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Research paper

Comparison between anthelmintic treatment strategies against *Ascaridia galli* in commercial laying hens

B. Tarbiat[a,*], D.S. Jansson[b], E. Tydén[a], J. Höglund[a]

[a] Department of Biomedical Sciences and Veterinary Public Health, Section for Parasitology, Swedish University of Agricultural Sciences (SLU), P.O. Box 7036, SE-750 07, Uppsala, Sweden
[b] Department of Animal Health and Antimicrobial Strategies, National Veterinary Institute (SVA), SE-751 89, Uppsala, Sweden

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ABSTRACT

The efficacy of a sustainable deworming strategy based on targeted treatments (TT) against *Ascaridia galli* was investigated for the first time in laying hen flocks on a Swedish commercial farm. Three experimental protocols with different levels of treatment, e.g. targeted treatment (TT), conventional treatment (CT) and untreated (UT), were tested in randomly allocated flocks of two different bird hybrids. Every second week faecal egg counts (FECs) were determined from pooled faecal materials collected on trays (20 × 27 cm) placed for a maximum of 12 h on the litter belts. In the TT, anthelmintic administration (fenbendazole, 1 mg/kg body weight for 5 days) started at 22 weeks post placement (wpp) and was repeated twice when the pooled FECs surpassed the assigned threshold of 200 egg per gram faeces (EPG). The CT flocks were treated once at 27 wpp using the same anthelmintic. Hens in the UT were not dewormed and served as controls. Additionally, FECs on cloacal contents, worm fecundity and worm burdens were determined at 19, 35 and 45 wpp. None of the flocks became infected until after 16 wpp. The cumulative pooled FECs at the end of the study were significantly ($p < 0.01$) lower in the TT compared to both CT and UT. Although repeated treatment in the TT protocol did not affect the fecundity, a worm density-dependent increase in fecundity was observed. Cloacal FECs and the number of adult *A. galli* in TT at 35 and 45 wpp were significantly lower compared to other flocks. This study provides evidence that the TT strategy is better in terms of lower worm burden and decreased cumulative environmental parasite egg numbers compared to CT strategy. The TT strategy should be considered as an alternative to the CT strategy with regard to *A. galli* control in commercial laying hens.

1. Introduction

The prevalence of the poultry roundworm *Ascaridia galli* has increased within the past decade in laying hens on commercial farms due to the European Union (EU) ban on non-enriched battery cages (Martín-Pacho et al., 2005; Kaufmann and Gauly, 2009; Jansson et al., 2010; Kaufmann et al., 2011; Sherwin et al., 2013). Infection with *A. galli* has been associated with reduced animal health and economically important egg production losses in laying hens (Kilpinen et al., 2005; Gauly et al., 2007). Extensive studies have been carried out to explore different aspects of parasite-host interactions between *A. galli* and hens such as, genetic resistance (Permin and Ranvig, 2001; Gauly et al., 2002; Abdelqader et al., 2007), host immune responses (Martín-Pacho et al., 2005; Marcos-Atxutegi et al., 2009; Schwarz et al., 2011), longevity of the parasite eggs under different environmental conditions (Tarbiat et al., 2015) and the effect on host behavior and health (Kilpinen et al., 2005; Gauly et al., 2007). Studies on sustainable worm control strategies in commercial laying hen flocks are however scarce.

Different management strategies have been suggested to enhance parasite control such as, interruption of the parasite life cycle by using chemical disinfectants between consecutive flocks (Höglund and Jansson, 2011) and good sanitation and improved biosecurity by reducing the contact with the source of infection, and by controlling personnel and equipment movement (Permin and Hansen, 1998; Ruff, 1999). In addition, there have been some reports on the nematodicidal activity of natural plant extracts such as *Allium sativum*, *Acacia oxyphylla* and papaya latex against *A. galli* (Singh and Nagaich, 2000; Lalchhandama et al., 2009; Mursof and Simon, 2012), however, with varying success. Despite these efforts, synthetic anthelmintics still remain the most essential part of the parasite control against *A. galli*.

* Corresponding author at: Department of Biomedical Sciences and Veterinary Public Health, Section for Parasitology, Swedish University of Agricultural Sciences (SLU), P.O. Box 7036, SE-750 07, Uppsala, Sweden.
E-mail address: behdad.tarbiat@slu.se (B. Tarbiat).

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Unlike in ruminants, only benzimidazoles (BZ) are currently available for treatment of nematode infections in laying hens in the EU member states. This highlights the importance of optimal use of this drug class. Among the BZs both flubendazole (FLBZ) and fenbendazole (FBZ) (drugs with the same mode of action) have been approved for use in chickens against *A. galli* (EMA/42178/2014). Even though a recent study on the efficacy of FLBZ showed that it is fully effective against all internal stages of *A. galli* (Tarbiat et al., 2016), there are major issues with how these drugs are being used. First, many farmers in Sweden deworm their laying hens once or twice (Höglund and Jansson, 2011). This is usually performed after 35–40 weeks of age when routine parasitological screening has shown a positive result or expelled worms and/or suboptimal performance or health impairment have been observed irrespective of EPG levels [In this paper we refer to this approach as the conventional treatment strategy (CT)]. Hence, deworming with FLBZ is often initiated when worm loads and EPG levels are already high. Thereby, the farm environment becomes heavily contaminated with parasite eggs. Second, it has been shown that FLBZ has a very short-lived effect (Höglund and Jansson, 2011) and chickens are susceptible to reinfection soon after treatment (Tarbiat et al., 2016). Despite successful elimination of intestinal worms, parasite eggs are likely remain in chicken barns and accumulate over time as reinfection occurs. It has been suggested that these eggs are resistant (Christenson et al., 1942) and are likely to remain viable and become infective all year round under natural conditions and in laying hen barns (Velichkin and Merkulov, 1970; Tarbiat et al., 2015). This makes these eggs the main source of infection for consecutive flocks as previously suggested (Höglund and Jansson, 2011).

The CT strategy for laying hens fails to provide efficient, long-lasting parasite control (Tarbiat et al., 2016). The study presented in this paper is a step towards developing a sustainable and more efficient deworming strategy that addresses the shortcomings of the conventional treatment method. The aim of this study was to compare the efficiency of a targeted treatment (TT) strategy where the flocks were treated more or less immediately when parasite eggs were observed in the faeces, with the conventional treatment strategy. By treating the birds soon after patency of the worms, we hypothesized that the environmental contamination with parasite eggs would decrease which in turn would lead to decreased infection pressure and lowered worm burdens.

2. Materials and methods

2.1. Study farm

The study was carried out between August 2014 and June 2015 in six flocks (flock number F 1–6) on a commercial farm with laying hens with a history of *A. galli* infection. All flocks (each containing ≈7000 hens) were housed on concrete indoors in separate but identical barns with a common service corridor. The barns were equipped with NATURA-Nova aviaries (big Dutchman) and fresh wood shavings were added as litter material before placement. Only soiled litter material was replaced during production. Two different chicken hybrids were used, Hy-Line (F 1–3) and Bovans (F 4–6) and were fed a commercial layer ration (Fenix Topp pk bk, Lantmännen Lantbruk). The barns had not been in use for 9 months prior to this study. Before placement of pullets, litter and faeces were removed and the empty barns were thoroughly cleaned with high pressure hot water, disinfected (Virkon® S and formaldehyde fogging), and dried. The pullets had been raised on another farm and were transferred helminth-free at 12 weeks of age.

2.2. Experimental outline and sampling

The materials sampled and the timing of sampling are summarized in Fig. 1. The flocks were confirmed to be mono-infected with *A. galli* before the beginning of the study by running an in-house species-specific multiplex qPCR targeting both *A. galli* and *Heterakis gallinarum* on faecal samples from each flock (data not shown).

Four faecal samples (pooled samples ≈800 g each) were collected bi-weekly starting at 8 weeks post placement (wpp) on four plastic trays (20 × 27 cm) placed on the manure belts under the slats in each of the six barns. The content of each tray was thoroughly mixed and then 3 g of faeces were analyzed with McMaster with a diagnostic sensitivity of 50 parasite eggs per gram faeces (EPG) (Foreyt, 2001). This was to assess the overall environmental contamination with *A. galli* eggs. When the infection was patent in all six flocks (18 wpp), the three flocks of the respective bird hybrid were randomly allocated among three treatment protocols; conventional treatment (CT), untreated treatment (UT), and targeted treatment (TT). The baseline flock EPGs before allocation were 300 ± 140, 130 ± 85, 237 ± 98 in CT, TT and UT respectively. Hens in the CT were dewormed once at 27 wpp according to the conventional deworming strategy explained above. In the TT, hens were dewormed as soon as the bi-weekly EPG values surpassed the pre-assigned threshold of 200 EPG. Therefore, the hens in the TT were dewormed three times at 22, 27 and 36 wpp. On each deworming occasion, the hens were treated daily for five days with fenbendazole (Panacur AquaSol®, Intervet AB, 1 mg/kg body weight) according to the manufacturer's recommendation. Hens were deprived of water for 2 h before FBZ was administered orally via the drinking water using a medicator. Hens in the UT were not dewormed and served as infection controls.

On three occasions (19, 35 and 45 wpp), irrespective of the deworming status of the flocks, the parasite worm burden was assessed. On each occasion, ten randomly selected hens per flock were euthanized (180 hens in total) by stunning and cervical dislocation according to Swedish regulations. The hens were necropsied and their intestines and cloacal contents were collected. All samples were transported to the laboratory at a temperature of around 5 °C. Handling and euthanasia were approved by the Swedish Ethical Committee for Scientific Experiments (C108/14).

2.3. Parasitological study

The intestines were cut open and the contents of the small intestines were washed with 1 L tap water into glass jars. The jars

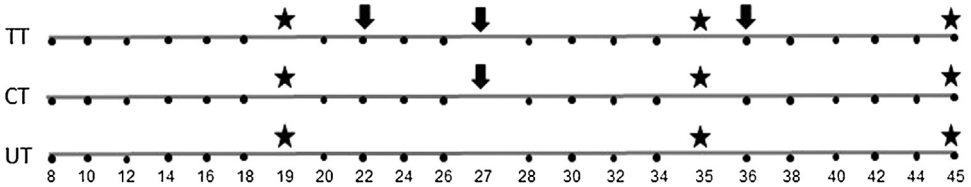

Fig. 1. The materials sampled and the timing of samplings weeks post placement for the three treatment groups (conventional treatment-CT, targeted treatment-TT, untreated treatment-UT). The black circle indicates the time faecal materials were collected from the manure belts. The star indicates the timing of cloacal faeces (10 samples) and intestine (10 samples) collections. The black arrows indicate fenbendazole administration (1 mg/kg body weight for 5 days).