. 255:38–42.

Thapa, S., L. K. Hinrichsen, C. Brenninkmeyer, S. Gunnarsson, J. L. T. Heerkens, C. Verwer, K. Niebuhr, A. Willett, G. Grilli, S. M. Thamsborg, J. T. Sørensen, and H. Mejer. 2015. Prevalence and magnitude of helminth infections in organic laying hens (*Gallus gallus domesticus*) across Europe. Vet. Parasitol. 214:118–124.

Wongrak, K., M. Gauly, and G. Daş. 2015. Diurnal fluctuations in nematode egg excretion in naturally and in experimentally infected chickens. Vet. Parasitol. 208:195–203.