



# Poultry litter as a source of gastrointestinal helminth infections

V. Maurer<sup>\*</sup>, Z. Amsler, E. Perler, F. Heckendorn

Research Institute of Organic Agriculture (FiBL), Ackerstrasse, CH-5070 Frick, Switzerland

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## ABSTRACT

The aim of this study carried out in 6 commercial layer houses was to examine the effect of litter management on water content, helminth egg count and litter infectiousness with the intestinal nematodes *Ascaridia galli*, *Heterakis gallinarum*, and *Capillaria spp.* Three types of litter management were established in each layer house in parallel: in compartment A, litter was left undisturbed, in compartment B, wet litter was replaced and in compartment C, new litter material was added weekly. Dry matter (DM) contents of the litter and parasitological parameters (helminth egg concentration in litter samples, faecal egg counts (FECs) in the permanent layer flocks, helminth prevalence and burdens in two series of tracer animals) were determined every 4 weeks during the first 32 weeks of one laying period. DM contents of the litter varied in a broad range (48–95%); 8 weeks after onset of the study, there were significant differences between sites ( $P < 0.001$ ) but not between management regimes. *A. galli*/*H. gallinarum* eggs were isolated from 91% of the litter samples, whereas eggs of *Capillaria spp.* were only extracted from 13% of the samples. Egg concentrations in litter remained at a similar level during the observation period. Neither management regime reduced helminth egg concentrations in the litter compared to the unmanaged regime. Laying hens started excreting helminth eggs 8 weeks after introduction to the layer house. In treatment C (litter added) FECs were lower than in the unmanaged treatment A in weeks 8 ( $P < 0.0001$ ), 20, and 28 (both  $P < 0.1$ ). There was no correlation between the concentration of helminth eggs in the litter and the FECs of the layer flocks. The prevalence of *A. galli* in tracer animals was lower (<10%) than the prevalences of *H. gallinarum* (68–80%) and *Capillaria spp.* (30–58%). Prevalences and *H. gallinarum* burdens did not differ significantly between management regimes. Although high helminth egg concentrations were found in litter, the prevalence and worm burdens in tracer animals were low compared to a similar study with tracers kept in poultry runs. The reasons for this may be that poultry litter negatively affects viability and infectiousness of helminth eggs. However, underlying mechanisms need to be clarified.

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## 1. Introduction

Aviary and deep-litter systems with or without access to a free-range area are increasingly used in European egg production units. On one hand it is recognised that these systems promote natural behaviour and increase animal welfare. On the other hand, the litter area represents an important site of infection with helminths, coccidia and

other pathogens (Malone, 2004; Permin et al., 1999). Among the poultry helminths, *Ascaridia galli* and *Heterakis gallinarum* are the most common species (Permin et al., 1999). They can cause weight depression (Kilpinen et al., 2005) and damage the intestinal mucosa, sometimes leading to haemorrhages, anaemia and severe diarrhoea; heavy *A. galli* infections can obstruct the small intestine and cause death (Ramadan and Znada, 1991). Even sub-clinical infections can increase serum testosterone concentrations and have a multitude of effects on hen behaviour (Gauly et al., 2007).

<sup>\*</sup> Corresponding author. Tel.: +41 62 965 72 57; fax: +41 62 965 72 73.  
E-mail address: [veronika.maurer@fibl.org](mailto:veronika.maurer@fibl.org) (V. Maurer).

As other *Ascarid* species, *A. galli* eggs are highly resistant to environmental stress and have high survival rates (Tønner-Klank et al., 2007). These features have been investigated into detail in several studies using *Ascaris suum*, mainly with regard to sanitising sewage sludge or other biowaste (Eriksen et al., 1996; Hansen et al., 1953; Johnson et al., 1998; Sahlström et al., 2008). In contrast, only few studies (Hansen et al., 1953; Saunders et al., 2000) are available on the viability of *A. galli* and other poultry helminth eggs at different environmental conditions.

Excessively dry litter leads to dust and related problems in animals and in stockpersons, while wet litter increases ammonia fumes in the house (Bermudez and Stewart-Brown, 2003). Therefore, farmers aim at keeping the litter moderately dry (around 25% moisture; Malone, 2004) by replacing wet litter material or by adding dry material during flocks. However, little is known about the effect of such litter management strategies on litter humidity and the related risk of infection of laying hens with intestinal helminths although litter is expected to favour parasite transmission (Matter and Oester, 1989; Waldenstedt et al., 2001). The aim of the present study was therefore to examine the effect of two common litter management regimes (replace or add new litter material) on water content, nematode egg counts and litter infectiousness.

## 2. Material and methods

### 2.1. Experimental sites and litter management

The study was conducted in six commercial organic layer houses in Switzerland (farms 1–6) between August 2004 and October 2007. Housing and feeding of the hens were according to the Swiss organic regulations (BioSuisse, 2008). In brief, the houses were equipped with aviary systems with a minimum of 0.07 m<sup>2</sup> of littered scratching area per bird; dung below the elevated resting and feeding area was removed weekly by means of a dropping belt. Each layer house comprised a flock of 2000 animals. The houses were subdivided into four compartments for groups of 500 birds of which 3 compartments were used for the experiment. Groups remained separated in the wintergarden and in the outdoor run.

Three types (A, B, C) of litter management were carried out in parallel in three compartments on each farm during one production cycle starting upon arrival of the pullets in the layer house. The treatments were allocated to the compartments at random. In compartments A litter was left undisturbed during the experimental period (control). In compartments B wet and compacted litter material was replaced weekly, and in compartments C new litter material was added weekly on the whole surface. Straw was used as litter material.

Farm visits took place every 4 weeks starting in week 4 after arrival of the flocks. The experiment was finished after 8 visits in week 32 on all farms except for farm 5, where the farmer decided for an anthelmintic treatment in week 20 and the experiment was finished after 5 visits.

### 2.2. Layer flocks and tracer chicken

Layer pullets were brought to the layer houses at the age of 18–20 weeks. Previous flocks on all the farms had been positively tested for the intestinal helminths *A. galli* and *H. gallinarum*, assuring a nematode positive litter environment. No anthelmintic treatment was applied during the experimental period.

In each layer flock, two series (T1 and T2) of 5-week-old parasite naïve female tracer chickens (Hybrid: Lohmann Tradition) were used to determine the infectivity of the litter. In each compartment, a litter sample representing a cross-section through the whole litter layer was taken from twelve predefined, evenly distributed places. Six tracer chickens per farm and treatment group were kept on those litter samples during 4 weeks and subsequently for 8 weeks in a parasite-free environment until slaughter. T1 were installed on litter samples taken in week 20 and T2 on litter of week 32 of the layer flocks. On farm 5, only T1 were used because of early termination of the experiment (see Section 2.1).

Layer and tracer chickens had been vaccinated by the Swiss standard procedure (Hoop, 2006) and additionally immunised against *Eimeria* spp. with Paracox<sup>®</sup>-8 at the age of 5–9 days.

### 2.3. Parasitological measurements

At each farm visit, faecal and litter samples were taken from each compartment for determination of helminth eggs (litter and faeces) and coccidian oocysts (faeces). The proportion of intact and defective *A. galli* and/or *H. gallinarum* eggs was determined in both, faecal and litter samples. Eggs were classified as 'intact' if the three covering layers were distinguishable and the contents were structured (cells or larvae visible); otherwise, eggs were classified as 'defective'.

In each compartment, a total of 20 fresh faecal samples were randomly collected from the dropping belt and pooled to obtain one bulk sample per compartment. After thorough mixing, three sub-samples per compartment were examined using a McMaster method as described by Schmidt (1971) and faecal egg counts (FEC) were calculated as the average of three examinations per compartment.

Litter samples were taken from twelve predefined, evenly distributed places in each compartment. On each place, a sample (approximately 100 g) representing a cross-section of the whole litter layer was removed. The 12 samples of one compartment were then pooled for (i) determination of dry matter (DM; 12 h at 105 °C) and (ii) parasitological analysis as described by Roepstorff and Nansen (1998) and modified according to Heckendorn et al. (2009). Helminth eggs were determined in two sub-samples of the pooled sample of each compartment. Average concentrations of intact and defective helminth eggs/were then calculated per g litter DM.

The gastrointestinal tract of tracer chickens was opened immediately after slaughter and the contents were washed over a sieve (200 µm) for determination and counting of the helminths present. The small intestine and caeca were

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