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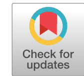
Research paper

Detection of *Ascaridia galli* infection in free-range laying hens



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ABSTRACT

Reliable methods for detection of *A. galli* infection using excreta egg count (EEC) and ELISA assays to determine *A. galli* specific IgY levels in serum and yolk samples were compared from hens infected naturally and artificially. Artificially infected hens were used to generate samples for analysis of preferred detection methods and to generate contaminated ranges for use in the naturally acquired infection study in which Lohmann Brown hens (n = 200) at 16 weeks of age were randomly assigned to four treatments with five replicate pens. Hens of negative control (NC) ranged on a decontaminated area, hens of low infection, medium infection and positive control (PC) ranged on the areas previously contaminated by hens artificially infected with 250, 1000 and 2500 *A. galli* eggs/hen, respectively. Additionally, hens of PC were orally infected with 1000 *A. galli* eggs/hen. Anti *A. galli* antibody levels in hen serum (SIgY) and yolk (YIgY) were measured before range access, and 2, 7 and 12 weeks after access to the contaminated ranges. In a natural infection study, eggs were detected in the excreta of all hens 4 weeks after range access, with the exception of NC in which no eggs were detected. EEC increased to reach maximum value (2204 ± 307 eggs/g) after 11 weeks of range access and then declined at 12 weeks (905 ± 307eggs/g) ($p < 0.01$). While SIgY OD values were not different in hens between any groups before range access, after 2 weeks, both SIgY and YIgY gradually increased in hens of PC (1.17 ± 0.03 and 0.88 ± 0.04) and medium infection (1.07 ± 0.03 and 0.96 ± 0.04) compared to low infection (0.38 ± 0.03 and 0.29 ± 0.04) ($p < 0.01$) and NC. After 12 weeks, SIgY were similar in hens of PC, medium and low groups whereas YIgY was higher in hens of low infection group ($p < 0.01$). Sensitivity of the serum and egg yolk antibody levels assay to detect *A. galli* infection was 100% and 96%, respectively, whereas the pooled EEC method yielded a sensitivity of 93%. The results of this study suggest that hens naturally infected with *A. galli* produce both SIgY and YIgY at different levels depending on the infection intensity and duration of exposure which allows the diagnosis of prior infection or early diagnosis of current infection. Use of the practical and non-invasive method of yolk sample analysis for detecting IgY can be just as informative as using serum samples to detect *A. galli* infection.

1. Introduction

Increased attention to hen welfare has resulted in the number of hens housed in non-caged husbandry systems to increase. Free-range production system are widely accepted as being more welfare friendly for hens allowing them to express natural behaviour and increase their physical activity (Castellini et al., 2006; Shimmura et al., 2010; Gauly et al., 2007). However, hens in free-range system are frequently in contact with the excreta and prone to gastro-intestinal parasitic challenges (Kaufmann et al., 2011). *A. galli* has been reported to be the most abundant gastrointestinal parasite in free range systems with its prevalence ranging from 22 to 92% of parasites observed (Martin-Pacho et al., 2005; Sherwin et al., 2014). In addition, Phiri et al. (2007) confirmed that free-range birds have a high incidence of helminth infections which may compromise their productivity. *A. galli* damages the gastrointestinal tract, impairs digestion and absorption of nutrients, obstructs the lumen of the intestine and contributes to increased flock mortality (Hurwitz et al., 1972; Luna-Olivares et al., 2015; Hinrichsen et al., 2016). Infection with *A. galli* also increases the vulnerability of hens to secondary bacterial infections by impairing their immune capacity to fight disease (Eigaard et al., 2006; Permin et al., 2006; Horning et al., 2003). Production losses have been associated with both changes in natural behaviour and significantly increased prevalence of cannibalism and incidence of *A. galli* infection in free range systems (Permin et al., 2006; Gauly et al., 2007). Therefore, early detection of parasitism is necessary not only to minimise the spread of infection but

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also to reduce the impact of disease on hen health, welfare and productivity.

Various methodologies for detecting *A. galli* infection in laying hens have been investigated including excreta egg counts (EEC) (MAFF, 1986), identification of anti- *A. galli* antibodies (IgY) in hen serum (Norup et al., 2013; Ruhnke et al., 2017; Martin-Pacho et al., 2005), and in hen egg yolk (Marcos-Atxutegi et al., 2009; Rahimian et al., 2017; Daş et al., 2017). Upon ingestion of infective *A. galli* eggs, it takes around 5–8 weeks for shedding of worms eggs in the excreta allowing detection of infection via EEC (Anderson, 2000; Taylor et al., 2007; Höglund and Jansson, 2011).However, following *A. galli* infection, antibodies (IgY) can be detected in serum usually within the first two weeks, or even as early as 10 days, after the initial contact (Schwarz et al., 2011; Dalgaard et al., 2015; Ruhnke et al., 2017). Recent reports support the notion that *A. galli* infection can be detected by measuring antibodies in serum and yolk samples (Daş et al., 2017; Sharma et al., 2018a). This study was conducted to determine the most effective methods for detecting *A. galli* infection in laying hens. The ability of EEC, at both the individual level and with pooled samples (PEEC), and anti- *A. galli* IgY in both serum (SIgY) and yolk (YIgY) to identify birds currently (or previously) infected either, artificially or naturally, with *A. galli*, was investigated by comparing parameter values to intestinal worm counts at necropsy. Data was then used to assess the usefulness of these various measurements for evaluating hen health in free range egg production system in Australia.

2. Materials and methods

2.1. Ethics approval

All experimental procedures were approved by the Animal Ethics Committee of the University of New England, Armidale, Australia (approval No AEC 16-075). Hens were housed in accordance with the Model Code of Practice for the Welfare of Animals, Australia (Primary Standing Committee, 2002).

2.2. Study 1- Artificial infection study

Two hundred Lohmann Brown laying hens at 17 weeks of age were obtained from a commercial pullet rearing facility. The hens were randomly assigned to 20 identically constructed pens, with 10 hens per pen. The indoor area of each pen was composed of 9 m^2 of a slatted floor and 1 m^2 of solid floor which was covered with wood shavings. Housing and rearing details have been described previously (Sharma et al., 2018a,b). Hens were treated with Levamisole (Kilverm, Vetsense, Jimboomba, Queensland, Australia, 4 ml/hen in drinking water) on the day of arrival. Levamisole is the only drug registered to be used to control internal parasites in commercial chickens in Australia. The experiment employed a stratified randomised design with 5 replicate pens of 10 hens for each of the 4 treatments. Treatment 1 served as a negative control group where hens were sham infected with saline. In treatment groups 2–4, hens were orally inoculated with different doses of embryonated *A. galli* eggs with treatment group 2 receiving: a low dose (250 *A. galli* eggs/hen), treatment group 3 a medium dose (1000 *A. galli* eggs/hen), and treatment group 4 a high dose (2500 *A. galli* eggs/hen). To prepare the embryonated *A. galli* eggs for hens in treatment groups 2–4, mature *A. galli* nematodes were collected from the intestine of naturally infected laying hens from a commercial farm. Infective material was prepared as previously described (Sharma et al., 2018a). *A. galli* eggs after harvesting were stored at 26°C for 3 weeks and embryonation confirmed by microscopy prior to infection. Hens were inoculated at 19 weeks of age with respective doses given as 6 applications over the duration of a two week time period using an inoculation method as previously described by Sharma et al. (2017, 2018a,b). For example, in low group a total dose 250 eggs/ hen was given over 6 applications (i.e ~42 eggs per dose). Hens were kept on the ranges for a period of 14 weeks where they contaminated the ranges by shedding the *A. galli* eggs. Contaminated ranges generated in study 1 were used to establish natural infection (study 2) as described below.

2.3. Study 2- Natural infection study

Two hundred 16 week old Lohmann Brown laying hens were obtained from a commercial pullet rearing facility. Hens were treated with Levamisole (4 ml/hen per o.s.) on the day of arrival. Details on housing conditions and preparation of infective oral inoculums used in the current study have been described in Sharma et al. (2018a,b).The experiment employed a stratified randomised design with 5 replicate pens of 10 birds for each of 4 treatments. Treatment group 1 served as a negative control (NC) where hens ranged on a decontaminated area (ranges were covered with bitumen one week prior to the access) and were treated with Levamisole (4 m L/hen) every 3 weeks throughout the experiment, treatment group 2 (low infection) and treatment group 3 (medium infection) birds ranged on the areas previously contaminated by hens artificially infected with 250 and 1000 embryonated *A. galli* eggs respectively (Sharma et al., 2018a,b). Hens of treatment group 4 were orally inoculated at 18 weeks with 1000 embryonated eggs and served as a positive control (PC). These hens ranged on an area previously contaminated by the hens artificially infected with 2500 embryonated *A. galli* eggs as described below.

2.4. Parasitological measurements

2.4.1. Infection intensity - Artificial infection study

Excreta analysis was performed at 8, 11, 15 and 20 weeks post infection. Serum samples were collected at random from 4 hens/pen to detect antibody levels before infection and 6, 11, 15 and 20 weeks post infection. Similarly, yolk samples were also collected at 11 and 20 weeks post infection. Details of samples collection and analysis are described in Sharma et al. (2018a). Hens were sacrificed at the end of the experiment after 14 weeks post infection and both intestinal *A. galli* worms and worms egg shed in excreta of individual birds counted as described in Sharma et al. (2018a). Parasitological data such as EEC, PECC and serum and yolk antibody levels were collected and used for correlation analysis with the data from naturally infected hens.

2.4.2. Infection intensity - Natural infection study

Infection intensity was assessed in the natural infection study by counting the number of *A. galli* eggs in a pooled hen excreta sample collected from each pen at 2, 4, and 6 weeks after access to the ranges, and weekly until 12 weeks post-access to the range. The number of *A. galli* eggs was counted in fresh excreta samples using a modified McMaster flotation method adapted from MAFF (1986) as described in (Sharma et al., 2018a,b). Eggs were counted in the fresh sample collected between 8 to 11 a.m. in the morning.

After 12 weeks of access to the ranges (30 weeks of age), intestinal *A galli* worm counts (n = 200) and EEC (n = 80) were performed on randomly selected individual birds after necropsy. The intestinal adult *A. galli* worms were counted in all the hens by direct observation of the intestinal content following splitting of the intestine longitudinally using blunt scissors. The immature worms were recovered from the intestine by a method adapted from (MAFF, 1986). Briefly, the intestinal content was rinsed through a 1000 μm mesh sized metal sieve and collected on a 250 μm sieve for immature worm counts by microscopy at 40× magnification (Stereo compound microscope Olympus CX31, Tokyo, Japan). Coprodeum content was collected from 4 hens per pen (n = 80) and counted for *A. galli* eggs using McMaster flotation method.

2.5. Determination of A. galli specific antibody in serum and yolk

Blood was collected from wing vein of hens (n = 200) on arrival,

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