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Diurnal fluctuations in nematode egg excretion in naturally and in experimentally infected chickens

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ABSTRACT

We investigated whether nematode egg excretion through feces of naturally or experimentally infected chickens follow certain patterns within a day, which may allow determining the most appropriate sampling time for the highest parasite egg concentration. Feces samples ($n = 864$) from chickens ($n = 36$) with naturally occurring mixed nematode infections (trials N1, N2) or with an experimental *Ascaridia galli* infection (E) were collected quantitatively every 4 h for four consecutive days. Number of eggs per gram of feces (EPG) was determined, and accumulative egg output (AEO) at each sampling time as well as total number of eggs excreted within 24 h (eggs per day, EPD) were then estimated. At the end of the collection period, the hens were necropsied and their worm burdens determined. Naturally infected hens harbored *Heterakis gallinarum* (100%), *Capillaria* spp. (95.7%) and *A. galli* (91.3%). The experimental *A. galli* infection produced patent infections in all the birds.

In general, both fecal egg concentration (EPG) and the amount of feces increased ($P < 0.05$) sharply from the early morning to early-noon (10:00 a.m.) and remained at a high level until evenings which thereafter decreased to their initial levels during the night both in naturally and experimentally infected birds. This resulted in a more apparent increase or a decrease in AEO at the corresponding time points, respectively, and led to much higher egg excretions during the daytime than the nights. Despite the apparent within day fluctuations in egg excretion, neither EPG ($P = 0.704$) nor AEO ($P = 0.499$) nor EPD ($P = 0.149$) was significantly different among the four collection days. Similarly, there was no significant interaction ($P > 0.05$) between effects of sampling hours and days on EPG and AEO, suggesting the existence of repeatable diurnal fluctuations within each day.

Although an association between climatic parameters (e.g., ambient temperature and relative humidity) and the nematode egg excretion was quantified, a causal relationship could not be demonstrated. We conclude that nematode egg excretion through chicken feces in both natural and experimental infections shows repeatable diurnal fluctuations, which may indicate adaptive strategies by nematodes and eventually favor parasite spread. Since analytic sensitivity of fecal egg counts suffers from low egg concentrations in feces, samples taken during the daytime have a higher diagnostic value.

1. Introduction

Nematode infections of chickens may cause a variety of problems and are responsible for economic and production losses (Ruff, 1999; Permin and Ranvig, 2001) due

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to a decrease in growth and egg production (Permin and Hansen, 1998; Gauly et al., 2005) and behavioral changes (Gauly et al., 2007). In modern non-cage housing systems, particularly in free-range systems, the infections are very common (Permin et al., 1999; Häne et al., 2000; Kaufmann et al., 2011; Hussen et al., 2012; Wongrak et al., 2014). Chickens in such systems are in close contact with their feces due to the access to runs and pastures, which ensure completion of nematode life cycles. The most prevalent nematode species in free-range chickens are *Heterakis gallinarum*, *Ascaridia galli* and *Capillaria* spp. (Permin et al., 1999; Kaufmann et al., 2011; Wongrak et al., 2014). *A. galli*, *H. gallinarum* and the most important *Capillaria* species (e.g., *Capillaria obsignata*) have direct life cycles and the eggs from these parasites are highly resistant to external conditions and can survive for an extended period of time (Ruff and Norton, 1997; Permin and Hansen, 1998).

It is likely that during the course of evolution parasites may have developed strategies to increase their spread with the propagule excretion patterns, which may eventually increase spatial contamination of environment with infective stages and thereby favor transmission success of parasites. For a couple of host–parasite systems, it has been reported that parasite eggs are periodically released by day-to-day fluctuations (Engels et al., 1996; Yu et al., 1998; Giver et al., 2000). More interestingly, diurnal periodicity in excretion of parasite propagules has been demonstrated for various species from different taxa (Oju and Mpoame, 2006; Villanouía et al., 2006; Dolnik et al., 2011; Coelho et al., 2013). In most cases parasite propagules are shed at a higher rate during the day, particularly late afternoons, than during the night (Villanouía et al., 2006; Dolnik et al., 2011; Coelho et al., 2013). It however remains largely unknown whether and how parasites benefit from these egg excretion patterns.

Apart from biological importance of the diurnal periodicity in the egg excretion phenomenon, there is a practical diagnostic concern. As the parasite propagules are excreted to the external environment through feces, their presence and/or concentration in feces is of diagnostic importance. It is known that information on quality of fecal egg counts (FEC) is influenced by a couple of feces related factors, e.g., daily amount (Daş et al., 2011a), consistency (Le Jambre et al., 2007) and feces flow (Michael and Bundy, 1988). Furthermore, FEC are variable due to variation in worm fecundity, uneven distribution of eggs in feces, host resistance, and a possible low sensitivity of egg counting techniques (Michael and Bundy, 1988). In this study we investigated whether nematode egg excretion in naturally or experimentally infected chickens follow certain patterns within a day, which may allow determining the most appropriate sampling time for the highest parasite egg concentration.

2. Materials and methods

2.1. Chickens and feces collection procedures

Three trials, two including naturally infected (N) chickens and one with experimentally (E) infected chickens were performed. A total of 23 naturally infected brown hens (Lohmann Brown) at the end of laying period were purchased in June 2012 (N1; $n = 10$) and in April 2013 (N2; $n = 13$) from a free-range farm with a known history of nematode infections (Wongrak et al., 2014). In the third trial (October 2012) experimentally infected white Leghorn chickens (Lohmann Selected Leghorn) (E; $n = 13$) were used. These hens had been inoculated with 300 embryonated eggs of *A. galli* 11 weeks before the start of the trial, and were kept indoors under group conditions.

In each trial, the birds were placed into individual cages in a half-open barn three days prior to the start of feces collections. Individual feces were then collected quantitatively for 4 consecutive days following a 3-days adaptation period to the cages. On each day, the total amount of feces produced by each hen was collected from the cages every 4th hour (i.e., at 06:00, 10:00, 14:00, 18:00, 22:00 and 02:00 h). During the entire study period the hens had free access to water and were fed ad libitum a commercial laying hen diet containing 185 g CP kg feed. Individual water and feed troughs were re-filled twice a day at 6.00 a.m. and 6:00 p.m. Lighting was supplied by natural light during the daytime and by artificial light with LED light bulbs between 18:00 and 22:00 h. At the time of feces collection during the nights light had to be turned on (less than 30 min) to remove all feces from the ground of cages without disturbing the hens. The average day length was approximately 14 and 16.5 h in the first and second trials with natural infections, whereas it was 11 h during the experimental infection. The sunrise was recorded at 05:03 and 06:18 in the first and second natural infections, and at 07:41 in the experimental infection (Anonymous, 2014). The daily ambient temperature (T_a) and relative humidity (RH) in the barn were measured continuously by temperature/humidity data loggers (Tinytag®). The barn provided protection from the sun and rain, but T_a and RH were similar to outside climatic conditions.

2.2. Parasitological examinations

Feces from individuals were weighed, mixed and labeled at each collection time and stored at 4 °C until examination (<24 h). A thoroughly mixed fecal sample (4 g) was then analyzed to determine the number of eggs per gram of feces (EPG) using a modified McMaster egg counting technique with saturated sodium chloride solution ($d \geq 1.2$ g/ml) which yielded in a minimum detection limit of 50 EPG (Sloss et al., 1994). Because of morphological similarities, eggs of *A. galli* and *H. gallinarum* were counted together. Similarly, eggs of different *Capillaria* spp. were not further differentiated and counted together as *Capillaria* eggs. A total of 864 feces samples were analyzed during the entire study period. During the second trial (N2), 252 fecal samples were analyzed for dry matter content (DM).

At the end of each trial (i.e., N1, N2, E), all the hens were slaughtered for post-mortem parasitological examinations. The gastrointestinal tracts were opened with scissors in a longitudinal section from the esophagus to the cloaca, including the caeca. The tracheas were examined macroscopically for the presence of *Syngamus trachea*. The contents were washed out separately with tap water into