# The impacts of *Ascaridia galli* on performance, health, and immune responses of laying hens: new insights into an old problem

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**ABSTRACT** Gastrointestinal nematodes are re-emerging in countries where the popularity of free-range poultry production systems is increasing. Amongst all gastrointestinal nematodes, *Ascaridia galli* is of significant concern due to the parasite's direct life cycle and ability to survive extreme environmental conditions. In laying hens, *A. galli* parasites have been associated with reduced health, welfare, immunity, and egg production. Direct losses are caused by obstruction and damage of the intestinal tract in hens when high worm burdens are present. These result in reduction in egg production and body weight of infected laying hens, consequently leading to significant economic losses for farmers. Furthermore, heavy infections with *A. galli* may lead to increased mortality within the flock. Indirect losses are due to suppression of immune system function which can increase susceptibility to secondary infections. Infection with *A. galli* can also alter nutrient utilization and absorption. Levels of anti- *A. galli* serum and egg yolk antibodies increase following *A. galli* infection. Elevated antibodies can be used as an indicator of current or previous infections and therefore can be used as a diagnostic tool. The impact of *A. galli* on hen health and welfare manifests through the depletion of liver lipid reserves and increased use of energy reserves to mount immune responses against the parasite. This review highlights the variable effects of *A. galli* infection on the performance, health, egg quality, and emphasizes especially on immune responses of free-range laying hens as well as it evaluates various potential detection methods and preventive and control measures of this parasitic disease.

**Key words:** anthelminitics, immunity, parasites, productivity, poultry

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## INTRODUCTION

*Ascaridia galli* is often the most prevalent (22 to 84%) gastrointestinal nematode in laying hen production systems with access to outdoor areas (Kaufmann et al., 2011; Wongrak et al., 2014; Thapa et al., 2015). Changes in consumer demands and banning of conventional cages by European regulatory authorities to improve the welfare of laying hens can be considered as major reasons for the re-emergence of nematode infections in Europe (European Commission, 1999; Wongrak et al., 2014). In the EU, there are almost 400 million laying hens, among which 15% are kept in free ranges and 5% in organic holdings (EC, 2019). This increase in free-range egg production can be observed worldwide. For example, in the UK, free-range eggs account for 48% of the total egg production, and in the USA, 12.5% eggs are from free-range production (Department for Environment, Food and Rural Affairs, 2017; USDA, 2017). In Australia, free-range egg production is rapidly increasing and in 2017 grew by 10.2% with an estimated grocery market value share of 52% (Australian Eggs, 2017). Additionally, in Australia, egg consumption has increased from 183 eggs per person in 2007–08 to 231 in 2016–17 (Australian Eggs, 2017). This increased consumption of eggs is, at least in part, associated with an increased awareness of the health benefits of egg consumption and improved willingness of the consumer to support the industry based on increased animal welfare standards. As a consequence, the trend of increasing barn and free-range egg production is expected to be ongoing. Furthermore, commercial flocks are usually segregated in groups of >3,000 hens, allowing individual birds to interact in depth with each other and the environment. However, the adoption of outdoor housing systems can reduce protection against biosecurity and safety hazards including an increased exposure to parasites and predators. This is mainly due to increased contact with excreta and wild birds in the outdoor environment, increasing exposure to pathogens. The most prevalent nematodes reported in free-range systems are *Ascaridia galli, Heterakis gallinarum*, and *Capillaria spp.* In conventional free-range laying hen systems, anthelmintic drugs


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can be used without mandatory egg withholding periods where approved. This is unlike organic productions systems in which the use of anthelmintics as prophylactic treatments are prohibited, can only be used when prescribed by a vet, and the product from the birds cannot be sold as organic for 60 days after administration (ACOS, 2019). Therefore with the increase number of hens in these production systems, the prevalence of helminths in poultry production systems is expected to increase.

There is widespread anthelmintic drug resistance in many parts of the world for some parasites of livestock (Craig, 1993; Sangster, 1999). In EU member states, benzimidazole drugs (flubendazoles and fenbendazole) have been commonly used for treatment of *A. galli* (EMA/42178/2014). However, a recent study indicated a lack of information regarding resistance to benzimidazoles in *A. galli*, indicating the need to optimize the tools for detecting and monitoring anthelmintic resistance in parasites of poultry (Tarbiat et al., 2017). In Australia, only 2 registered anthelmintic products (piperazine and levamisole) have been used for decades in commercial poultry. The widespread use of these products, with limited rotation with other anthelmintics, is expected to have increased the likelihood of development of drug resistance by parasites (Sutherland and Leathwick, 2011). Therefore, development of methods for early infection detection are required to monitor the efficacy of anthelmintics in specific production settings. This review will focus on the immune responses induced in the host during *A. galli* infection and the effects of infection on the production performance, energy reserves, and egg quality of free-range laying hens. Practical and reliable methods for detection of *A. galli* infection using serum and yolk antibodies along with prevention and control measures will also be discussed.

## *Ascaridia galli* Infection—Effect on Performance

The life cycle of *A. galli* is direct and involves a single host. Eggs need to be embryonated in the litter or soil to become infective. Hosts become infected by ingesting the embryonated eggs containing the infective larvae at either stage 2 (L2) or stage 3 (L3) of their development (Herd and Mc Naught, 1975). There are few epidemiological studies carried out to investigate the infection and transmission cycle of *A. galli*. Generally, it is accepted that host infection can be influenced by many factors such as age, sex, diet, and genetics of the host, as well as the age and dose of the infective eggs (Permin and Hansen, 1998; Das et al., 2010).

*A. galli* infection in chickens are accompanied by various clinical signs including loss of appetite and body weight, ruffled feathers, drooped wings, retarded muscular and osteological development, altered hormone levels, anorexia, depression, and increased mortality (Ackert and Herrick, 1928; Dahl et al., 2002). When hens were repeatedly infected with a dose of 250 *A. galli* eggs at the age of 6, 12, 18, and 24 wk, no clinical signs or symptoms of infection were observed during the period of 25 wk post-inoculation. Even following repeated inoculation with high numbers of embryonated eggs (250, 500, and 1,000 embryonated *A. galli* eggs, respectively), no clinical signs were observed for 24 wk post-inoculation (Sharma et al., 2018a). However, co-infected hens with *A. galli* and *P. multocida* showed clinical signs such as depression, anorexia, ruffled feathers, and mortality (Dahl et al., 2002). Infections with *A. galli,* in the absence of other gastrointestinal nematodes are rare in free-range farms. A prevalence study conducted on 19 free-range farms detected *Heterakis* eggs in 17 farms, *Ascaridia* eggs in 16 farms, *Trichostrongylus* eggs in 9 farms and *Syngamus* eggs in 6 farms. Similarly, another study reported *A. galli* (69.5%) to be highly prevalent helminth followed by *Heterakis spp* (29.0%) and *Raillietina spp* (39.6%) in the organic laying hens flocks (Thapa et al., 2015). This highlights the need to investigate the impacts of different levels of *A. galli* infection under commercial conditions, where chances of co-infections are increased, to more accurately assess potential impacts of *A. galli* infection on productivity. Results also suggest the impact of *A. galli* infection can be influenced by various aspects of hen husbandry (Sharma et al., 2018a, c). Other factors influencing the potential impacts of *A. galli* infection include the condition of birds when infected. Higher infection intensity and worm burdens have been observed in lighter as compared to heavier birds, possibly through different mechanisms acting on allocation of available nutrients towards immune system function versus production (Daş and Gauly, 2014). Also, more stressed/fearful hens are known to have higher parasite excreta egg counts (**EEC**) suggesting that stress can impact on immune system function influencing infection intensity (Sherwin et al., 2013). Naturally infected hens were found to have higher intestinal worm burden compared to artificially infected hens (Sharma et al., 2018b). Lower worm burden observed in the artificially infected hens might be due to increased worm expulsion after initial experimental inoculation (Stehr et al., 2018). Whereas re-infection of hens from the infected ranges might have contributed to higher worm burden in natural infection studies (Sharma et al., 2018b). Therefore, it is evident that allowing bird's access to ranges, previously housing infected birds, can lead to high intensity infections, an important consideration when implementing control strategies on farm.

Different studies have highlighted the variable impacts of *A. galli* infection on various performance parameters. Performance in laying hens can be assessed by measuring feed intake, body weight gain, egg production, FCR, mortality in the flocks and health and behavioral status. Gauly et al. (2007) observed increased feed intake in infected hens compared to control hens, an effect ameliorated by anthelmintic treatment. In

another study, no effects on feed intake was observed at 20, 25, 35, and 40 wk of age when hens were infected with different levels (250, 1,000, and 2,500 *A. galli* eggs/hens) (Sharma et al., 2018a). It is difficult to compare these 2 studies as different methods (pecking time vs. residual feed measurement), and different study designs (longitudinal vs. control group) were used. More research is necessary to establish the relationship between appetite, feed intake, and *A. galli* infection.

*A. galli* infection can also reduce the ability of infected birds to absorb and utilize nutrients, subsequently suppressing growth rates (Daş et al., 2010). Body weight gain when measured every week from 0 to 10 wk post-infection was found to be depressed in *A. galli* infected hens (Malviya et al., 1988). Whereas hens treated with anthelmintics were found to have higher weight gain by 39% after 6 wk of treatment in commercial farms compared to non-treated controls (Skallerup et al., 2005). Heavier hens were more resistant to infection compared to lighter hens. Lighter hens had higher worm burden and infection intensity (excreta egg counts; Daş and Gauly, 2014). Once infected with *A. galli,* infection status did not affect the body weight gain of birds when assessed at 20, 25, 35, and 40 wk of age (Sharma et al., 2018a).

Equivocal results about the impact of gastrointestinal parasite on laying hen egg production have been reported: while egg production was decreased from 84 to 60% after 6 wk of concurrent infection of *A. galli* with *P. multocida.* (Dahl et al., 2002), others have demonstrated no significant difference in egg production between non-infected hens and hens with a high prevalence of *A. galli* infection. (Gauly et al., 2007; Sherwin et al., 2013).

*A. galli* infections can impact on the welfare, behavior and social status of laying hens. Infections with *A. galli* were found to induce behavioral changes and increase the likelihood of severe feather pecking and cannibalism, thereby compromising the welfare of birds (Gauly et al., 2007).

Locomotion activity in Lohman Brown hens was decreased during pre-patent and patent periods associated with *A. galli* infection, and the hens increased activity once more after anthelmintic treatment. The proportion of ground pecking decreased during both the prepatent and patent periods compared to the control birds. After anthelmintic treatment, ground pecking activity was comparable to the control group. *A. galli* infected hens spent more time in the nests during the prepatent and patent periods compared to the controls. After anthelmintic treatment, this behavior was decreased but was still significantly above the level of control group (Gauly et al., 2007).

Helminth infection can also increase mean increased mortality rates. For example in a field study, a mean mortality rate of 1.7% was observed in organic layers at the age of 30 to 37 wk due to helminth infection. Further, on farms with a high prevalence of *A. galli* and *Heterakis* infection, mortality rates during the summer season were higher than on farms with low infection levels, whereas no difference was observed during winter (Hinrichsen et al., 2017). Currently, limited specific field studies have been conducted to demonstrate the effects of helminth infection on mortality rates in laying hens.

## Effect of A. galli Infection on Liver Lipid Reserves

Fat is the most economic energy reserve in animals and 3 organs have major involvement in fatty acid metabolism: the adipose tissue, the skeletal muscle, and the liver (Frayn et al., 2006). Mature hens increase their blood lipid concentration at the beginning of lay, which is suspected to be triggered by an increase in ovarian activity. Lorenz et al. (1938) observed more than double the fatty acid content in the liver of laying female white leghorn hens compared to non-laying hens. Hens began to increase fat deposition by the onset of maturity (Lorenz et al., 1938). Another study performed on the Lesser Scaup, a North American diving duck, showed that lipid reserves of females declined on average, 0.5 g for every 1 g of lipid deposited in yolk (Afton and Ankney, 1991). It has also been reported that *A. galli* infection reduces dietary metabolizable energy in chickens which might be due to reduced digestibility by the presence of parasites (Walker and Farrel, 1976). Similarly, *Ascaris* infection in humans disrupts liver lipid metabolism possibly due to a break down in liver function and subsequent changes in hormone secretion (Bansal et al., 2005). These studies suggest that *A. galli* infection may affect stored energy reserves such as liver lipids in laying hens. We recently demonstrated that hens with high *A. galli* burden had consistently lower lipid reserves compared to uninfected hens (Sharma et al., 2018b), suggesting energy reserves, stored in the form of liver lipids, are utilized by infected hens to maintain production in the face of infection. The effect of liver lipid depletion on hen health and production for the duration of an entire laying cycle needs further investigation.

## Ascaridia galli Infection—Effect on Hen Egg Quality

Maintaining the quality of hen eggs is important for sustainable farming because market access depends on demonstrable and constant quality assurance. The quality of eggs can be evaluated by assessing various internal and external parameters. External egg quality parameters include: egg weight, shell reflectivity, shell thickness, shell weight, shell percentage, shell-breaking strength, shell translucency, and cuticle cover estimation. Internal egg quality parameters include albumen height, haugh unit, and yolk color. Factors known to influence egg quality in laying hens include: hen

breed, strain, age, nutrition, stress, disease, production systems, transportation, and storage (Roberts, 2004). Maintaining egg quality is especially important when hens are raised in production systems where exposure to parasites can occur. Rarely, Ascarids may migrate to the oviduct and become enshelled in hen eggs. Cases of commercial hen eggs containing *A. galli* larvae in the albumen have been reported (Reid et al., 1973; Fioretti et al., 2005). Although these reports provide evidence that *A. galli* infection can impact on internal egg quality, there is limited information describing the prevalence of this problem and the risk factors involved. In a recent study, neither artificial nor natural infection with *A. galli* was found to influence external and internal egg quality, irrespective of infection intensity (Sharma et al., 2018a, b). Further studies to investigate the impact of *A. galli* on egg quality over the whole production cycle are recommended.

## IMMUNE RESPONSES AGAINST *A. galli*

Host responses to parasitic infections are complex and involve many aspects of the humoral and cellular immune systems. When developing control strategies, it is important to understand the immune response induced in the host by these parasites. Failure to control nematode infections can compromise hen health by increasing susceptibility to other diseases in highly infected birds. (Horning et al., 2003). Helminths such as *A. galli* induce both cellular and humoral immune responses in their hosts (Degen et al., 2005; Marcos-Atxutegi et al., 2009; Schwartz et al., 2011). Elevation of serum antibodies, IgY and infiltration of both $CD4^+$ and $CD8^+$ positive T-cells at the site of infection have been observed during *A. galli* infection (Schwarz et al., 2011; Norup et al., 2013).

The prevalence of parasitic infections in commercial layer flocks has received little attention despite the economic, health, welfare, and behavioral consequences. In avian species, following nematode infection, polarization of immune response towards a helper T- cells type 2 (Th2) generally occur (Degen et al., 2005). Indeed, *A. galli* infections have been shown to stimulate classical Th2 immune responses in the host (Schwarz et al., 2011; Balqis et al., 2013). The cytokine environment established during parasitic infection may alter outcomes for birds suffering from viral infections and fungal toxins (Dänicke et al., 2013; Pleidrup et al., 2014). Recently, it has been reported that an increase in intraepithelial $CD8^+$ cytotoxic and $CD4^+$ T helper populations in blood occurs following experimental infections with *A. galli* (Ruhnke et al., 2017). Further investigation is required to understand the biological implications of these changes in cell populations and to characterize the Th1 and Th2 immune responses induced by *A. galli,* to better understand the regulatory mechanisms associated with this specific helminth infection in hens.

### *Immunoglobulin (Ig) Y*

Immunoglobulins are secreted by plasma cells in response to antigen exposure. These antibodies are major effector products of humoral immunity (Tizard, 2002). IgY is the major antibody isotype present in the blood of oviparous animals such as birds, reptiles, and lungfish (Warr et al., 1995). Among 3 main immunoglobulins in birds, IgY is found in higher concentration (5 to 15 mg/mL) than IgA (13 mg/mL) and IgM (0.3 to 0.5 mg/mL) in serum (Rose et al., 1974; Kowalczyk et al., 1985). IgY is the predominant immunoglobulin isotype found in egg yolk whereas IgM and IgA are present in egg white due to mucosal secretion from the oviduct (Rose et al., 1974). IgY has a higher molecular weight of 180 KDa than mammalian IgG (160 KDa) (Warr et al., 1995). Similar to mammalian IgG, IgY is composed of 2 heavy and 2 light polypeptide chains and 2 antigen binding sites (Warr et al., 1995). Interestingly, IgY is capable of mediating anaphylactic reactions, a function attributed to mammalian IgE (Carlander, 2002). The Fc region (hinge region with heavy chain) of IgY mediates effector functions such as complement fixation, opsonization, and anaphylactic reactions. Whereas, the Fab (light chain) regions contain the antigen binding sites (Schat et al., 2013). Until the immune system of the chick matures, absorbed IgY from egg yolk provides effective humoral immunity against pathogens, protecting the chick from disease (Schade et al., 2005).

Experimental infection of hens with *A. galli*, has been shown to induce specific antibody responses in broilers as well as in layers (Marcos-Atxutegi et al., 2009; Ruhnke et al., 2017; Sharma et al., 2017, 2018a, b, c). In these studies, serum and yolk IgY have been found to increase with age, possibly due to (1) increased immune responsiveness of the young bird due to maturation of the immune system with age, (2) the natural profile of antibody responses to the parasite, (3) age-related changes in the efficiency and selectivity of antibody transfer from serum to yolk, or (4) a combination of above. However, the effectiveness of anti-*A. galli* antibodies to protect hens against parasitic infection is questionable as correlations between antibody responses and infection levels in hens are not generally observed (Norup et al., 2013). Similar observations have been reported in hens which were naturally infected with *A. galli* where elevated antibody levels in hens were not correlated with parasite burden, as measured by worm's egg count (Sharma et al., 2018a, c). Recently, it has been shown that it is the larvae stages of *A. galli* which induce strong serum and yolk antibody responses in the host, rather than mature worms (Daş et al., 2018). Moreover, plasma antibody levels were found to initially correlate with infection dose but then reflected reinfection levels thereafter (Daş et al., 2018). To improve our understanding of antibody profiles in response to *A. galli* infection, further studies are recommended to quantify both tissue and lumen worm

burdens through regular necropsies in *A. galli* infected hens accompanied by measurements of serum and yolk antibody levels at pre-defined time points.

## DETECTION AND MONITORING OF *A. galli* INFECTION

To improve health of hens, the ability to detect parasite infection and mitigate the spread of disease on-farm is crucial. For this to occur, it is necessary to develop diagnostic methods; ideally methods which enable early detection of infection. Early detection will maximize the chances of successful intervention, decreasing production losses and range contamination for subsequent production cycles.

### *Excreta Egg Counts and Intestinal Worm Counts*

Currently, the diagnosis of *A. galli* infection is predominantly based on EEC or via direct worm identification in the intestine during bird necropsy (Permin and Hansen, 1998). Excreta egg count is an easy and commonly practiced method for diagnosing *A. galli* infection in laying hens. The number of nematode eggs in the excreta is regarded as a reasonable indicator of adult worm burden. Equivocal results on the reliability of this method are reported by various researchers. For example, a strong correlation between worm burden and EECs in birds artificially infected with *A. galli* has been reported (Permin and Ranvig, 2001; Thapa et al., 2015; Sharma et al., 2018a). In addition, other researchers have reported a significant positive correlation ($r = 0.42$) between *A. galli* worm burden and EEC in experimentally infected chickens whereas correlation between worm burden and EEC was not significant for *H. gallinarum* (Daş et al., 2017). In that report, hens with increased worm burden (high number of female worms) were found to have decreased eggs shed in excreta for *H. gallinarum*. This was hypothesized to be due to a crowding effect which might have lowered the fecundity of individual female worms (Daş et al., 2017). Therefore, EECs may not provide an accurate indication of infection intensity information. Moreover, there are other logistical limitations of this diagnostic technique. For example, it takes more than 5 wk from the time of infection for parasite eggs to appear in the excreta (Permin et al., 1998). Furthermore, EEC is not only influenced by host factors such as host age, host sex, host immunity, and consistency of excreta but also by parasite related factors such as number of adult *A. galli* present in the intestine, *A. galli* age and fecundity, and stage of infection (Permin and Hansen, 1998; Wongrak et al., 2014). Environmental factors, such as season, can also influence EEC along with factors associated with storage of samples prior to analysis including storage temperature, times of excreta collection, storage temperature, and storage time of excreta (Nielsen et al., 2010; Kaufmann et al., 2011).

### *Detection of A. galli Antibody in Serum and Egg Yolk*

An ELISA (enzyme linked immunosorbent assay) test can be used to determine levels of antigen-specific antibodies in biological samples. ELISA tests to detect *A. galli*-specific serum IgY levels in hens, have been developed as an indirect method of detecting infection (Marcos-Atxutegi et al., 2009). Collection of egg yolk is a non-invasive and practical method to obtain samples for ELISA testing, in comparison to serum samples which requires blood to be collected from hens (Daş et al., 2017; Sharma et al., 2018c). *A. galli* can co-exist with other helminth species infecting the bird, so cross-reactivity to common parasite antigens is an important consideration when interpreting serological data. The use of an ELISA for detection of disease has several limitations: ELISA assays based on antibody response detect only the magnitude of the host's response rather than the intensity of the infection. Furthermore, antibodies can stay elevated for months post-infection, meaning that antibodies will be detectable long after the infection has been cleared. Recently, it was reported that a coproantigen ELISA (faecal ELISA) method had higher sensitivity compared to faecal egg count method for diagnosing *Fasciola hepatica* infection in red deer (French et al., 2016). Similarly, coproantigen had higher diagnostic sensitivity (93%) compared to serum ELISA (88%) for diagnosing *Fasciola hepatica* in goats (Villa-Mancera et al., 2016). Such findings suggest that the use of a coproantigen ELISA, to detect *A. galli* antigen in the excreta of hens, may provide an improved method of detecting disease and warrants further investigation.

### *Indirect Methods of Evaluating Infection Status*

The use of inflammatory markers such as acute phase proteins or the levels of intraepithelial lymphocytes, can be used as indicators of hen health, however, changes in these parameters are not disease specific and would only be indicative of general health. The acute phase protein response is an early and non-specific systemic reaction of the innate immune system to the local or systemic disturbances caused by trauma, infection, stress, inflammation, etc. (Eckersall and Bell, 2010). Changes in acute phase protein profiles can be used to identify physiological responses to a challenge in the host before the appearance of visible clinical signs (Hong et al., 2006). In ruminants, the level of acute phase proteins (serum amyloid A, haptoglobin, $\alpha$ acid glycoprotein, and lipopolysaccharide binding protein) can be used to generally evaluate the health status of the herd (Gånheim et al., 2007). Using these markers as an early

indication of disease could potentially be useful to monitor the disease status of commercial hens, however, the potential contribution of stress and other environmental challenges to changes in acute phase protein levels requires consideration. On this basis, acute phase proteins (such as serum amyloid A, transferrin and C-reactive proteins, and $\alpha$-1-glycoprotein) and blood hemoglobin levels may be useful as an early indicator of parasitism (Stadnyk et al., 1990). Acute phase proteins can be measured in plasma and are secreted from the liver in response to infection and inflammation. Concentrations of serum amyloid A have been found to increase in the plasma of hens infected with viral diseases, such as infectious bursal disease and infectious bronchitis (Nazifi et al., 2011a, b). The response of serum amyloid A towards parasite infections has not been studied. The acute phase protein, $\alpha$-1-glycoprotein, when measured in laying hens kept in different housing systems at 4 wk and 4 mo post-arrival, was found to be significantly higher in hens housed in conventional cages in comparison to free-ranges (Salamano et al., 2010). These researchers hypothesized that differences were likely due to increased stress imposed by housing hens in conventional cages. In contrast, when investigating acute phase protein responses in *A. galli* infected hens, $\alpha$-1-glycoprotein levels were not significantly different in *A. galli* infected and non-infected broilers, even though in the same experiment, levels were higher in hens challenged with *Clostridium perfringens* (Ruhnke et al., 2017). Further experiments are required to assess the potential benefits of measuring acute phase proteins responses in infected hens as an indicator of disease.

## PREVENTION AND CONTROL

### *Pasture Rotation, Disinfectants, and Nutritional Management*

In ruminants, pasture rotation is considered as a non-chemical method that can be adopted to reduce environmental contamination with free-living stages of parasites (Thamsborg et al., 2010). In laying hen flocks, pasture rotation did not reduce Ascarid infections significantly (Maurer et al., 2013; Thapa et al., 2015). Recently, it has been reported that about 2 to 3% of *A. galli* eggs remained viable and infective for up to 2 yr in the pasture (Thapa et al., 2017). This indicates that used pastures or areas can harbor a large number of residual eggs from the previous flock thus reducing the effectiveness of pasture rotation management in *A. galli* (Heckendorn et al., 2009).

Disinfecting the empty barns with adequate active components before hen's placement can help in preventing the transmission of *A. galli* infections to some extent. Use of chlorocresol disinfection solution of 1 to 2% on the shed was found to be ovicidal, and delayed the onset of parasite eggs expulsion by 10 wk in a commercial farm (Höglund and Jasson, 2011). It also reduced the worm burden and EECs. However, incomplete disruption of the life-cycle observed in the study might be due to continued exposure to the infection on the range. Methods of applying the chemicals such as chlorocresol in the field need to be developed (Tarbiat et al., 2015).

The nutritional status of the host can influence interactions between the host and parasites as well as the interaction between various parasites sharing the same host. Dietary fibers which contain non-starch polysaccharides (**NSP**) impact digesta viscosity (Daenicke et al. 1999). Reports have shown that diets high in insoluble NSPs can reduce aggressive pecking behavior in hens by increasing feed intake time (Van Krimpen et al., 2008). It has also been reported that successful establishment of the caecal nematode, *H. gallinarum,* in the host can be influenced by fermentable NSP supplementation of diets (Daş et al. 2011a). Additionally, highly fermentable NSP diets can also alter the interactions between *H. gallinarum* and *Histomonas meleagridis* (Daş et al. 2011b). However, care must be taken to feed an adequate percentage of NSPs, as hens fed with insoluble NSPs were subsequently shown to consume more feed per unit body weight gain and had retarded body weight development compared to control birds (Daş et al. 2012). The same study suggested that NSP supplemented diets altered gastrointestinal environment favoring establishment of nematode infection therefore had no potential to control *A. galli* infection but (Daş et al. 2012). This information is relevant mostly in organic poultry where medical treatment options are limited (ACOS, 2019).

### *Selective Breeding for Disease Resistance*

Lohmann Brown laying hens are considered more resistant to parasites than other breeds (Gauly et al., 2007; Permin and Ranvig, 2001). A study conducted in 2 brown genotypes (Lohmann Brown plus and Lohmann Brown classic) showed the heritability for worm burden to be 0.55 and 0.56, respectively (Wongrak et al., 2015). This indicates the possibility of selecting hens based on superior genetics for parasite resistance. Further research could investigate the genetic background of resistance mechanisms at the molecular level (Kaufmann et al., 2011). Similarly, another study was conducted to compare resistance to *A. galli* infection in Lohmann Brown and Danish Landrace laying hens (Permin and Ranvig, 2001). In this experiment, significantly lower worm burden and nematode eggs shed were observed in Lohmann Brown hens, indicating that breeding for resistance to *A. galli* is possible (Permin and Ranvig, 2001). A self-cure mechanism was observed in both of these breeds when infected at a week old. These hens could expel infections when they were challenged secondarily (Permin and Ranvig, 2001).

### Biosecurity

Adherence to strict biosecurity measures can be helpful in preventing transmission of parasitic infections. Infective eggs which are present in the soil/pasture can be easily transmitted between farms or between areas within farms because these eggs can adhere to people, machinery, or other materials. There are some husbandry-based control measures that can reduce the level of parasitic infections such as dead bird disposal, preventing rodents and wild bird entrance, supplying clean feed and water, and restrictions of entry for personnel and vehicles (National Farm Biosecurity Manual for Poultry, 2010).

### Anthelmintics and Immunization

In Australia, chemicals, anthelmintics, and pesticides are registered for the control of internal or external parasites in avian species. Levamisole and piperazine are currently the registered drugs for treatment of nematode infections in commercial layers in Australia with a nil withholding period for eggs. Commonly used anthelmintics from the benzimidazole group in Australia include mebendazole and fenbendazole which are mostly applied during rearing as they have an egg withholding period. There is widespread anthelmintic drug resistance in many parts of the world for some parasites of livestock (Craig, 1993; Sangster, 1999). No data for drug resistance in *A. galli* populations is available. The fact that this registered product has been commonly used for decades may indicate that selection for resistance has already occurred (Sutherland and Leathwick, 2011). Therefore, it is important that methods for early infection detection are available to monitor the efficacy of anthelmintics in the production setting. The reliance on one active compound, levamisole to control *A. galli* in laying hens is a concern. Should resistance arise, control will become very difficult, so the registration of additional products or chemicals is desirable for the future of the free-range egg industry.

Benzimidazole (flubendazoles and fenbendazole) is commonly used in Europe by applying in drinking water to control nematode parasites in egg-laying birds. It was found effective in killing adult worms and also interrupted the worm's egg output temporarily. Some of the parasites may survive benzimidazole treatment and can later mature and reappear to cause damage (Höglund and Jasson, 2011). However, no evidence of resistance of benzimidazole in *A. galli* infections has yet been documented (Tarbiat et al., 2017).

Vaccine development for nematode parasites like *A. galli* can be challenging due to the presence of the parasite using several life stages within the host and secondly due to their location in tissues with less accessibility to immune effector cells such as the intestinal lumen and mucosa. It has been reported that there is no protective maternal immunity against *A. galli* parasite (Rahimian et al., 2017). Chickens immunized with a crude extract of *A. galli* following an oral or intramuscular route of immunization developed a humoral and cell-mediated immune response. However, to date the immune response initiated using *A. galli* vaccines failed to protect against *A. galli* infection (Andersen et al., 2013). *A. galli* infection at the time of vaccination influenced the development of both humoral and cell-mediated immune responses induced by a live attenuated commercial Newcastle Disease Virus vaccine (Pleidrup et al., 2014). In contrast, another field study showed no effects of helminth infection on the vaccine induced immune response in chickens (Saasa et al., 2014). These findings highlight the critical need for more studies to confirm the influence of gastrointestinal nematode infection on the magnitude and type of responses achieved through vaccination.

### Anthelmintic Potential of Different Medicinal Plants Against A. galli in Poultry

Various in vivo studies examined the effectiveness of extracts from plants (*Allium sativum*, *Cassia occidentalis*, *Piper betle*, *Tribulus terrestris*, *Morinda citrifolia*, *Psorelia corylifolia*, *Anacardium occidentale*, *Pilostigma thonningi*, and *Ocimum gratissimum)* against *A. galli* infection (Chakraborty et al., 1979). *Tribulus terrestris* (Zygophylaceae) contains various biologically active ingredients like steroids, alkaloids, flavonoids, saponins, tannins, and unsaturated fatty acids (Adaikan et al., 2000), and evaluation of an alcohol extract obtained from this plant demonstrated anti-ascarid activity in vitro (Chakraborty et al., 1979). Aqueous extract of *Basa latifolia* was more effective against adult *A. galli* compared to alcohol extracts. Investigations of the use of these botanical extracts could be beneficial to complement the use of conventional synthetic drugs (Chakraborty et al., 1979).

## CONCLUSIONS

*A. galli* is a common roundworm of relevance for laying hens worldwide. The phasing out of conventional cage systems and adoption of new alternative outdoor systems has resulted in the re-emergence of parasite infections in commercial egg-laying hens. The impact of *A. galli* on hen health and welfare manifests through the depletion of liver lipid reserves and increased use of energy reserves to mount immune responses against the parasite. The effects of this parasite on the production, health, and welfare of hosts appear to be well-documented, however many studies have been conducted under non-commercial conditions, and others have not controlled for potential confounding effects. Moreover, the effect of *A. galli* infection on production has not yet been fully evaluated with conflicting results reported from various studies. The detection of current or previous *A. galli* infection in hens can be achieved by measuring antibody levels in egg yolk samples. Moreover, yolk samples are as informative as serum

samples when detecting disease and collection of yolk samples is easier and more welfare friendly for birds. Following strict biosecurity measures and good farm management can help in preventing spread of the parasitic infections.

## FUTURE PERSPECTIVES

Further research is required to investigate the impact of *A. galli* infection on hens across the entire laying cycle. The impact of infection on the full production cycle and the critical threshold at which infection impacts on production levels and/or egg quality still needs to be determined.

Further information regarding *A. galli*-specific antigenic proteins suitable for use in ELISA methods will improve the specificity of disease detection using antibody levels in serum and egg yolk. Results suggest that yolk samples can be used for detecting yolk antibody levels. This method is non-invasive and reliable although it can be applicable only after hens start laying eggs. Although ELISA assays can identify a current or previous exposure to *A. galli* it cannot be used for quantitative information regarding the intensity of infection.

Concurrent nematode infections might influence vaccine-induced immune responses in chickens but further research is required to understand such effects and provide guidelines on optimal gastrointestinal nematode infection status of birds at the time of vaccination with specific vaccines. It would be wise to ensure more compounds are registered for control of parasites in hens, in case resistance to the drugs being used now emerges. Selective breeding of hens for disease resistance and evaluating anthelmintic potential of medicinal plants might be of benefit to the poultry industry in the future.

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