Ascaridia galli Adult Nematode in Chicken Egg from a Commercial Organic Farm in Israel

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ABSTRACT

Ascaridia galli (Ascaridida: Ascaridiidae) nematodes are intestinal parasites of domestic chickens and other Galliform species. They are ubiquitous worldwide, and perpetuation of their direct life cycle is favored by constant exposure of birds to infective worm eggs present in fecal material. Rarely, adult worms may be found in intact chicken eggs. We hereby describe a case of such a finding in a chicken egg from a commercial organic farm in Israel. Morphologic parameters, as well as sequences obtained following amplification of segments of the cytochrome C oxidase subunit 1 and 18S small subunit ribosomal RNA loci were used to confirm the diagnosis. Subsequent testing of the respective flock has confirmed the presence of infection, and effective anthelmintic treatment has been applied. Although the eggs are non-aesthetic for consumers, they do not present a risk for human infection. Nevertheless, such an infection may negatively affect health and productivity of the respective flock, and should be addressed accordingly.

Keywords: *Ascaridia galli*; Eggs; Domestic Chickens; Israel; PCR.

INTRODUCTION

Ascaridia galli nematodes are large (1.4-12 cm), whitish roundworms, reported mostly in domestic chickens (*Gallus gallus domesticus*), but also found at times in turkeys, geese, guinea fowl and a number of wild birds (1). They are closely related to *A. columbae* and *A. dissimilis,* which infect pigeons and turkeys, respectively. Together with *Heterakis galliinarum* (Ascaridida, Heterakidae), they are the most prevalent nematodes found in laying hens worldwide (2, 3). The adult nematodes inhabit the small intestine of their host, producing eggs that are expelled with feces in the environment. The relatively large (70-90 x 40-50 μM) ascarid eggs are extremely resistant in the environment, and may remain viable for up to two years given sufficient moisture. Unembryonated eggs sequentially develop through two larval stages (L1 and L2), until an infective L3 develops in about 3 weeks in favorable ambient conditions (23°C, high humidity). Following the ingestion of an infective egg by a suitable host, the L3 develop into L4 in the intestinal wall. The L4 return to the intestine lumen, and mature into reproductive adult worms in about 5-8 weeks (1, 4). Earthworms, which ingest embryonated eggs and are predated by chickens, may also play a role in the life cycle. However their relevance in the life cycle is thought to be minor (1). High worm burdens may incur in birds, resulting in severe damage to the small intestine, especially in young birds. *A. galli* adults can perform extra-intestinal migration, to end-up at various organs in the abdominal cavity, where they persist for a week or longer. This latter property leads

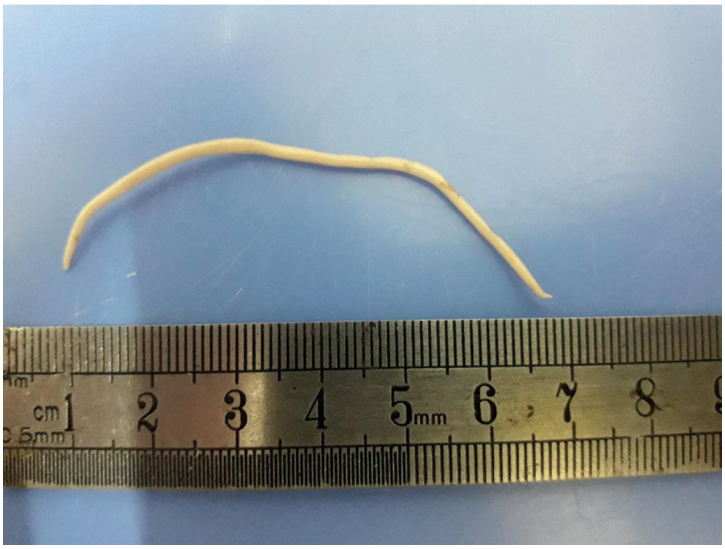

Figure 1. The adult *A. galli* found inside a free-range chicken egg.

to the finding of young adults or mature worms inside a seemingly normal chicken egg. Such cases are discerning for consumers of infected eggs, raising concerns as to possible human infection, as well as to poor standards of hygiene at the respective egg layer farms. These concerns may be reduced after information is provided by a trusted source, such as food inspection officers or veterinarians. However, the finding of an infected egg also draws attention to the subclinical presence of the worms in the respective chicken flocks. Infection with *A. galli* may have a negative impact on a flock's health and productivity (2), that may be otherwise overlooked since overt clinical disease is rarely seen.

In this report, we describe a case of *A. galli* infection in an egg purchased from a commercial organic farm, including its morphological and molecular identification, and discuss the implications of such a finding.

CASE REPORT

A dozen chicken eggs were purchased at a grocery store in the latter part of 2021. The source of the eggs was a licensed, organic, commercial layer chicken farm from Israel. The farm houses about 12,000 birds, and the respective flock was 40 weeks old at the time. The animals were kept according to local organic farming regulations, and were housed in a closed facility on raised plastic slates. The flock was regularly visited by a poultry veterinary clinician, and birds were reported to be in good condition at the time. No antimicrobial or anthelminthic agents were administered to the flock prior to the described event, and no prior events of helminth infections were recorded at this farm.

Upon opening one of the purchased eggs, a relatively large (about 6 cm), milky white elongated narrow object was seen in the albumen (egg white) of one of the eggs, without any further abnormal findings (Fig. 1). On visual inspection prior to the opening of the shell, the egg appeared normal. Two other eggs that were examined from the same package were normal. The concerned client contacted the Department for Control of Animal Products in the Israeli Veterinary Services. The remaining intact eggs from the aforementioned package were collected by veterinary officers, alongside the suspected helminth sample. Eggs were examined by bright illumination (5) without any abnormal findings, and the helminth was submitted to the parasitology laboratory at the Kimron Veterinary institute for identification.

A single sample preserved in 70% ethanol was received for inspection. The sample was washed three times and soaked in distilled water for 8 hours prior to microscopic examination. A light microscope coupled to a digital camera (Leica DM500/ICC50E, Leica Microsystems, Switzerland) was used. Following this, a segment of the median part of the helminth was taken for DNA extraction, using a commercial kit (DNeasy Blood & Tissue Kit, Qiagen, Germany) according to manufacturer's instructions. A polymerase chain reaction (PCR), targeting a 420bp segment of the cytochrome C oxidase subunit 1 (CO1) gene was performed, using primers

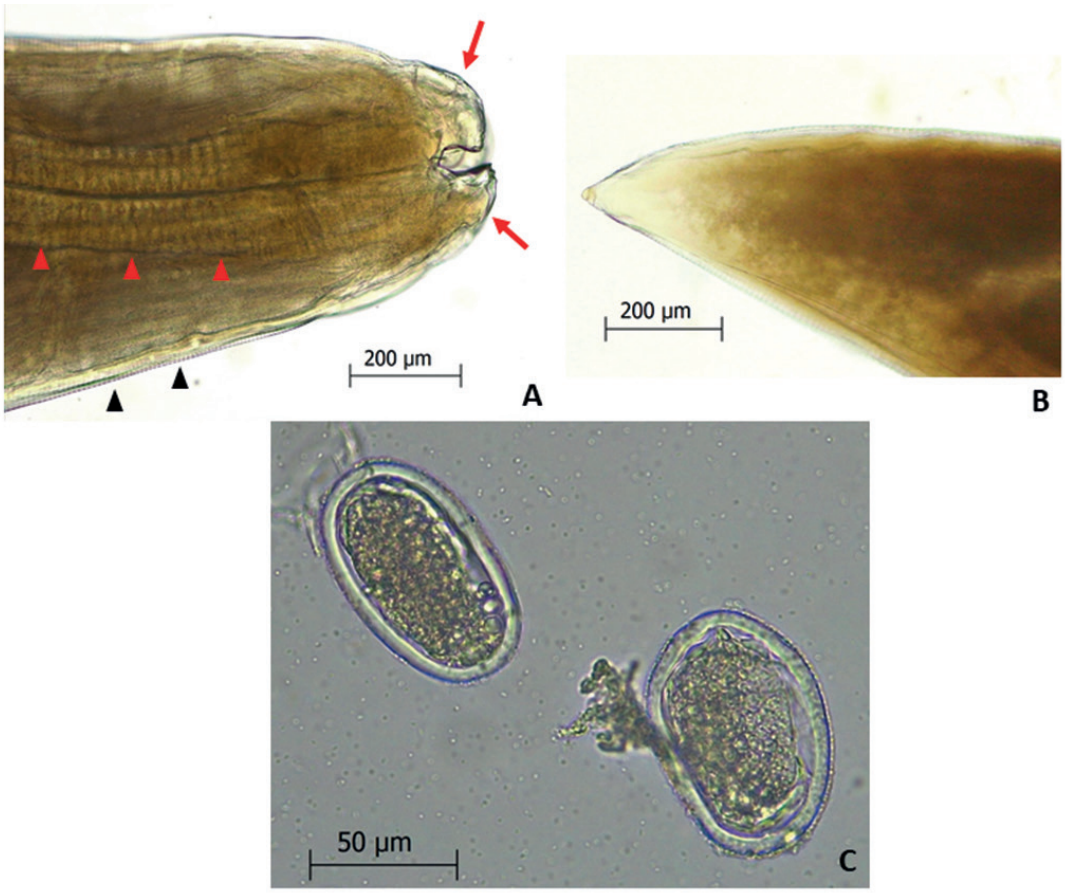

Figure 2. Morphologic characteristics of the adult nematode. A. anterior part including two lips (arrows) with the third seen in the background, oesophagus (red arrowheads) and part of the cervical alae (black arrowheads). B. posterior part. C. eggs found after dissection of the median part of the worm. All structures are distorted to various degrees due to dehydration.

JB3/JB4.5 (6). An additional PCR targeting an 800bp segment of nematode 18S small subunit ribosomal RNA, was performed using primers Nem18S_F/R (7). PCR amplicons were examined on a 1.5% agarose gel, and sequenced (Hylabs, Israel).

The length of the worm was 7.1 cm, however since the specimen was kept in 4˚C for at least 24h without any medium, prior to its transfer to 70% ethanol, it has dehydrated and was somewhat deformed, and may well have been previously longer. Morphologic characteristics that were observed included three lips, narrow cervical alae and gravid uteri containing a large number of thick shelled, ascarid type eggs, measuring 90x52 µM (Fig. 2). Based on these findings the specimen was classified as an adult female of *Ascaridia galli* (Schrank, 1968) (8, 9).

The CO1 PCR resulted in a 400bp long sequence, that yielded 97.43% identity to a sequence of *A. galli* full mitochondrion (10, accession number JX624728.1), and slightly higher (up to 97.94%) identity to multiple other *A. galli* sequences deposited in NCBI GenBank (https://blast.ncbi.nlm.nih.gov). The sequence was deposited as accession number OK501301. Since only a single *A. galli* sequence that targets the 18S gene was present in GenBank, we amplified part of this locus as well, resulting in a 854bp long sequence found to have 99.77% identity to the previously published sequence (11, accession number EF180058.1). The next best matches were to a sequence from *Ascaridia nymphii* (98.74% identity, accession number LC057210.1) *Heterakis gallinarum* (98.62% identity, accession numbers MK844591.1 and DQ503462.1), and *Heterakis spumosa* (98.39% identity, MH571872.1). The sequence was deposited under accession number OK501308.

Following this finding, the egg layer flock was identified using traceability labeling on the eggshells. In a post mortem examination performed on five birds from this flock, three were found to be infected with adult nematodes and cestodes. The flock was consequently treated with flubendazole (Flubengal, BIOVAC LTD., Israel) at 300ppm in the feed, for seven consecutive days. In a post-treatment fecal testing done two weeks after completion of the treatment, no infected birds were found.

DISCUSSION

The described finding of an adult *A. galli* helminth within a chicken egg is highly uncommon; however, it has been previously reported in several publications (5, 12). Two routes by which adult worms may end up in an intact egg have been suggested; the first is by penetration of the gut wall, followed by travel via the abdominal cavity and penetration of the reproductive tract, possibly through the infundibulum and into the oviduct, where the worm attaches to the albumen prior to shell secretion. The other suggested route is by traveling via the distal colon and into the cloaca, from where the worm ascends the reproductive tract via the vagina (5).

In the absence of well-preserved male or female adult worms for definite morphological identification, molecular tools present an alternative diagnostic option. Biswas *et al.* (13) have demonstrated that the use of the nuclear ribosomal internal transcribed spacer 1 (ITS1) and ITS2 for this purpose may not be adequate in *Ascaridia*, since they lack ability to distinguish between *A. galli* and *A. columbae* species. The CO1 locus however, was suggested to provide a target with sufficient discriminatory power. The CO1 sequence from the worm submitted to us for identification was highly similar to multiple sequences of *A. galli* deposited in GenBank, supporting the preliminary diagnosis based on host species and helminth morphological characteristics.

The infection of chicken eggs with *A. galli* is not of public health concern, since humans are not a suitable host, and such cases do not present a zoonotic risk. However, there is significant evidence for a negative impact of *A. galli* on the health and productivity lying hens (reviewed in 2). High infection loads may result in a reduction in egg production and body weight of infected laying hens, and increased mortality may occur.

Current trends, including the rising public awareness and demand for improved animal welfare practices in farm animal husbandry, result in an increase in the number of commercial flocks raised under free-range protocols. In these systems, chickens have access to soil, in yards shared by a large number of animals, and where other farm and wild birds may have access. These conditions favor the establishment of *A. galli* infection (3). Management practices such as rotational use of yards, good biosecurity and hygiene measures and use of disinfectants may be implemented in order to reduce the incidence and burden of infection; however, use of anthelmintic drugs is still the mainstay for controlling *Ascaridia* infection in poultry (14). Benzimidazole drugs, namely fenbendazole and/or flubendazole are the two drugs registered for use in chickens in many countries, including Israel, and are considered effective. Information on ascarids anthelminthic resistance in domestic poultry is still scarce (15, 16, 17). However, it should be noted that benzimidazoles, together with macrocyclic lactones are the two anthelmintic groups to which resistance is increasingly reported from domestic ruminants (reviewed in 18). Therefore, sustainable use of anthelmintic drugs in commercial poultry farming, and development of practices such as targeted treatments, should be a priority. Findings of studies aimed at this direction show positive initial results regarding both effectiveness of control of *A. galli* infection, as well as positive impact on productivity (14, 19).

In conclusion, the finding of *A. galli* adult nematode in chicken eggs is rare, and does not pose any human health concern. However, its implications on the flock of origin should be recognized, and properly addressed. The increased public demand for free-range eggs may result in an increase in the prevalence of helminths such as *A. galli* in chickens, highlighting the importance of future development of sustainable anthelmintic strategies in this sector.

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CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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