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Short Communication

Observations on the occurrence of *Heterakis gallinarum* in laying hens kept on soil

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ABSTRACT

The occurrence of *Heterakis gallinarum* infection in a flock of Rhode Island Red laying hens is described. These hens were entirely kept in houses on a farm for commercial egg production, where a deep litter production system was adopted. Faecal samples from 120 hens selected at random were examined by common flotation technique and modified McMaster's technique. *H. gallinarum* eggs were detected in 50% of the examined samples with very low faecal egg counts (<50 eggs per gram of faeces). There was no evidence of clinical signs, gross pathological lesions, and consequences on production level linkable to heterakiasis. *H. gallinarum* is transmitted by direct ingestion of infective eggs from the soil and is one of the most important intestinal helminths of poultry due to the role it plays as vector of histomoniasis. In accordance with European legislation on the welfare of laying hens, a progressively increasing number of farmers can adopt breeding programs on soil. Periodic coprological examinations of chickens reared on commercial farms from areas throughout Italy are thus advisable to determine the exact distribution of *H. gallinarum* and the extent to which heterakiasis may influence health status and production of chickens in this country.

Key words: Heterakis gallinarum, Prevalence, Laying hens, Welfare.

RIASSUNTO

OSSERVAZIONI SULLA PRESENZA DI *HETERAKIS GALLINARUM* IN GALLINE OVAIOLE ALLEVATE A TERRA

Viene descritta la presenza dell'infestazione da Heterakis gallinarum *in un gruppo di galline ovaiole di razza Rhode Island Red. Le galline erano tenute costantemente all'interno dei capannoni di un'azienda per la produzione commerciale di uova, nella quale era stato adottato un sistema di allevamento su lettiera spessa. I campioni di 120 galline selezionate a caso sono stati esaminati con la comune tecnica per flottazione e con il metodo modificato di McMaster. Uova di* H. gallinarum *sono state individuate nel 50% dei campioni esaminati con conteggi di uova sempre molto bassi (<50 uova per grammo di feci). Non risultavano essere evidenti segni clinici, lesioni patologiche macroscopiche e conseguenze sul livello di produzione riferibili*

alla parassitosi. H. gallinarum *viene trasmesso per ingestione diretta di uova infestanti dal suolo ed è uno dei più importanti elminti intestinali del pollame per il suo ruolo di vettore di istomoniasi. In seguito alla legislazione europea sul benessere delle galline ovaiole, un numero crescente di allevatori può adottare sistemi di allevamento a terra. Periodici esami coprologici sono quindi auspicabili in tutto il pollame allevato nelle aziende commerciali del nostro paese, per determinare l'esatta distribuzione di* H. gallinarum *e in che misura la heterakiasi può influenzarne lo stato sanitario e il livello di produzione.*

Parole chiave: Heterakis gallinarum*, Prevalenza, Galline ovaiole, Benessere.*

Introduction

The nematode *Heterakis gallinarum* is a common caecal pinworm (1-2 cm in length) of birds found in many gallinaceous species in the world. Eggs are shed in faeces and heterakiasis occurs when infective eggs are ingested by definitive hosts. Earthworms may concentrate and transport eggs. Larvae may hatch in earthworms remaining infective for at least one year. *H. gallinarum* is not regarded as a serious threat for chickens, but it is one of the most important nematodes of poultry due to its role in the epidemiology of the flagellate *Histomonas meleagridis*, which in turn infects many gallinaceous species (mostly turkeys), causing necrosis of the caecal mucosa, swelling of the caecum, and liver necrosis. The disease is called blackhead or histomoniasis. *H. meleagridis* is ingested by *H. gallinarum* within the intestinal tract of birds and is released outside within the nematode eggs. *H. gallinarum* females produce 34,000 to 86,000 eggs in a lifetime (Fine, 1975), depending on chicken breeds (Chute *et al.*, 1976). *H. meleagridis* can remain viable in *H. gallinarum* eggs for as long as these remain viable. Hatching of *H. gallinarum* eggs within a new host releases the protozoan. *H. gallinarum* eggs can survive and remain infective for years in the soil. Due to longevity of the eggs and ability of the earthworms as paratenic hosts, *Heterakis* is difficult to eliminate from a flock. Clinical signs like thyplitis and diarrhoea associated with heterakiasis are rarely seen.

Reported prevalence rates in chickens range from 10.2% to 72.5% in Europe (Kokozidou and Zafeires, 1996; Permin *et al.*, 1999), <1 to 84% in the U.S.A. (Waters *et al.*, 1994), and 17.28 to 78.8% in Africa (Permin *et al.*, 1997; Eshetu *et al.*, 2001), with 1.9-7.5% of the farms (Wilson *et al.*, 1994) and 7-100% of the flocks (Waters *et al.*, 1994) being infected. *H. gallinarum* infections linked to histomoniasis have been well documented in chickens (Homer and Butcher, 1991; Permin, 2003). However, little is known about the prevalence of *H. gallinarum* in chickens in Italy, since available reports are mostly restricted to partridges and pheasants (Rizzoli *et al.*, 1999; Tampieri *et al.*, 2005). The current short communication describes the local occurrence of *H. gallinarum* infection in a flock of laying hens (*Gallus gallus domesticus* Linnaeus, 1758) and is designed to provide some initial data in this country.

Material and methods

Aim of the study

Between May and June 2007, a coprological survey was carried out in a flock of Rhode Island Red hens from a layer farm for commercial egg production in the province of Pisa (Tuscany, Central Italy), as part of a longitudinal study to assess the occurrence of soil transmitted helminths in several commercial farms.

Animals sampled and breeding conditions

General information about husbandry,

hen health status, productivity levels and so on were gathered from an interview with the farmer. Layer replacement pullets were purchased from a commercial grower and brought to the layer houses at the age of 17 weeks. In accordance with the grower's specifications, the pullets received periodic administration of piperazine given in drinking water, and were correctly vaccinated following a recommended schedule for laying hens, including Marek's and New Castle diseases, Infectious Bronchitis, Laryngotracheitis, and so on. A deep litter system of egg production had been adopted on the farm. The laying hens were entirely kept in houses (no access to the external environment) where maximum stocking density was 7 hens per m^2, and all the floor area was solid with a litter of straw. Water and feeding troughs were raised, and one nest box per 5 hens was provided. Laying hens were kept in houses with windows and received additional artificial light to provide 16 h light and 8 h dark daily. Insulation, heating and ventilation of the houses ensured that air circulation, dust levels, temperature, relative humidity, and gas concentration were kept within limits which were not harmful to animals. Strangers were not allowed to enter houses due to biosecurity. No anthelmintic control program was undertaken in the layer phase. At the end of the laying period, the houses were completely cleared and disinfected. Measures for insect and rodent pest control on the farm premises were periodically taken during the year. At the time of sampling, laying hens were 72 weeks old and were going to be sold and slaughtered, because they were at the end of their career after a 52-week laying period.

Faecal examination

Approximately 5 grams of individual faecal samples were collected from 120 randomly selected laying hens. The samples were collected from the soil in clean plastic containers immediately after defecation, brought to the laboratory on the day they were obtained, and stored at +5C° until processed. Each sample was mixed thoroughly using a commercially available sodium nitrate solution with 1.2 specific gravity (Coprosol®, Candioli Farmaceutici, Beinasco, TO, Italy) to yield a homogenous suspension which was filtered through a 60 mesh sieve. The flotation technique was performed with test tubes filled to the top with the faecal suspension. A cover glass was placed on top for 15 minutes, then removed, placed on a microscope slide, and examined under 100x magnification. Worm eggs were identified using the keys described by Thienpont *et al.* (1986). Although *H. gallinarum* and *Ascaridia galli* eggs are very similar in appearance and not easily distinguishable, the side-wall of the latter tend to be more barrel shaped. Faecal egg counts (FECs) were undertaken within 24 hours by a modification of the McMaster technique with a sensitivity of 50 eggs per gram of faeces (Thienpont *et al.*, 1986).

Data analysis

To calculate the prevalence rate, each sample containing at least one egg was considered as positive. Prevalence was calculated as the number of positive faecal samples/the total number of samples examined x 100. Relative 95% confidence intervals (95% CI) were also calculated.

Results and discussion

Worm eggs were found in coprological tests of 50% (95% CI=41-59%) of the 120 Rhode Island Reds examined at random. Unsegmented ellipsoidal eggs with smooth and thick shell, measuring 62-77 µm in length and 33-47 µm in width, were detected and identified as *H. gallinarum*

eggs based on their morphological characteristics. No other worm eggs besides *H. gallinarum* eggs were found by coprological analysis. *H. gallinarum* is currently associated with free-ranging and organic poultry production systems where the animals have free access to outdoor areas (Permin *et al.*, 1999). However, Waters *et al.* (1994) disclosed a prevalence of 12% in laying hens kept in battery cage systems. Later, results of a cross-sectional prevalence study in different poultry production systems clearly showed that *H. gallinarum* was the most common gastrointestinal helminth found and was harboured in 19.4% of hens kept in deep litter systems (Permin *et al.*, 1999). With respect to data on *H. gallinarum* prevalence in hens from closed production systems, the present 50% prevalence rate is considerably higher than those previously reported. Therefore, although the present survey only covers one egg layer farm, it indicates that *H. gallinarum* infection may be higher and better established than it was previously thought to be in laying hens kept on deep litter and reared under confined management regime. In addition, it gives a baseline indication of the level of infection and importance of this intestinal helminth in poultry farming in a geographical area of Italy. In the present report, worm eggs other than *H. gallinarum* eggs were not identified by coprological analyses at all. This finding is in contrast to the observations of Permin *et al.* (1999), who found that laying hens kept on deep litter were infected by *Ascaridia galli* and *Capillaria obsignata* too. Taking into account the low host's specificity of *H. gallinarum*, allowing direct and/or indirect contacts of infected chickens with other birds can promote cross-infections. Harbouring viable eggs of the worm, untreated litter from infected flocks should not be used as fertilizer in areas where domestic and/or wild populations of susceptible avian species might be exposed to the infection. The interview with the farmer could not explain how and when the infection was introduced into the farm. Provided layer replacement pullets received adequate prophylactic anthelmintic treatment before being purchased, it is possible that the hens were already infected before they were moved to the layer houses, as a result of failure of the treatment to eliminate the total caecal worm burden. Or, it is also possible that *H. gallinarum* viable eggs were already present in the layer houses due to their persistence in the soil, as a result of failure to completely remove the litter from previous flocks, one of which was presumably infected.

The farmer had not noticed any particular problem about the hen health status or the egg quality (i.e., eggshell pigmentation, eggshell thickness, and so on) during the laying period. Based on his statements, mortality and egg loss rates were around 6% and 8%, respectively. According to the farmer, the most important and recent problems encountered in the flock during the preceding months were cannibalism, egg prolapse, and thermal stress as major causes of mortality, while the egg loss was mostly attributed to cracked and broken eggs. Thorough clinical examination of each hen for any sign of disease could not be performed. However, clinical signs attributable to *H. gallinarum* infection were not seen at visual inspection carried out before slaughtering. A high number of hens showed extensive feather loss especially in the neck and chest areas. In many cases, tail feathers were missing or broken too. Some hens showed signs of lameness probably caused by limb lesions or by muscle and joint pains. No gross lesion was observed in the caeca of hens at post-mortem examination. Neither attempts to find adult helminths by scraping of the caecum content nor his-

topathological investigations were made, since these did not fall within the aim of the present survey. The present lack of impact on production level, clinical signs, and gross pathological lesions linkable to heterakiasis may probably be explained by low and particularly insidious worm burdens as shown by very low FECs (<50) detected in all the faecal samples examined. The failure to note such effects is not indication that they did not occur, but rather that they could not be determined in this survey. According to Pennycott and Steel (2001), as flocks grow older they are more likely to test positive for worm eggs, but FECs tend to be highest around the time of peak egg production in the flock. Therefore, the time of sampling probably accounted for the present finding of high infection rate but low FECs, since the examined hens were 72 weeks old and were at the end of their laying period. The lack of appreciable clinical signs, gross pathological lesions, and impact on production level in course of heterakiasis has also been reported in commercial broiler chickens (Wilson *et al.*, 1994) and in turkeys parasitized by *H. gallinarum* alone (Brener *et al.*, 2006). Nevertheless, the pathogenicity of *H. gallinarum* may vary among hosts as documented in various avian species. Under experimental conditions, studies on growth rate and pathological changes in 1-month-old White Leghorn chickens showed a significant reduction in the average rate of the weekly weight gain from 2 weeks post-infection. Pathological changes included congestion, haemorrhages and catarrhal enteritis of the small intestine, nodules with necrotic centre in the caecum, diffuse infiltration of lymphocytes, macrophages and heterophils in the intestinal mucosa, and desquamation of the caecal epithelium (Choudhury and Das, 1993). Haemorrhages in the myocardium, liver and kidneys, atrophy of mucous membrane of the caecum, hyperplasia of lymphoid follicles, and granuloma formation were reported following natural and experimental infection in guinea fowls (Khan *et al.*, 1994). Congestion, thickening, petechial haemorrhages, intussusception, nodules in the caecal wall, chronic diffuse thyplitis, haemosiderosis, granulomas with necrotic centre in the submucosa, and leiomyomas in the submucosa, muscular layer and serosa were observed in 82% of naturally infected pheasants (Menezes *et al.*, 2003). Intense, chronic, diffuse inflammatory processes with mononuclear and polymorphonuclear leucocytes infiltrations were detected in the caecum of turkeys exposed to natural infection (Brener *et al.*, 2006). Data of the previous studies indicate that some hosts can be more severely affected by *H. gallinarum* than were hens of the present survey. Severity of helminth infections may be influenced by several factors of the parasite and host. Our findings suggest that either the present *H. gallinarum* strain was well adapted to chickens or that hens of the current report were more resistant than other hosts to the infection. Higher resistance of the examined hens could be innate or could be caused by general conditions such as a better plane of nutrition. Experimental infection with *H. gallinarum* thrived best in White Leghorn than New Hampshire chickens, with respect to length, survival, and reproductive capacity (Chute *et al.*, 1976). *H. gallinarum* worm burden was slightly higher in backyard chickens with poor body conditions (Jansson *et al.*, 2004). The possibility that the lack of appreciable effects of heterakiasis observed in this study was caused by a higher resistance of the examined hens compared with other hosts or by management factors could not be ruled out. Longitudinal studies carried out in different breeds of chickens and other susceptible hosts are required to allow definitive conclusions of these observations.

Conclusions

The European Union directives on the welfare of laying hens (1999/74/EC, 2002/4/EC) distinguish three rearing systems: a) enriched cages with at least 750 cm² of area per hen; b) non enriched cages with at least 550 cm² of area per hen; and c) non-cage systems with at least one nest for 7 hens, adequate perches, and stocking density not exceeding 9 laying hens per m² usable area. Non-cage systems represent a considerably higher risk of exposure to soil transmitted helminths due to direct contact with faeces. As our report shows, *H. gallinarum* can be highly prevalent in a deep litter production system, even if laying hens are entirely kept indoors. This may reflect an important aspect in the epidemiology of heterakiasis, which could serve as silent source of *H. melagridis* and economic losses. Infections with intestinal worms including *H. gallinarum* might cause losses of 10-20% due to impaired feed conversion, reduced growth and egg production, and increasing mortality (Schou and Permin, 2003). Investigations are thus advisable to know the exact distribution of *H. gallinarum* and the extent to which heterakiasis may influence health status and productivity in poultry farms in Italy.

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