Veterinary Parasitology 214 (2015) 118–124

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

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

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ARTICLE INFO

*Article history:*
Received 14 July 2015
Received in revised form 5 October 2015
Accepted 7 October 2015

*Keywords:*
*Ascaridia galli*
*Heterakis* spp.
*Raillietina* spp.
Prevalence
Worm burden
Management
Organic laying hens

ABSTRACT

Helminths are associated with health- and welfare problems in organic laying hens. The present observational 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 conclusion, 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 welfare (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.

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http://dx.doi.org/10.1016/j.vetpar.2015.10.009
0304-4017/

*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 vaccination against Newcastle disease (Pleidrup et al., 2014).

Studies from some European countries have shown that prevalence 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 parasite 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 material 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 contrast, 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 general, management factors (e.g. nutrition, housing system, pasture use, etc.) differ within and between countries, e.g. due to differences 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 European 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 criteria: 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 contacting 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 several management factors were recorded during a questionnaire based interview. Data included pasture rotation management, litter 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 surface) 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 cervical 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, gastrointestinal 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 intestine 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, specific 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