Prevalence and magnitude of helminth infections in organic laying hens (*Gallus gallus domesticus*) across Europe

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Abstract

Helminths are associated with health- and welfare problems in organic laying hens. The present observa- tional cross-sectional study therefore aimed to estimate the prevalence and worm burdens of intestinal helminths in organic flocks of laying hens in 8 European countries, and to identify management factors that might be associated with helminth infections, with emphasis on *Ascaridia galli*. Data on flock-level management factors (e.g. nutritional factors, litter quality, housing system, opening- and closing hours of popholes, pasture rotation and provision of occupational materials) were collected during a farm visit when the hens were on average 62 weeks old. Worm counts were performed for 892 hens from 55 flocks and the number of ascarid (presumably primarily *A. galli*) eggs per g faeces (EPG) for 881 hens from 54 flocks. The association between parasitological parameters (prevalence, worm burden and EPG) and the management factors were analysed by multivariate models. Results showed that *A. galli* was highly prevalent across Europe with an overall mean prevalence of 69.5% and mean worm burden of 10 worms per hen. The overall mean prevalence and worm burden for *Heterakis* spp. were 29.0% and 16 worms per hen, respectively, with a large variation between countries. On average, the hens excreted 576 ascarid EPG. The mean prevalence of *Raillietina* spp. was 13.6%. A positive correlation was found between mean *A. galli* worm burden and ascarid EPG. Of the analysed management factors, only pasture access time had a significant negative association with *A. galli* worm burden which was in contrast to the general belief that outdoor access may increase the risk of helminth infections in production animals. In conclu- sion, the complexity of on-farm transmission dynamics is thus a challenge when evaluating the relative importance of management factors in relation to helminth infections.

1. Introduction

Helminth infections are common worldwide in all types of poultry production systems.

Infections such as ascaridioses are important as they can be associated with production losses (Reid and Carmon, 1958; Toledo and Castell, 1981; Skallerup et al., 2005) and behavioural changes which could indicate reduced animal wel- fare (Gauly et al., 2007) in chickens (*Gallus gallus domesticus*). This is partly due to direct effects on the host, but helminths may also increase the risk of chickens becoming infected with secondary pathogens such as *Pasteurella multocida* (Dahl et al., 2002) and *Escherichia coli* (Permin et al., 2006). Furthermore, helminths (e.g. *Ascaridia galli*, *Heterakis* spp.) can serve as vectors for transmission of pathogenic infections, e.g. *Histomonas meleagridis* (McDougald, 2003) and *Salmonella enterica* serovar Typhimurium (Chadfield et al., 2001), the latter being a major zoonosis. *A. galli* may also interfere with the development of immunity in chickens after vac- cination against Newcastle disease (Pleidrup et al., 2014).

Studies from some European countries have shown that preva- lence and magnitude of several helminth infections (e.g. *A. galli*, *Heterakis* spp., *Capillaria* spp.) may be high in laying hens kept in organic and free-range production systems (Permin et al., 1999; Pennycott and Steel, 2001; Jansson et al., 2010; Kaufmann et al., 2011a; Sherwin et al., 2013; Bestman and Wagenaar, 2014). Non-cage systems thus provide favourable conditions for para- site transmission as parasite eggs can accumulate and potentially develop to infectivity in the litter indoors or soil on pastures and eggs can remain infective in the environment for at least a year (Farr, 1956). Indoors, the use of litter and perhaps bedding mate- rial makes it difficult to maintain a high level of hygiene and ascarid eggs can become infective within only 14–21 days, as observed for A. *galli* eggs under optimal conditions (Tarbiat et al., 2015). In con- trast, helminth transmission in cages is low (Permin et al., 1999) as most of the faeces with parasite eggs are easily removed before the eggs become infective to hens. Studies from Switzerland (Maurer et al., 2009, 2013) and United Kingdom (Sherwin et al., 2013) have found associations between husbandry factors (e.g. litter management, pasture management) and parasite egg counts in organic/free-range laying hens. In gen- eral, management factors (e.g. nutrition, housing system, pasture use, etc.) differ within and between countries, e.g. due to differ- ences in organic farming standards, but the extent to which these factors may influence helminth infections in laying hens on-farm is not well documented. Identification and verification of the relative importance of management factors could aid in control of poultry parasites in organic systems.

Therefore, the present study aimed to estimate prevalence and worm burdens of intestinal helminths in organic egg producing flocks distributed across 8 European countries, and to analyse the association between selected management factors and helminth infections, with special emphasis on *A. galli*.

2. Material and methods

2.1. Study design and flock selection

An observational cross-sectional study was carried out in 8 Euro- pean countries, Austria (AT), Belgium (BE), Denmark (DK), Germany (DE), Italy (IT), The Netherlands (NL), Sweden (SE) and the United Kingdom (UK), from February 2012 to March 2014. The study included 55 flocks in different farms selected amongst organic layer farms with more than 500 hens. Flock selection was based on the voluntary participation of the farmers and a set of exclusion cri- teria: flocks with more than one hybrid and flocks with mobile housing with relocation more frequently than every two weeks were excluded. Each country was responsible for recruiting farmers to participate within their country, and this was done by contact- ing the farmers by phone, email or letter. Collected data included information on management factors and parasite infection levels through necropsy of randomly selected hens and faecal samples.

2.2. Recording of flock and management factors

Each participating flock was visited when the birds were on average 62 weeks of age (range: 54–68 weeks) and sev- eral management factors were recorded during a questionnaire based interview. Data included pasture rotation management, lit-

ter quality, housing system, layer hybrid, provision of occupational material (e.g. silage or hay), deworming, the opening- and closing hours of the popholes (used to calculate pasture access time), the nutrient contents (crude protein percentage (CP%) and crude fibre percentage (CF%)) of the feed given around the necropsy period, and necropsy season. Pasture rotation was categorised as either 'yes' (within or between production cycles) or 'no'. Litter quality was categorised as being either 'good' (dry and good free-flowing, or conglomerates if present on less than 33% of the littered sur- face) or 'poor' conglomerates or plaques present on at least 33% of the littered surface. Housing system was recorded as 'single- tier' or 'multi-tier'. The provision of occupational material was also categorised into two levels ('yes' and 'no'). The necropsy season was categorised into 4 levels: 'spring' (March–May), 'summer' (June–September), 'autumn' (October–November) and 'winter' (December–February). Deworming was recorded as 'yes' or 'no' and a total of nine of the 55 flocks were treated. In DK 5 flocks were treated either 11, 17, 22, 31 or 40 weeks before necropsy, whereas two flocks were treated either 11 or 29 weeks before necropsy in NL. In DE and BE, one flock was treated 13 and 37 weeks, respectively, before necropsy.

2.3. Parasitology

2.3.1. Post mortem worm counts

Intestinal tracts were obtained either from hens killed directly at the farm or from slaughtered hens at the abattoir. In 31 flocks, live hens (BE: n = 14–16 per flock; DK: n = 19–21 per flock; NL: n = 10–15 per flock; SE: n = 15 per flock; UK: n = 10–19 per flock) were randomly selected from different areas of the henhouse. On average, the hens were 65 (BE), 72 (DK), 59 (NL), 59 (SE) or 65 (UK) weeks old at necropsy. The hens were stunned and killed by cer- vical dislocation. In 24 flocks, gastrointestinal tracts (AT: n = 15 per flock; DE: n = 15–19 per flock; IT: n = 15 per flock) were randomly selected at the abattoir when the hens were sent for slaughter at 69 (AT), 74 (DE) and 85 (IT) weeks of age on average. Whole carcasses and gastrointestinal tracts were either stored at 5 °C and examined within 48 h, or frozen (18 °C) for later examination. In total, gas- trointestinal tracts of 892 hens from 55 flocks were examined. The gastrointestinal tract was placed on a tray and separated from the mesentery by gentle tearing or with the help of scissors. The intes- tine was opened along its entire length with a pair of scissors and the contents in the small intestine were inspected for *A. galli* and *Raillietina* spp. The intestinal content was spread on the tray and the mucosa was washed gently with tap water to release worms. Using a protocol with morphological characteristics of chicken intestinal helminths, all macroscopically visible *A. galli* (1 cm) were counted while *Raillietina* spp. were recorded as present or absent. The caeca were also opened longitudinally and the contents were spread out, washed with tap water and examined for *Heterakis* spp.

2.3.2. Faecal worm egg counts

A. galli infections were also assessed indirectly by estimating the number of ascarid eggs in faecal samples of intestinal faeces (which are normally solid in consistency, contain white crystals of urates and are expected to contain mainly *A. galli* eggs) and care was taken not to include caecal faeces (which are brownish, more pulpy and contain mainly *Heterakis* spp. eggs) (Lapage, 1956). For this purpose, 14–15 fresh individual faecal samples per flock (20–21 in DK) were collected from the floor of the housing facilities during the farm visit and analysed individually for ascarid eggs by a simple McMaster technique (Sensitivity: 50 eggs per g faeces (EPG); Flotation fluid: 500 g glucose monohydrate/1000 ml saturated NaCl solution, spe- cific gravity of 1.27 g/ml) (Roepstorff and Nansen, 1998). As the eggs of *A. galli* and *Heterakis* spp. are too similar in morphology to be clearly differentiated, the eggs were counted together as ascarid eggs.

Table 1
Prevalence (%) of *Ascaridia galli, Heterakis* spp. and *Raillietina* spp., as determined by post mortem worm counts, in organic layer flocks ($n = 55$) in 8 European countries. Country Flocks(n) Hens(n) *Ascaridia galli* *Heterakis* spp. *Raillietina* spp.

Mean ± S.D.				Range	Mean ± S.D.	Range	Mean ± S.D.	Range
Austria	10	150	60.6 ± 21.4	26.7–93.3	46.0 ± 30.7	6.7–100	14.7 ± 16.9	0.0–53.3
Belgium	5	75	54.3 ± 30.9	0.0–81.2	77.3 ± 39.9	6.7–100	13.6 ± 15.0	0.0–35.7
Denmark	13	259	76.6 ± 18.2	35.0–95.0	1.6 ± 3.2	0.0–10.0	1.9 ± 4.2	0.0–14.3
Germany	7	114	89.5 ± 4.5	82.4–94.1	87.4 ± 13.7	60.0–100	26.8 ± 73.3	0.0–73.3
Italy	7	105	50.5 ± 28.8	13.3–86.7	0.0 ± 0.0	0.0–0.0	33.3 ± 18.5	0.0–60.0
Netherlands	2	25	96.7 ± 4.7	93.3–100	100 ± 0.0	100–100	33.3 ± 47.1	0.0–66.7
Sweden	9	135	72.6 ± 39.5	0.0–100	0.0 ± 0.0	0.0–0.0	0.0 ± 0.0	0.0–0.0
United Kingdom	2	29	73.3 ± 37.7	46.7–100	0.0 ± 0.0	0.0–0.0	10.7 ± 15.2	0.0–21.4
Overall (Europe)	55	892	69.5 ± 27.5	0.0–100	29.0 ± 39.9	0.0–100	13.6 ± 20.6	0.0–73.3

Table 2
Mean worm burden of intestinal *Ascaridia galli* and *Heterakis* spp. in organic layer flocks ($n = 55$) in 8 European countries.

Country	Flocks(n)	Hens(n)	*Ascaridia galli*		*Heterakis* spp.	
			Mean ± S.D.	Range	Mean ± S.D.	Range
Austria	10	150	5 ± 4	0–12	16 ± 19	0–56
Belgium	5	75	8 ± 8	0–21	50 ± 43	0–109
Denmark	13	259	6 ± 4	1–16	0.03 ± 0[a]	0–0
Germany	7	114	21 ± 16	3–56	58 ± 39	23–121
Italy	7	105	7 ± 7	0–17	0 ± 0	0–0
Netherlands	2	25	33 ± 15	23–44	45 ± 12	37–53
Sweden	9	135	13 ± 11	0–31	0 ± 0	0–0
United Kingdom	2	29	8 ± 5	5–12	0 ± 0	0–0
Overall (Europe)	55	892	10 ± 11	0–56	16 ± 30	0–121

[a] Presented as unrounded figure as this is the only data with missing agreement between the mean worm burden and the prevalence.

Table 3
Prevalence (%) of ascarid species (presumably primarily *Ascaridia galli*), as determined by the number of ascarid eggs per g faeces (EPG), in organic layer flocks ($n = 54$) in 8 European countries.

Country	Flocks(n)	Faecal samples (n)	Prevalence (%)		EPG	
			Mean ± S.D.	Range	Mean ± S.D.	
Austria	10	150	49.3 ± 32.1	0.0–100	492 ± 665	0–2057
Belgium	5	74	53.3 ± 36.2	0.0–93.3	465 ± 505	0–1213
Denmark	13	267	66.3 ± 25.4	0.0–95.0	705 ± 439	0–1378
Germany	7	105	92.4 ± 14.6	60.0–100	594 ± 339	156–1163
Italy	7	105	60.0 ± 29.1	20.0–100	249 ± 213	31–643
Netherlands	1	15	100	–	2450	–
Sweden	9	135	73.3 ± 38	0.0–100	585 ± 564	0–1656
United Kingdom	2	30	76.7 ± 14.1	66.7–86.7	557 ± 160	443–670
Overall (Europe)	54	881	66.7 ± 31.0	0.0–100	576 ± 540	0–

2.4. Data management and statistical analyses

All descriptive and analytical statistics were carried out using the software R (version 3.1.1.). Worm counts were performed for 892 hens from 55 flocks and faecal egg counts of ascarid worms (*A. galli/Heterakis* spp.) for 881 faecal samples from 54 flocks (one flock from NL was excluded because of the missing data). Flock preva- lence for each worm species (or ascarid species in case of faecal samples) was calculated as the percentage of hens positive for a par- ticular worm species (or ascarid species eggs) within a flock. Mean worm burden (or mean ascarid EPG) at flock level was calculated as a mean of worm burdens (or ascarid EPGs) of all hens within a flock. For each parameter (prevalence, worm burden, EPG), country specific values were calculated as a mean of all flock values within a country. Overall means were calculated for each parameter based on the respective country values.

The relationship between parasitological parameters (*A. galli* prevalence, *A. galli* worm burden and ascarid EPG and EPG based ascarid prevalence at flock level) and management factors were analysed using linear mixed effect models, with hybrid as a random variable (lme function, nlme package) (Pinheiro et al., 2014). The management factors analysed were dietary CP%, dietary CF%, occu- pational material, pasture access time, pasture rotation, necropsy season, housing system, deworming and litter quality. Univariate analyses were performed initially for each variable and the vari- ables with $P < 0.2$ were included in a full multivariate model. The variables with $P > 0.05$ in the full model were removed sequentially by a backward elimination procedure to obtain a reduced model. Only CP% and CF% ranging between 14.6–22.2% and 3.0–6.5%, respectively, in the feed given at the time around slaughter were included in the analyses, while 4 flocks with uncommonly high CP% (29.1%: DK; 31%: DK) or CF% (8%: DE; 10.1%: DK) were excluded. One

flock in the NL was also excluded as the CP%, CF% and EPG data were lacking. The reduced dataset consisted of 812 hens from 50 flocks. For *Heterakis* spp., many flocks and countries were totally negative and risk analysis was not performed for this parasite.

The relationship between mean *A. galli* worm burden and ascarid EPG was analysed at flock level (dataset containing 54 flocks) using Spearman's correlation coefficient at 95% confidence interval.

Table 4

Descriptive statistics of prevalence, worm burden and eggs per g faeces (EPG) of *Ascaridia galli* or ascarid species (*A. galli*/*Heterakis* spp.) in relation to management factors. The mean values are calculated using data from 50 organic layer flocks (reduced dataset) included in the risk-factor analyses.

Management factors	Flocks (*n*)	Hens (*n*)	Mean *A. galli* prevalence% ± S.D.	Mean *A. galli* burden ± S.D.	Mean EPG based ascarid prevalence% ± S.D.	Mean ascarid EPG ± S.D.
Necropsy season						
Spring	13	207	72.5 ± 30.3	8 ± 9	59.4 ± 30.6	565 ± 647
Summer	14	239	66.6 ± 20.4	10 ± 7	68.4 ± 30.2	678 ± 507
Autumn	10	164	75 ± 21.5	8 ± 5	66.3 ± 26.1	507 ± 327
Winter	13	202	58.9 ± 36.9	8 ± 9	72.2 ± 34.0	507 ± 674
Occupational material						
Yes	23	404	76.6 ± 27.8	10 ± 9	74.1 ± 26.8	742 ± 594
No	27	408	60.3 ± 26.7	7 ± 6	60.2 ± 31.6	423 ± 468
Pasture rotation						
Yes	13	234	78.9 ± 21.5	10 ± 8	71.9 ± 25.7	808 ± 634
No	37	578	63.9 ± 29.4	8 ± 8	64.8 ± 31.5	486 ± 497
Housing system						
Single-tier	27	439	64.2 ± 27.3	8 ± 7	64.8 ± 30.9	624 ± 567
Multi-tier	23	373	72.1 ± 29.1	9 ± 8	68.7 ± 29.5	506 ± 531
Litter quality						
Good	31	600	66.9 ± 31.0	8 ± 8	68.3 ± 28.9	623 ± 575
Poor	19	212	69.3 ± 23.5	9 ± 7	63.9 ± 32.4	483 ± 504
Deworming						
Yes	7	128	74.9 ± 17.9	9 ± 7	80.9 ± 13.8	1012 ± 738
No	43	684	66.6 ± 29.5	9 ± 8	64.3 ± 31.4	498 ± 485
Hen hybrid						
Lohmann Brown	16	245	69.8 ± 21.5	8±6	61.2 ± 32.2	447 ± 543
Dekalb White	9	135	72.6 ± 39.5	13 ± 11	73.3 ± 38.0	585 ± 564
Hyline Brown	7	104	53.3 ± 32.9	6 ± 6	57.1 ± 26.9	291 ± 265
Lohmann S. Leghorn	5	95	72.8 ± 14.9	8 ± 6	73.3 ± 18.3	869 ± 497
Hisex White	5	99	74.9 ± 25.2	4 ± 2	61.0 ± 17.8	453 ± 376
Dekalb Amber	1	15	46.7	5	86.7	443
Hyline Silver	1	15	93.3	23	100	2450
Isa Brown	1	16	81.2	21	93.3	1213
Lohmann Brown Lite	4	69	49.4 ± 37.7	5 ± 3	61.6 ± 41.6	724 ± 642
Tetra SL	1	19	89.5	19	93.3	435
Crude protein (CP) %[a]						
14.6–16.1	6	101	66.9 ± 21.0	10 ± 10	63.9 ± 24.1	477 ± 479
16.1–17.6	19	301	63.8 ± 33.6	9 ± 8	70.8 ± 29.7	544 ± 560
17.6–19.2	17	285	7 ± 27.3	9 ± 7	69.9 ± 33.0	638 ± 522
19.2–20.7	7	110	65.7 ± 21.6	6 ± 4	51.7 ± 30.9	565 ± 756
20.7–22.2	1	15	46.7	3	53.3	497
Crude fiber (CF) %[a]						
3.0–3.6	3	45	77.8 ± 26.9	15 ± 12	82.2 ± 25.2	744

±458						
3.6–4.2 ±272	11	165	68.5 ± 30.3	8 ± 6	67.9 ± 32.5	335
4.2–4.8 ±474	12	204	74.6 ± 19.8	9 ± 6	71.9 ± 27.1	690
4.8–5.4 ±593	12	197	53.7 ± 34.2	5 ± 4	54.2 ± 34.8	452
5.4–6.0 ±852	8	140	71.2 ± 27.1	11 ± 11	67.3 ± 29.7	787
6.0–6.5 ±486	4	61	73.4 ± 28.4	9 ± 9	71.7 ± 24.0	648
Pasture access time (h)[a]						
0–4.8 ±564	9	135	72.6 ± 39.5	13 ± 11	73.3 ± 38.0	585
4.8–9.6 ±410	14	217	67.9 ± 28.1	9 ± 6	48.2 ± 33.3	313
9.6–14.4 ±591	24	405	67.8 ± 25.3	8 ± 7	75.8 ± 21.6	751
14.4–19.2 ±77	2	35	47.5 ± 17.7	3 ± 2	55.8 ± 13.0	238
19.2–24	1	20	65.0	2	65.0	365

[a] Variables analysed as continuous variables in the risk factor analysis, but presented as categorical to give an overview of the distribution of parasitological data among different categories.

3. Results

3.1. Prevalence and magnitude of intestinal parasites

The prevalences of *A. galli*, *Heterakis* spp. and *Raillietina* spp. based on the post mortem worm counts are presented in Table 1. The most common parasite was *A. galli* (69.5%), followed by *Heterakis* spp. (29.0%) and *Raillietina* spp. (13.6%). There was a large variation in prevalence of the 3 groups of parasites within country and between countries. The parasite composition also varied between the flocks: 17 (30.9%) of the 55 flocks had all 3 parasite species, 9 (16.4%) had only *A. galli* and *Heterakis* spp., 9 (16.4%) had only *A. galli* and *Raillietina* spp., 18 (32.7%) had only *A. galli*,1 (1.8%) had only *Heterakis* spp. and 1 (1.8%) had none of the three species of parasites. Of the 55 flocks, only 2 (3.6%) flocks were negative for *A. galli*, 28 (50.9%) flocks were negative for *Heterakis* spp. and 29 (52.7%) flocks were negative for *Raillietina* spp.

The overall mean worm burden across Europe was somewhat higher for *Heterakis* spp. compared to *A. galli*, even though none of the hens in the UK, IT and SE were found to be positive for *Heterakis* spp. (Table 2). The country specific mean worm burden for *Heterakis* spp. and *A. galli* ranged between 0 and 58 and 5-33 worms per hen, respectively (Table 2).

Table 5
Association between *Ascaridia galli* worm burden (at flock level) and management factors in organic layer flocks (n = 50) across Europe.

Management factors	Estimate	Standard error	P-Value
Intercept	12.456	2.074	
Fixed effect			
Pasture access time (h)	−0.439	0.206	0.040
Random effect			
Hybrid variance	1.89 × 10−07	0.00043a	
Residual variance	53.38	7.24a	

a Standard deviation

Of the 881 faecal samples examined from the 54 flocks, 589 samples were positive for ascarid eggs, yielding an overall mean prevalence of 66.7% across Europe (Table 3). At country level, the mean prevalence was between 49.3 and 100% and the mean coun- try specific ascarid EPG ranged between 249 and 2450 (Table 3). The mean *A. galli* worm burden was significantly correlated with the mean ascarid EPG (r_s = 0. 50; $P < 0.001$).

3.2. Association between management factors and parasitological parameters

The descriptive statistics of the parasitological data from the 50 flocks that were included in the analyses are presented in relation to the nine management factors in Table 4. However, only pasture access time was found to have a significant ($P = 0.04$) negative asso- ciation with the *A. galli* worm burden in laying hens (Table 5). None of the nine factors had a significant association with *A. galli* preva- lence (based on worm burden), ascarid prevalence based on EPG or ascarid EPG.

4. Discussion

The current study is the first to report prevalences and worm burdens of intestinal helminths in organic layer flocks across 8 European countries, demonstrating that *A. galli* is by far the most common helminth. There are not many studies for comparison, but the current occurrence of *A. galli* (or ascarids in general) in Denmark, Sweden and Germany was similar to earlier reports (Permin et al., 1999; Jansson et al., 2010; Kaufmann et al., 2011a). In contrast, *Heterakis* spp. seems to have almost disappeared in Danish organic layers within the last 20 years (Permin et al., 1999).

Of the tested management factors, only pasture access time had a significant association with *A. galli* worm burden. Similarly, in pigs, a lack of association between ascarid infections (*Ascaris suum*) and a number of management factors has been reported by three Danish studies using multivariate models (Roepstorff and Jorsal, 1990; Dangolla et al., 1996; Roepstorff et al., 1999). This may reflect that the on-farm transmission dynamics of ascarid infections are too complex to be linked with only a limited num- ber of management factors that may further interact with biotic and abiotic factors in ways that are not fully understood. In addi- tion, host immune responses may modulate ascarid worm burdens thus obscuring exposure levels and their relation to management practices and environmental contamination with infective eggs. Acquired immunity is thus very important in regulation of *A. suum* burdens in pigs (Miquel et al., 2005), but the relative effect of immu- nity on *A. galli* establishment and persistence in poultry has not yet been elucidated.

The current negative association between *A. galli* worm bur- den and pasture access time contradicts the general expectation that the risk of helminth infections increases with increased out- door access. As current EU regulations require a maximum stocking density of 6 hens per m^2 indoors and a maximum of one hen per four m^2 outdoor area, faecal material may potentially be spread over a larger area outdoors compared to indoors, thus theoretically decreasing the risk of animals becoming infected. However, fae- ces and *A. galli* eggs may not be spread evenly over the pastures as the majority of hens have been shown to primarily forage on areas close to the henhouse (Hegelund et al., 2006; Heckendorn et al., 2009), possibly depositing most of the eggs within a small area. To further complicate our understanding of transmission pathways, survival and development of *A. galli* eggs to infectiv- ity may differ between the indoor and outdoor areas. The indoor areas may provide more favourable conditions (e.g. temperature) for egg development throughout the year (Tarbiat et al., 2015) thus facilitating a faster embryonation of eggs excreted by hens dur- ing the production cycle compared to the outdoor areas. However, very little is known about how oxygen availability, temperature fluctuations and ammonia in the bedding material may affect sur- vival and development of chicken ascarid eggs indoors, though high ammonia and temperature levels may inactivate *A. galli* eggs in slurry (Katakam et al., 2014b). Indoors, bedding material has been suggested to favour transmission of ascarid worms in pigs (Sanchez-Vazquez et al., 2010), but it has also been shown that survival and development of pig ascarid eggs to infectivity is not clearly associated with the level of litter soiling (Katakam et al., 2014a). This may be why litter quality (e.g. soiled litter) did not have had an impact on helminth transmission and infection levels in laying hens in our study. Similarly, a Swiss study

(Maurer et al., 2009) did not find a difference in the number and infectivity of ascarid eggs between managed litter (weekly removal of old litter or addition of new litter material on top of old litter for 32 weeks) and unmanaged litter (no removal or addition of new litter mate- rial) in 6 layer flocks. Few studies have dealt with the ecology of *galli* eggs, but if deposited outdoors during the winter months the eggs cannot develop to infectivity in colder climates where temperatures drop below 15 °C (Tarbiat et al., 2015). Spring and summer may provide better conditions for development, though it may still be slow in northern Europe, as demonstrated for *A. suum* eggs (Larsen and Roepstorff, 1999; Kraglund, 1999). Further- more, ascarid eggs deposited in pastures are likely to experience a high mortality due to adverse environmental factors such as direct exposure to sunlight (Brown, 1927; Bray and Lancaster, 1992) and desiccation (Caldwell and Caldwell, 1928; Seamster, 1950). In addi- tion, biotic factors (bacteria, fungi and mites) in the litter and soil environment may influence the survival of ascarid eggs but these factors have not yet been examined on farm. Many factors may thus influence the survival and development of eggs both indoors and outdoors but it is difficult to predict which factors are most impor- tant in relation to the transmission and infection dynamics of *A. galli* in non-cage production systems.

It is not well documented if pullets are first infected with helminths in the egg production units or in the rearing houses. If the pullets are worm free on arrival at the production units, the initial source of infection post placement must be residual infec- tive ascarid eggs present in the environment (e.g. pastures and henhouse if not cleaned thoroughly) from the previous flock(s) (Höglund and Jansson, 2011). This is because avian ascarid eggs are thick-shelled (Christenson et al., 1942; Wharton, 1980) and can remain infective in the environment for many months (Farr, 1956; Bray and Lancaster, 1992) and thus ensure the persistence of para- sites outdoors and possibly indoors. Pasture rotation is considered as an important non-chemical method to reduce the environmental contamination with free-living stages (primarily larvae) of rumi- nant nematode parasites because the larvae have a limited life span (Thamsborg et al., 2010). However, pasture rotation did not appear to reduce the ascarid infections significantly in the current study, a result similar to a study on organic layer flocks by Maurer et al. (2013). It is possible that rotation cycles (e.g. up to one year in the present study) are too short to be effective as it has been shown that pastures contaminated with *A. galli*/*A. dissimilis* eggs can remain infective for at least one year (Farr, 1956), though it may be even longer as seen for other thick-shelled nematode eggs (Müller, 1953; Burden et al., 1987). Secondly, access to the different pas- tures around to the henhouse is often through permanently used areas just in front of the house. These permanently used areas can harbour a large number of infective residual eggs from the previous flocks (Bray and Lancaster, 1992; Heckendorn et al., 2009) and may therefore reduce the effectiveness of pasture rotation management in *A. galli* control.

The EU organic regulations require that hens should have access to high-fibre occupational materials (e.g. roughage, silage, car- rots) to support natural foraging behaviour. Experimental studies have shown some association between nutrition and dynamics of helminth infections in hens. Supplementation with fibre rich maize silage or carrots resulted in higher faecal *A. galli* egg counts in experimentally infected organic layers compared to hens given only a standard diet (Idi et al., 2005; Shrestha et al., 2013). Simi- larly, Daş et al. (2011, 2012) reported higher establishment rates, faecal egg counts and worm burdens of ascarid (*H. gallinarum*/*A. galli*) infections in chickens that were given high fibre diets (rich in insoluble non-starch polysaccharides). Increased worm burdens and faecal egg counts of the nematode, *Oesophagostomum den- tatum*,has similarly been reported in pigs given a diet with high levels of insoluble dietary fibres (Petkevicius et al., 1999). An exper- imental study by Permin et al. (1998) further showed that *A. galli* worm burdens were lowered in hens if the dietary proteins level was reduced. However, our on-farm study was not able to docu- ment a significant relationship between worm infections and any of the measured nutritional parameters (CF%, CP% or the provision of occupational material). It is likely that the CP% of standard com- mercial diets in most of the flocks varied too little (mainly between 16.1 and 19.2%) to exert any influence. Fibre intake from the litter material and outdoor vegetation may also have influenced the results.

In EU, routine preventive deworming is not allowed in both organic and conventional egg production but birds may be treated if they are diagnosed to be infected with parasites; however, the drug withdrawal period in the organic systems has to be twice long as compared to the conventional systems. In the current study, deworming had no significant effect on the parasitological param- eters which is likely due to the large variation in the time of deworming within and between the countries. To the best of our knowledge, there are no official deworming

guidelines (e.g. cut-off EPG) in most of the European countries and the deworming prac- tices therefore vary largely even within a country. For example in Denmark, based on our personal experiences, some organic farms do not treat the birds at all whereas some farms follow structured parasite monitoring and treatment programs.

It is generally thought that there is a genetic element involved in host resistance to parasites, and differences in ascarid fae- cal egg counts have been observed between different hybrids of laying hens (e.g. Schou et al., 2003; Kaufmann et al., 2011b). How- ever, these studies could not demonstrate a significant association between hybrid and *A. galli* prevalence and worm burden. In our study, we found a low level of variation between hybrids for the outcome variables, possibly because of the small number of flocks per hybrid as some hybrids were present only in one flock (Table 4). In the current study, the ascarid eggs in the faecal samples are very likely to be primarily *A. galli* eggs as the samples contained only intestinal faeces. A strong positive correlation was found between the mean ascarid EPG and *A. galli* worm burden. The correlation could potentially have been further stronger if the faecal samples were collected at the same time as the animals were necropsied.

Similar to our study, a significant positive correlation between worm burden and EPG has been reported for *A. galli* by experimental studies by Train and Hansen (1968) and Gauly et al. (2005).

5. Conclusions

The results from the present study documented that *A. galli* was highly prevalent in organic layer flocks across 8 European coun- tries while a large variation existed in the prevalence of *Heterakis* spp. between countries. *Raillietina* spp. occurred in most countries at moderate level. We could only demonstrate an epidemiological relationship (negative) between pasture access time and *A. galli* intestinal worm burden. This indicates that the transmission of helminth infections in non-cage systems are very complex and fur- ther studies on egg ecology and transmission patterns both indoors and outdoors are needed to improve management guidelines.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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