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Original Scientific Article

THE FIRST DETECTION OF *Acuaria spinosa* IN PHEASANTS

Ivan Pavlović[1], Iulia Floristean[2], Viorel Floristean[3], Snežana Ivanović[1], Zoran Kulišić[4], Živka Ilić[1], Dubravka Jovičić[5]

[1]Scientific Veterinary institute of Serbia, V. Toze 14; [4]Faculty of Veterinary Medicine Belgrade; [5]Faculty Futura, University Singidunum, 11000 Belgrade, Serbia, [2]Directia Veterinara si pentru Siguranta Alimentelor Iasi; [3]Faculty of Veterinary Medicine Iasi, Iași, România

*Corresponding author, E-mail: dr_ivanp@yahoo.com

Summary: Parasitoses caused by the *Acuariidae* helminths sporadically occur in both free-living and artificially bred pheasant populations (*Phasianus colchicus* L.). In order to verify this situation, an investigation with the aim of detecting the *Acuaria species*. infection on seven pheasant farms in Serbia and Romania, was carried out. A total of 127 adult birds were examined. The collected nematodes were identified by their morphometric characteristics. The three different *Acuaria* species were identified including: *Cheilospirura (Acuaria) hamulosa, Dyspharinx (Acuaria) spiralis* and *Acuaria spinosa*. The parasites identified as the *Acuaria spinosa* species had equal lips and furrow cuticula. Both parasites had four spiny cordons 'which did not exceed a third of the anterior of the esophagus. The tail was short and rounded. Eggs were ellipsoid, thick shelled, embryonated, 0.039-0.41 µm long by 0.025-0.027 µm wide.
Furthermore, thefirst parasite's body length was 32 µm, with a width of 0.336 µm (maximal width at the middle of the body). Four spiny cordons were 0.797 µm long, and the cylindrical pharings were 0.018 µm long. The vulva lies just posterior to the middle of the body, 3.56 µm from the anterior end, 51 % of the total body length (TBL) from the anterior end. The anus lies at 0.25 µm from the posterior end. The body length of the second parasite was 34 µm with a width of 0.367 µm. Spiny cordons 'which did not exceed a third of the anterior of the oesophagus were 0.809 µm long, and the cylindrical pharings were 0.021 µm long. The vulva lies just posterior to the middle of the body, 5.64 µm from the anterior end, 57 % of the total body length (TBL) from the anterior end. The anus lies at 0.28 µm from the posterior end.
According to the authors knowledge this is the first detection of the *Acuaria spinosa* in pheasants (*Phasianus colchicus* L.).

Key words: *Acuariidae; Phasianus colchicus; Acuaria spinosa*

Introduction

Parasitoses caused by nematodes produceshealth problems in both the free-living and artificially bred pheasant populations. The nematode infection is the most frequent infection transmitted through an intermediate host in the pheasant population. There are several papers regarding helminthoses in freeliving and farm bred pheasants. Most of these investigations were performed in Europe, namely in Czechoslovakia, Lithuania, Poland, Greece, Germany and Italy (2, 8, 10, 12, 17, 25).

In the West Balkan countries, numerous investigations were conducted in Romania (5, 14) and Serbia (20, 22, 23, 24). During those examinations parasitoses caused by the *Acuariidae* helminthes occurred sporadically, in both free-living and farm bred populations of pheasants (9, 16, 29). Therefore, this investigation was carried out on seven pheasant farms in order to establish the helminth fauna with an emphasis on the presence of the *Acuaruidae* species in the artificially bred pheasant population in Serbia and Romania.

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Material and methods

The investigation was carried out on seven pheasant farms for hunting/restocking purposes including four farms in Serbia and three farms in Romania. The helminthes were collected from adult pheasants that had died of natural causes by parasitological necropsy. A total of 127 adult birds were examined. The collected nematodes were fixed in glacial acetic acid and preserved in 70 % ethanol. For further examination, parasites were clarified in lactophenol and nematodes were identified by their morphometric characteristics described by Skrjabin et al. (26) and Chabauda (4). All the laboratory techniques used were described by Šibalić and Cvetković (28).

Gizzards were dissected in a 0.85 % NaCl solution and the cuticle was removed for helminth investigation. Fragments of the parasitized gizzards were removed and immediately fixed in formalin. The material was then processed for paraffin embedding. Five micrometres thick sections were stained with hematoxylin and eosin.

Micrographs were obtained using a Carl Zeiss bright field microscope, by means of Practica PLC3 and a Kodachrome 64 film.

Results

Infection with nematodes was found in 37.79 % (48/127) cases. Polyparasitism involving two species was detected in 28.34 % (36/127) of pheasants (13,21).

A total of 14 of 127 (11 %) examined pheasants were found to have been infected with the *Acuariidae* sp. During necropsy, specimens were found lying freely under the gizzard cuticle, partially or fully burrowed in the walls of the organ, mainly in the region of the caudal blind sac. There, specific macroscopic lesions were observed as present in the muscular portion of the stomach and included sclerotic and nodular lesions accompanied by a fine-whitish granular residuum, located underneath the corneous lining of the gizzard, at the points of nematodes implantation (15). Three different species were collected and identified: *Cheilospirura hamulosa, Dyspharinx spiralis*, and *Acuaria spinos*, which was found in onlyt one bird examined, which was represented by two parasites. Furthermore, it was found that *C. hamulosa* and *D. spiralis* infections occurred in both countries (on one farm in Serbia and two in Romania), while A. spinosa infection occured only in Romania. *Acuaria spinosa* was found in only one bird, and two worms were found.

The parasites identified as the Acuaria spinosa species had equal lips and furrow cuticula. Both parasites had four spiny cordons 'which did not exceed a third of the anterior of the esophagus. The tail was short and rounded. Eggs were ellipsoid, thick shelled, embryonated, and 0.039-0.41 μm long by 0.025-0.027 μm wide.

Furthermore, the first parasite's body length was 32 μm, the width was 0.336 μm (maximal width at the middle of the body). Four spiny cordons were 0.797 μm long, and cylindrical pharings were 0.018 μm long (Fig. 1). The vulva lies just posterior to the middle of the body, 3.56 μm from the anterior end, 51 % of the total body length (TBL) from the anterior end. The anus lies at 0.25 μm from the posterior end.

The second parasite's body length was 34 μm, and the width was 0.367 μm. Spiny cordons did not exceed exceed a third of the anterior of the esophagus. Spiny cordons were 0.809mm long, and the cylindrical pharynx was 0.021 μm long (Fig. 2). The vulva lies just posterior to the middle of the body, 5.64 μm from the anterior end, 57 % of the total body length from the anterior end. The anus lies at 0.28mm from the posterior end.

The histological examination revealed traumatic lesions in hemorrhagic and necrotic routes and tunnels generated by cuticular cordons and spines of the adult nematodes, surrounded by a conjunctive reaction (Fig. 3). Nematodes were observed in tunnels with fibrous walls, impregnated with various mesenchimal cells, predominant lymphocytes and eosinophils. The muscular wall of the stomach was almost completely replaced by conjunctive tissue, in which diffuse lymphoid infiltration and lymphoid nodules, as well as parasitic granuloma were observed.

Discussion

Acuaria spinosa belongs to the *Acuariidae family*, the *Acuariinae subfamily*, and genus Acuaria. When first described in *Bonasa umbellus, the* species was named *Cheilospirura spinosa* (6, 7). In a later comparison withs nematode from the Acuariinea family it was concluded by Boughton (3) that they were the same species,

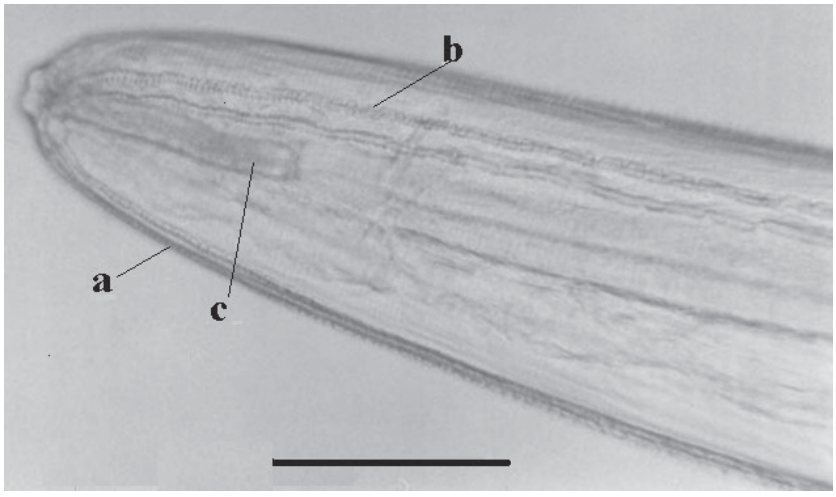

Figure 1: Anterior portion of a second sample of *Acuaria spinosa* ventral view, a) furrow cuticula, b) spiny cordons; c) pharings; Bar = 0.1 mm (Bar of 1 is common to 2 and 3)

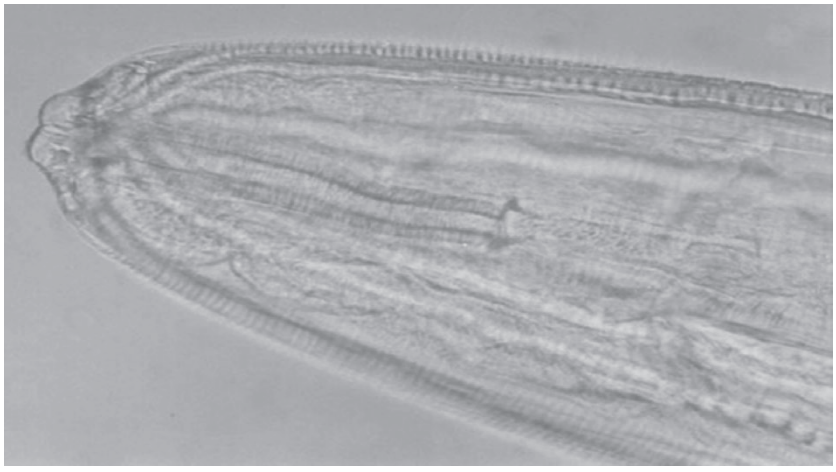

Figure 2: Anterior portion of a second sample of *Acuaria spinosa*, ventral view

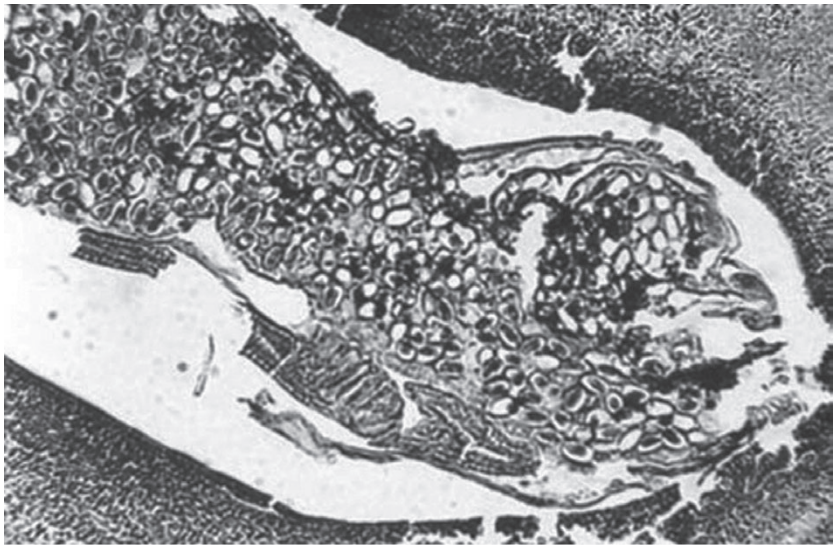

Figure 3: Perforated sites in the submucosa caused by *A. spinosa*

and that nematodes belong to the Acuaria genus. Finally, a complete description of parasites and their development was presented by Skrjabin et al. (25). According to Skrjabin et al., parasites measured 37-40 μm long and 0.31-0.34 μm wide and possessed two equal lips and furrow cuticula. These measurements coincide with the findings of this study (parasites body lengths were 32-34 μm and widths were 0.336- 0.367 μm; four spiny cordons were 0.797- 0.809 μm long).

Grasshoppers (*Melanopus femurrubrum* and *M. differentialis*) serve as intermediate hosts for Acuaria spinosa. In these hosts the infective larvae develop and the final host acquire the infection by ingesting grasshoppers (7, 26).

Acuaria spinosa (syn. *Cheilospirura spinosa*) was first described as a species of nematode found in the gizzard of ruffed grouse in Michigan (USA) (27). Cram (6) detected *A.spinosa* in the horny lining of the gizzard of *Bonasa umbellus*, *Colinus virginianus* and *Pediocetes* (T*ympanuchus*) *phasianellus campostris*. Later, *A. spinosa* was found in *Alectoris graeca* (1) and in *Tympanuchus cupido* (9). The results of this study conclude that this is the first occurrence of *Acuaria spinosa* in ring necked pheasants (*Phasianus colchicus* L.).

The helminthes from the Acuariidae family sporadically occur in pheasants, as first reported in the USA by Cram (6). Later examination pointed to the factthat Acuariidae can be found in free living and farm bred pheasants in numerous countries in Europe (10, 11, 12, 13). During those examinations the following species were found: *C. hamulosa,* followed by *A. spiralis* and *A. gruveli.* In Serbia and Romania *C. hamulosa* was the most frequent Acuariida species (13, 14, 19, 20, 21).

In the infected birds, parasites of the Acuaridae genus caused severe gross lesions in the gizzard such as hemorrhages, ulcers and thickening of the mucosa and cuticle, and single yellowish nodules on the caudoventral muscle (16). Histological examination of the infected gizzards revealed discrete and coalescing nodular and cystic lesions in the mucosa and musculature which contained sections of the parasite. Cellular reaction in the lesions was characterised by a large number of lymphocytes, monocytes, plasma cells, heterophils, and, in some of the sections, a severe eosinophilic reaction. The mucosa and submucosa showed markedly thickened and diffuse mononuclear infiltration as well as reactive lymphoid nodules (15). During examinations it was found that pathological changes caused by *A.spinosa* were similar to cases of infection with other Acuaria species.

The presence of *Acuaria spinosa* in pheasants or other birds, except *Bonasa umbellus, Colinus virginianus, Pediocetes* (*Tympanuchu*) *phasianellus, Alectoris graeca* and *Tympanuchus cupido* was not reported in available literature. Furthermore, the presence of adult nematodes in gizzard suggests the possibility of adaptation of *Acuaria spinosa* to parasitism in a new host – pheasants. This is not unique, because *Brachylaemus fuscatus* and *Plagorchis megalorchis* were found in pheasants in Scotland (24). *Zygocotyle lunata* was found in 1.6 % of pheasants in Nebraska (11). Infection with *Ornithostrongylus quadriradiatus* in pheasants was found in the Belgrade area (18). During 2002 the first occurrence of *Thominx cyanopicae was established* in the new host – pheasants and their full adaptation to it, confirmed by histological aspects which revealed the pathological effect of parasites (14).

The first occurrence of *Acuaria spinosa* in pheasants has an epidemiological importance and indicates the necessity for continued parasitological examination of this bird species.

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PRVO ODKRITJE *Acuaria spinosa* PRI FAZANIH

I. Pavlović, I. Floristean, V. Floristean, S. Ivanovic, Z. Kulišić, Ž. Ilić, D. Jovičić

Povzetek: Parazitoze, ki jih povzročajo črevesne gliste iz družine Acuariidae, naj bi se občasno pojavljale pri prosto živečih in gojenih populacijah fazanov. Opravili smo raziskavo, s katero smo želeli odkriti okužbo z *Acuaria species* na sedmih farmah fazanov v Srbiji in Romuniji. Skupno je bilo preiskanih 127 odraslih ptic. Zbrani nematodi so bili določeni glede na njihove morfološke lastnosti. Identificirali smo tri različne vrste *Acuaria: Cheilospirura (Acuaria) hamulosa, Dyspharinx (Acuaria) spiralis* in *Acuaria spinosa*. Zajedavci, ki so bili identificirani kot *Acuaria spinosa*, so imeli enake ustnice in nagubano kutikulo. Obe vrsti zajedavca sta imeli po 4 trnaste kordone, ki niso presegali tretjine sprednjega dela požiralnika. Njihov rep je bil kratek in zaokrožen. Jajčeca so bila embrionirana, elipsoidne oblike z debelo lupino, dolga 0,039-0,41 µm in široka 0,025-0,027 µm.
Telesna dolžina prvega zajedavca je bila 32 µm s širino 0,336 µm (maksimalna širina na sredini telesa). Štirje trnasti kordoni so bili dolgi po 0,797 µm, valjasto žrelo pa 0,018 µm. Vulva je ležala posteriorno na sredini telesa, oddaljena 3,56 µm od sprednjega konca in 51-odstotkov skupne dolžine telesa od anteriornega dela. Anus je ležal 0,25 µm od posteriornega dela. Telesna dolžina drugega parazita je bila 34 µm, širina pa 0,367 µm. Trnasti kordoni ki niso presegli tretjine anteriornega dela požiralnika, so bili dolgi 0,809 µm, valjasto žrelo pa 0,021 µm. Vulva je ležala posteriorno od sredine telesa, oddaljena 5,64 µm od anteriornega konca in 57-odstotkov skupne dolžine telesa od anteriornega dela. Anus je ležal 0,28 µm od posteriornega konca.
Po naših podatkih je to prvi primer odkritja *Acuaria spinosa* pri fazanih (*Phasianus colchicus* L.).

Ključne besede: *Acuariidae; Phasianus colchicus; Acuaria spinosa*