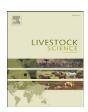
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Helminth infections in laying hens kept in organic free range systems in Germany

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ABSTRACT

This study describes the spectrum and intensity of helminth infections in laying hens kept in organic production systems in Germany. A total of 740 laying hens from 18 organic free range farms were collected between 2007 and 2010. The hens were sacrificed and the gastrointestinal tracts were examined for the presence and intensity of helminth infections with standard parasitological methods. Three nematode (Ascaridia galli, Heterakis gallinarum, Capillaria spp) and four cestode (Raillietina cesticillus, Hymenolepis cantaniana, Hymenolepis carioca, Choanotaenia infundibulum) species were found. Almost all hens (99.6%, N=737) harboured at least one helminth species. Average worm burden per hen was 218.4 (SD = 218.3) worms. The most prevalent species were the nematodes Heterakis gallinarum (98%) followed by Ascaridia gali (88%) and Capillaria spp. (75.3%). The overall prevalence of the cestodes was 24.9%. Total worm burden was significantly higher during the summer season when compared with animals slaughtered during winter season (254 vs. 191, P<0.0001). The most dominant helminth species was Heterakis gallinarum averaging 190 (SE=9.8) worms per hen in the summer and 129 (SE = 8.7) in the winter season (P<0.0001). Average Ascaridia galli burden was 25 (SE = 2.0) in summer and 26 (SE = 1.7) in winter (P = 0.1160). Risk of infection with any of the nematodes was 50% higher in summer compared to winter ($\Psi = 1.49$, P < 0.0319). Probability of infection with any of the tapeworm species was 3.5 times higher in summer than in winter (P<0.0001).

It can be concluded that the vast majority of hens are subclinically infected with at least one helminth species. The prevalence as well as intensity of the helminth infections, particularly with tapeworms, considerably increases in summer. The results indicate that it is essential to adopt alternative control strategies in order to lower infection risk in organic production systems which are gaining popularity.

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1. Introduction

Recent changes in consumer prospects regarding a sustainable animal production and welfare has led to the ban of the conventional cages for laying hens in Germany since 2010 and the European Union after 2012 (1999/74/EC, Anonymous, 1999). Thus, alternative production systems have gained popularity and percentages of hens kept in such systems increased over the last couple of years (MEG, 2010; ZMP, 2008).

There is evidence that different production systems bear different risks of parasite infections for animals. Parasitic infections, particularly in floor husbandry systems with or without outdoor access, are re-emerging. A study from Denmark showed that the prevalence of the nematode *Ascaridia galli* was 64% in free range/organic systems, 42% in deep-litter systems and 5% in conventional cages (Permin et al., 1999). The birds get infected by ingestion of infective parasitic stages present in soil and litter and/or by eating intermediate or transport hosts. Infections with endoparasites may have severe consequences on the host as well as on the production systems as reported by several studies. Parasites may for example

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obstruct the small intestine and cause death (Ramadan and Znada, 1991). They can act as vectors and lead to secondary infections, e.g. E. coli (Chadfield et al., 2001; Dahl et al., 2002; McDougald, 2005; Okulewicz and Zlotorzycka, 1985; Permin et al., 2006). Furthermore they have adverse effects on behavior patterns, growth and nutrient utilization of chickens (Chubb and Wakelin, 1963; Daş et al., 2010; Gauly et al., 2007). The control of endoparasites in various species is heavily dependent on the use of anthelmintics. In general, the use of anthelmintics in layers is very limited due to economic concerns as well as environmental and hygiene regarding development of drug resistance (Jackson and Miller, 2006) and chemical residues in animal products (Craig, 1993; Sangster, 1999; Waller, 1994). Particularly in organic production systems use of anthelmintics is strictly restricted as prophylactic treatments are prohibited. The changes in the production systems and in the climate may alter population dynamics of endoparasites which may accumulate the importance of helminth infections in the future. In order to improve and maintain the performance and productivity of hens, and to adopt alternative control strategies against endoparasites (Heckendorn et al., 2009) it is essential to determine the spectrum as well as intensity of the parasitic agents, which has not been done for organic layers in Germany, so far.

Therefore, the aim of the present study was to investigate the spectrum and intensity of helminth infections, as well as to estimate seasonal effects on the prevalence and burden of helminths in organic free range layers in Germany.

2. Material and methods

2.1. Farm and animal sampling

A total of 740 laying hens of 5 genotypes (Lohmann Brown, Lohmann Selected Leghorn, Isa Brown, Tetra Brown, Lohmann Tradition) were collected from 18 commercial free range farms in Germany. All farms were certified as organic farms according to the guidelines of the European Union and national guidelines (2092/91/EEC; 834/2007/EC; Bioland, 2010). Farms were located throughout Germany with a focus on the central region as one of the most intense poultry production area in Germany. The main farm selection criteria were two folds; (1) the farms had to fulfil the standards for the EU organic guidelines, and (2) willingness of the farmers for voluntary participation. Because the number of farms was limited, a relatively high number of chickens per farm (on average 41) was sampled to ensure a representative and reliably large sample size. Flock size of the sampled farms ranged between 750 and 3000 hens with the majority of farms approaching the maximum flock size according to the guidelines of the European Union. The age of the free range farms as well their runway management differed. Subjectively, appearance of the runway varied between optimal, as described in Hegelund et al. (2005), Keeling et al. (1988), and Zeltner and Hirt (2003, 2008) and far from being ideal. In most cases proper vegetation and a pasture rotation was lacking. The sampled hens were either in the last third or at the end of the laying period. Therefore, age of hens varied between 54 and 72 weeks. Hens slaughtered from October to March were included in the winter data set (N=417), whereas hens slaughtered from April to September were included in the summer data set (N = 323).

2.2. Necropsy, parasite processing and species identification

After slaughtering, the gastrointestinal tracts and tracheas were removed, opened longitudinally, and washed in tap water following the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the effectiveness of anthelmintics in chickens and turkey (Yazwinski et al., 2003). The separated contents were poured into a sieve with a mesh aperture of 100 µm, washed and examined for the presence of adult helminths.

All visible parasites were collected first and then the content of the gastrointestinal tract and the scraped mucosa were examined under $20\times$ dissecting microscope. All species were counted and stored in tap water until differentiation on the same day. Identification of nematodes started with cleaning the worms in physiological saline solution. Afterwards they were examined under a light microscope at $40\times$ magnification and differentiated based on the morphological characteristics as described by Norton and Ruff (2003) and Soulsby (1982).

Cestode harvest was done by submerging the intestine in water floating the worms to increase their visibility. In some cases, the scolices were strongly attached to the mucosa. To liberate the scolice, the attachment points were located; the intestine was cut around the attachment point and frozen in water for 2 h. After thawing, scolices could be removed easily from the mucosa using fine needles. Cestodes were identified using the helminthological keys according to Jones and Bray (1994), McDougald (2003), Schmidt (1986) and Soulsby (1982). Staining-destaining techniques with Carmine dye were performed for identification of testes and cirrus.

All adult *A. galli* and *H. gallinarum* worms found were sexed as determined by Hartwich (1975). Furthermore, for each hen, a maximum of ten randomly selected worms per worm species and sex were measured for length using a ruler.

2.3. Statistical analyses

Prevalences of mono species-specific and mixed helminth infections were calculated with the Freq procedure of SAS (2010). Effect of season on the incidence of each helminth species was analyzed using the GENMOD procedure of SAS with a logit link function as shown in the following model.

$$\eta_i = \text{log}[p_i / (1{-}p_i)] = m + \tau_i$$

i = seasons; winter, summer

where;

p_i the proportion of infected birds on season i

m the overall mean of the proportion on the logarith-

mic scale

 τ_i the effect of season i

Based on the output of the GENMOD procedure, the odds ratios (Ψ) as the probability of being infected with a given species at one of the seasons were estimated (Kaps and Lamberson, 2004).

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