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Prevalence and Molecular Characterization of *Anonchotaenia* Species from Quails in Elbehera Governorate, Egypt

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Abstract

Quail meat has gained a reputation as an outstanding source of protein and other essential nutrients, giving it numerous advantages over other poultry species. However, quail production has some limitations. One of them is vulnerability to parasitic infections that produce severe economic losses. Consequently, this study aimed to determine the prevalence and molecular characterization of *Anonchotaenia* species infecting quails in Elbehera Governorate, Egypt. A total of 239 quails were examined for gastrointestinal parasites. The total prevalence of *Anonchotaenia* infection was 0.83%. The prevalence was 1.11% in the Edko district, but no infection was recorded in Rashid. The prevalence of infection in migrant quails was 2.21%, while no infection was recorded in domesticated quails. The prevalence was higher in males than in females. The 18S rRNA sequence of Egypt's *Anonchotaenia* species has 99% identity with *Anonchotaenia brasiliensis*. The phylogenetic tree of the 18S rRNA showed that sequence of *Anonchotaenia* sp. from Egypt is in the same clade as *Anonchotaenia macrocephala* from Brazil and Chile. Molecular characterization using 18S rRNA gene sequencing is valuable for parasitic helminth genetic identification in quails. The results presented a novel member of the genus *Anonchotaenia* in quails from Elbehera governorate, Egypt for the first time.

KEYWORDS
Anonchotaenia species, Molecular Characterization, 18S rRNA, Quails, Elbehera, Egypt

INTRODUCTION

Quail production is considered an important branch of the modern poultry industry. It, like most animal production systems, necessitates continuous improvements in new technologies and sanitary control (Darwish *et al*., 2018; Garcia *et al*., 2002). Quail (*Coturnix coturnix*) is one of the smallest domesticated birds and has several benefits, including resistance to several poultry diseases, a higher ability to benefit from food, high production, low feed intake, a minimal death rate, and highly valuable eggs and meat. Furthermore, they are distinguished by their little primary expenditures and need a small area for farming, indicating a novel poultry production trend (Bashtar *et al*., 2010; Bahar *et al*., 2014).

Migratory quail (*Coturnix coturnix japonica*), also known as common quail, is a migratory bird migrating from Europe to Egypt through the autumn season and acting as a vector, contributing to the ecology and spread of several zoonotic pathogens that threaten human and animal health (Benskin *et al*., 2009). The northern coast of Egypt is a destination for many migratory birds, including quails, from the Saini peninsula in the east to Matrouh in the west. Elbehera governorate has two districts bordering the Mediterranean Coast, namely Edko and Rashid Cities. Several parasites infect the quail's internal organs. Worms are the main parasites that infect quails' digestive tracts (Garcia, 2001; Peterson, 2007). Few studies have investigated quail gastrointestinal parasites in this area of Egypt (Waheeb *et al*., 2022).

Anonchotaenia Cohn, 1900, is the only Paruterinidae species with an unarmed scolex, short, wide proglottids, and a septa-free uterus. It has a global distribution and 27 species that parasitize a wide range of passeriform hosts (Mariaux, 1991). The morphology helps in species differentiation in cestodes. However, the few available morphological characters are easily obscured or distorted in contracted material, making species delimitation within *Anonchotaenia* difficult. Although the number of testes per proglottid varies between conspecifics and even within an individual's strobila, it has traditionally been regarded as the most reliable character for distinguishing *Anonchotaenia* spe-

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cies (Mariaux, 1991). Molecular tools are effective in the identification of cestode species (Foronda *et al*., 2004). The 18S rRNA gene PCR was used in this context (Foronda *et al*., 2004). It was successfully used for the identification of *Anonchotaenia* species (Phillips *et al*., 2014). Cestodes of the genus *Anonchotaenia* have received scant attention and are not well-known among Egyptian birds; furthermore, it was not characterized on the genetic level. Therefore, the aim of this investigation was to determine the prevalence of *Anonchotaenia* species infection in domesticated and wild quails in two districts in Egypt's Elbehera governorate. Furthermore, PCR of the 18S rRNA gene was used to characterize *Anonchotaenia* species.

MATERIALS AND METHODS

Quail samples

From September 2020 to March 2022, 239 quails (94 migratory and 145 domesticated of various species) were collected alive and transported to Damanhur University's Faculty of Veterinary Medicine's Department of Parasitology for further examination.

Collection of helminths

Each bird's digestive tract was dissected and inspected under a dissecting microscope in a Petri dish having physiological saline. The worms were collected using a pasture pipette.

Preparation of permanent mounts

The collected helminths were washed several times with physiological saline to remove debris attached to the parasite. Cestodes were fixed by compressing them between two glass slides and immersing them in a 10% formalin fixative for 24 hours. Then they kept it in the fixative until it stained. The worms were washed with water to remove the fixative and then stained with acetic acid-alum carmine at 1%. At the end of the staining period, the stain remnants will be removed by washing in 30% and then 50% ethyl alcohol. Color differentiation of the samples will be done using an acid-alcohol mixture (1% HCl in 70% ethyl alcohol) under a dissecting microscope. Dehydration occurs by the serial passage of worms for 1-2 hours in ascending grades of alcohol: 30%, 50%, 70%, 80%, 90%, and absolute alcohol. A clearance occurs with xylene. Specimens were mounted in Canada balsam on clean, dry, and labeled slides according to Kruse and Pritchard (1982). The collected cestode species were identified according to Khalil *et al*. (1994). The helminth size was measured by the MCR method (Otify, 2012).

Polymerase chain reaction

G-spinTM Total DNA Extraction Kit, iNtRON Biotechnology, Seoul, Korea, was utilized to extract total genomic DNA. For DNA amplification using PCR, we used COSMO PCR RED Master Mix (W1020300X) (Willowfort, Birmingham, UK) and 1.5 µL of forward (5´-GGGAAATAGAGCAATAACAGGTC-3´) and reverse (5´-GACTTTTACTTCCTCTAAATGATC-3´) primers of the 18S rRNA gene. The primers were designed using the 18S rRNA gene sequence from GenBank (AJ 555174). The PCR amplification reactions were performed in a thermocycler, the TECHNE/TC 3000 (Barloworld Scientific Ltd., Staffordshire, UK). The PCR reaction conditions were as follows: initial denaturation at 95 °C for 2 min and 30 cycles consisting of the denaturation stage at 95 °C for 15 sec, the annealing stage at 55°C for 30 sec, extension at 72°C for 30 sec, and final extension for 10 min. After amplification, PCR products were combined with gel loading buffer and loaded into a 2.5% agarose gel stained with ethidium bromide. They were then electrophoresed in an electrophoresis unit with a 100 bp size marker DNA ladder from Nippon Genetics Europe in Düren, Germany. Electrophoresed gels were then pictured by a gel documentation system, the UVP Photo Doc-It Imaging System (Analytik Jena, CA, USA).

DNA sequencing and phylogenetic analysis

Sequencing was performed at the Animal Health Research Institute, Giza, Egypt. The gel PCR product was purified with the QIAquick Gel Extraction Kit. The sequencing reaction was performed with the Big Dye® Terminator v3.1 Cycle Sequencing Kit. After the run, the data was read with sequence analysis software V5.2. Blasting the sequence was applied with BLASTn at the National Center for Biotechnology Information versus previously published sequence data found at GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Finally, sequence data corresponding to cestodes of birds were downloaded and then applied to a neighbor-joining phylogenetic analysis by Mega 6 software (Tamura *et al*., 2013), including *Anonchotaenia cf. brasiliensis* (KF685938), *A. brasiliensis* (KF685936 and KF685937), *A. macrocephala* (KF685939 and KF685935), *Cladotaenia* sp. (KP241938), *Raillietina tetragona* (MH122786 and MH253901), *Choanotaenia infundibulum* (AJ55517), and *Homo sapiens* 18S rRNA (M10098) outgroup. The sum of branch length was = 0.79753495. The percentage of replicate trees from 500 bootstrap replicates in which the associated taxa clustered together were placed next to the branches. The tree was scaled, with branch lengths in the same units as the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were calculated using the Maximum Composite Likelihood method (Felsenstein, 1985; Tamura *et al*., 2004) and are in base substitutions per site unit. Eleven nucleotide sequences were examined. The analysis included codon positions 1st, 2nd, 3rd, and Noncoding. All positions with gaps and missing data were removed. The final dataset contained 264 positions.

Statistical analysis

To determine the effect of age, gender, and location on infection prevalence, SPSS software version 20 was used. $P < 0.05$ is regarded as significant.

RESULTS

Prevalence of Anonchotaenia species infection

The total prevalence of *Anonchotaenia* species in studied quails was 0.83%. The prevalence of infection in the Edko district was 1.11%, while no infection occurred in the Rashid district (Table 1). The migratory quails had a prevalence of 2.12%, but no infection was recorded in domesticated quails (Table 1). The infection rate in examined males was 0.85%, and that in examined females was 0.81% (Table 1). Locality, sex, and age did not significantly affect the prevalence of infection in quails.

The morphology of Anonchotaenia species

Scolex was globular, without a rostellum or hooks. Suckers were rounded, deep, and muscular. Mature segments were

broader than long, while the gravid ones were longer than wide. Genital pores alternated irregularly. Testes were found in a total of 10–14 per segment posteriorly in two groups. The ovary was compact, sac-like, and globular. The uterus is found as a transversely elongated sac. The par-uterine organ was tubular and spirally curved anterior to the uterus, with densely pigmented tissues covering the anterior end. Eggs moved into a par-uterine organ surrounded by round capsules in the final gravid segment (not shown).

Molecular identification

The primers designed for 18S rRNA used in this study successfully amplified a 360-bp fragment (Fig. 1). Amplicons were sequenced. From the analysis of the NCBI BLAST data concerning scores of identities, similarities, and query coverage, the sequence for the *Anonchotaenia* species was identified. *Anonchotaenia* sp. sequence from Elbehera, Egypt, was registered in GenBank with accession number LC738784. According to a partial 18S rRNA nucleotide sequence, homology analysis revealed that our isolate of *Anonchotaenia* sp. showed 99.31% identity with *Taenia caixuepenggi* in *Ochotona curzoniae* from China (MZ476189), *Taenia laticollis* in a man from Finland (AB731624), and *T. pisiformis* in dogs from China (JQ609339) and Spain (AJ555168), with 99% identity with *Anonchotaenia cf. brasiliensis* in *Ammodramus humeralis* from Brazil (KF685938), 98.86% with *Biuterina* sp. in *Andropadus latirostris* from Gabon (KF685932) and *Taenia hydati-*

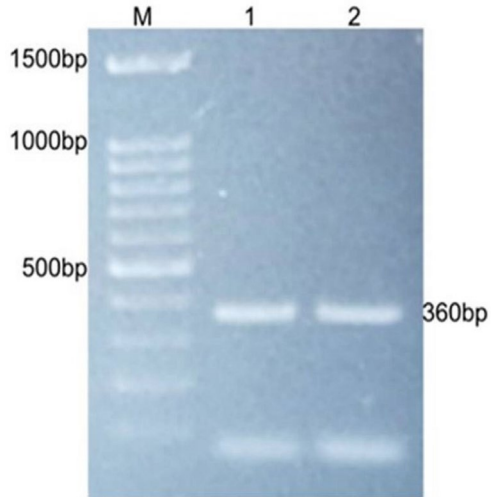

Fig. 1. 18S rRNA gene PCR gel electrophoresis. Lanes 1 and 2, amplified PCR products of the *Anonchotaenia* species 18S rRNA gene bands appeared at 360 bp. M is a molecular DNA ladder size marker of 100 bp.

Table 1. Risk Factors Affecting the Prevalence of *Anonchotaenia* Species Infection in Quails in Elbehera Governorate, Egypt.

Factor		No. examined	No. infected (%)	X^2	P
Locality	Edko	180	2 (1.11)	0.66	0.42
	Rashid	59	0 (0)		
Quail Type	Domesticated	145	0 (0)	3.11	0.08
	Migratory	94	2 (2.12)		
Sex	Male	117	1 (0.85)	0.00	0.98
	Female	122	1 (0.81)		

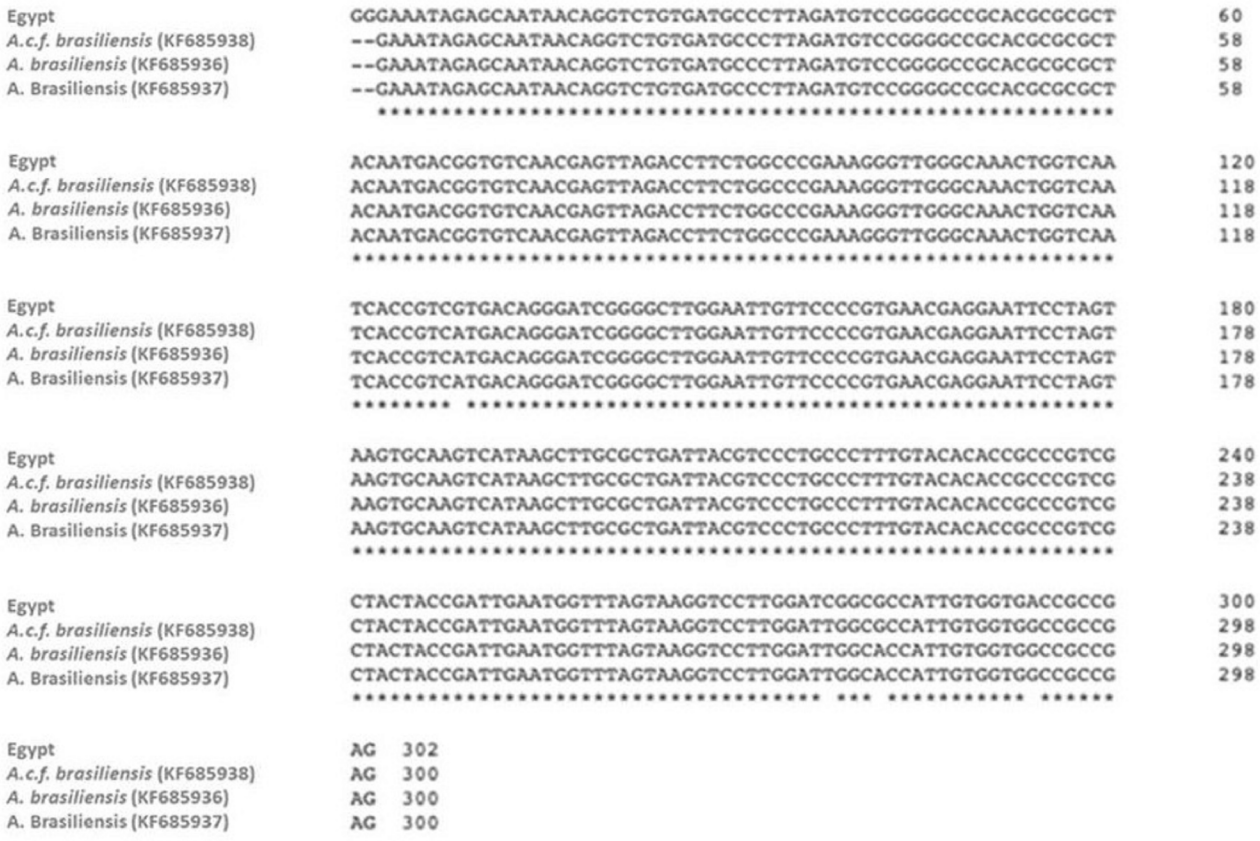

Fig. 2. Alignment of 18S rRNA Gene Sequences of *Anonchotaenia* Species. The other species include *A. cf. brasiliensis* (KF685938) and *A. brasiliensis* (KF685936 and KF685937) from Brazil and Chile. Stars, identical and space, unidentical nucleotides.

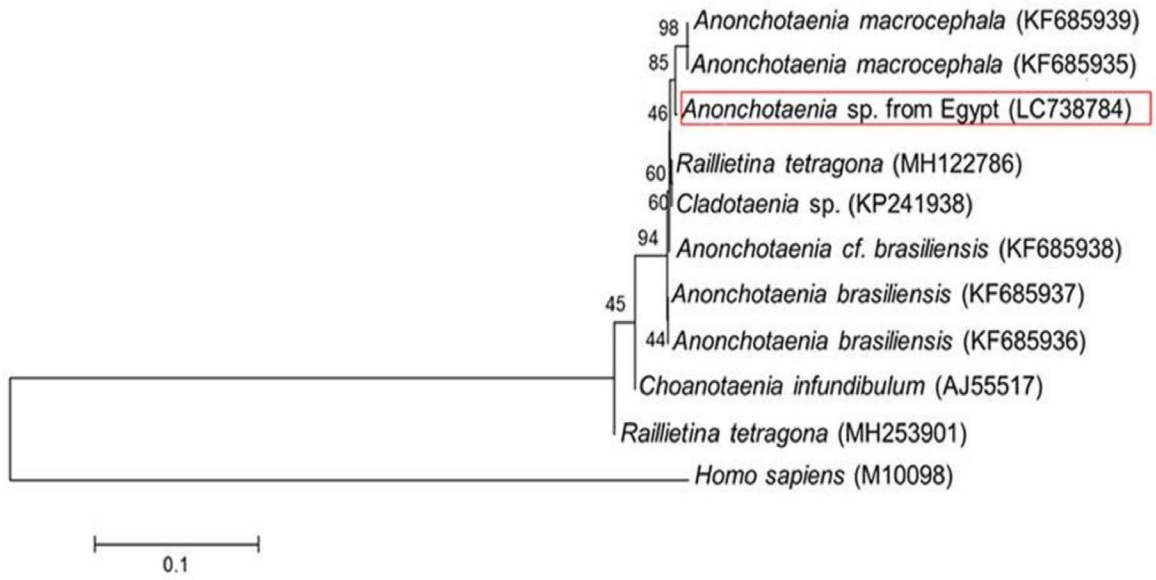

Fig. 3. Neighbor-Joining Phylogenetic Tree of Egyptian *Anonchotaenia* Species' 18S rRNA Gene. The Maximum Composite Likelihood method was used to calculate the evolutionary distances. The percentage of replicate trees from 500 bootstrap replicates that had the associated taxa clustered together and placed next to the branches. The tree was drawn to scale, with branch lengths measured in the same units as the evolutionary distances used to infer the tree.

gena (KX79210, AB731619, and GQ260090), 98.67% identity with *Anonchotaenia brasiliensis* in *Tachyphonus coronatus* (KF685936) and *Thraupis cyanoptera* (KF685937) from Brazil and *Raillitina tetragona* in *Pseudopodoces humilis* (MH122786) from China, 98.33% identity with *Cladotaenia* sp. in an Eagle from China (KP241938.1), *Anoplotaenia dasyuri* in *Sarcophilus harrisii* from Australia (MZ618885, MZ618886, and MZ618887), and 97.67% identity with *Anonchotaenia macrocephala* in *Tachycineta meyeni* from Chile (KF685935) and *Tachycineta leucorrhoa* from Brazil (KF685939). It had an identity of 97.33% with *Thysaniezia ovilla* in sheep (MF158841) and *T. connochaeti* in cattle (MF158839 and MF158840) from Senegal. Sequence alignment of 320 bp of the Egyptian sequence with three species of *Anonchotaenia*, namely *A. cf. brasiliensis* (KF685938) and *A. brasiliensis* (KF685936 and KF685937) using Clustal Omega showed that the common nucleotides were identical except at positions 128 G instead of A, 276 C replaced T, and 280 and 292 G replaced A (Fig. 2). Phylogenetic analysis of the partial 18S rRNA nucleotide sequence of *Anonchotaenia* species placed the current Egyptian isolate in the same clade as other isolates of *A. macrocephala* (KF685935 and KF685939) and closest to *A. cf. brasiliensis* (KF685938) and *A. brasiliensis* (KF685936 and KF685937) from Brazil and Chile (Fig. 3). All isolates in this clade were exclusive to different bird species.

DISCUSSION

Quail production is an important branch of the modern poultry industry. Quail's advantages include resistance to several poultry diseases, a higher food conversion rate, high production, low feed consumption, low mortality, and highly valuable eggs and meat. Furthermore, they are distinguished by low initial costs and small farming areas (El-Ghareeb *et al*., 2009; Darwish *et al*., 2018). There are some small farms rearing quail in Edko and Rashid, Elbehera governorate. The migratory quail is a migrant bird, migrating from the cold northern area of the earth to Egypt in the autumn. It acts as a vector, contributing to the spread of several zoonotic pathogens that threaten human and animal health. Migratory quails visit Egypt's northern coast, particularly the districts of Edko and Rashid, Elbehera governorate. As a result, we decided to investigate quail parasites in domestic and wild quail in these districts. We discovered a cestode belonging to the family Paruterinidae. In this study, *Anonchotaenia* species were only detected in migrant quails but not in domestic ones. This occurs because migratory quails are exposed to intermediate hosts such as snails or insects through migration. These hosts comprise the infective stage of helminths, so the prevalence was higher in the migrant quails than in farmed ones. *Anonchotaenia* sp. morphology agrees with that recorded by Fuhrmann (1908).

One *Anonchotaenia* species was detected in the migratory quail in this study. This was agreed upon with Saxena and Bauch (1978) in India, who discovered one species of *Anonchotaenia, A. globate*, during a *Passer domesticus* examination. Phillips *et al*. (2014) discovered two passerine bird species in Brazil and Chile in South America: The *A. prolixa* sp. n. from *Elaenia albiceps chilensis* and *A. vaslata* sp. n. from *Tyrannus melancholicus*. Furthermore, Mariaux (1991) discovered five *Anonchotaenia* species while studying birds from the Ivory Coast in Africa. This variation could be attributed to geographical distribution, the availability of intermediate hosts, and the number and species of birds examined.

The morphology of the recovered cestode is highly identical to members of the family Paruterinidae, especially members of the genus *Lyuterina*. We use molecular characterization to confirm the genus and remove any doubt of being a member of another genus. We designed primers based on the available sequence in GenBank of one Prautinid cestode, *Lyuterina nigropunctata*. Specific amplification of the 18S rRNA gene using primers showed the band at 360 bp. This is as expected for the designated amplicon size. This agrees with Foronda *et al*. (2004). BLASTn results of our *Anonchotaenia* sp. showed high sequence identity (99%) with *Anonchotaenia cf. brasiliensis* (KF685938) from Brazil, 98.67% similarity with *Raillitina tetragona* (MH122786) and *Anonchotaenia brasiliensis* (KF685936 and KF685937), 98.33% similarity with *Cladotaenia* sp. from Eagle (KP241938), and 97.67% similarity with *A. macrocephala* (KF685935 and KF685939) which were isolated from different bird species and different sites. While no sequences related to *Lyuterina* species were detected. Moreover, we check the similarity of our sequence with the sequence of *Lyuterina* that we used in the design of the primers, but the similarity was lower than 90%. This indicates that the worm is a cestode of birds from the genus *Anonchotaenia*. Phylogenetic analysis showed that *Anonchotaenia* sp. from the current study was placed in the same clade as the isolates of *A. macrocephala* (KF685935 and KF685939) isolated from different bird species and different places in Brazil and Chile. This confirms that the sequence is from the genus *Anonchotaenia*, possibly *A. macroceph-*

ala; however, more research is needed to confirm the species.

CONCLUSION

The study presented the prevalence, risk factors, and molecular characterization of a cestode of quail, *Anonchotaenia* species, for the first time in Elbehera Governorate, Egypt.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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