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MOLECULAR DETECTION OF *ASCARIDIA GALLI* IN LOCAL BREED CHICKENS (*GALLUS GALLUS DOMESTICUS*) IN BAGHDAD CITY

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Abstract

Ascaridia galli is the most prevalent and pathogenic species especially in domestic fowl which causes a disease called ascaridiasis. The present study was carried out to molecular detection of *Ascaridia galli* in the local breed domesticated chickens (*Gallus gallus domesticus*) at Baghdad city by using conventional PCR. A total of 52 adult chicken fecal samples were examined and the overall infection rate was 17.30% (9/52). In conclusion, we think this is the first report for molecular detection of *A. galli* at Baghdad city at Baghdad city and this parasite is still has a high infection rate in adult local breed domesticated chickens.

Keywords: *Ascaridia galli*, *Gallus gallus*, domestic chickens, PCR.

Introduction

Ascaridia galli is a parasitic roundworm belonging to the phylum Nematoda (Tarbiat, 2018) of the genus *Ascaridia* which are an essentially intestinal parasites of birds (Yamaguti, 1961). It is the largest nematode of small intestine of birds (Ramadan and Znada, 1991; Fioretti *et al*., 2005; Garedaghi, 2011). It may cause reduction in growth rate, and weight loss (Reid and Carmon, 1958; Ikeme 1971; Ntekim, 1983; Oniye *et al*., 2000; Jacobs *et al*., 2003; Adang *et al*., 2012), sometimes serious illness, pathological lesions and economic losses in native birds such as hens, turkeys, geese and some other birds (Ramadan, and Znada, 1991). Also It is the most prevalent and pathogenic species especially in domestic fowl (*Gallus domesticus*) and causes Ascaridiasis, a disease of poultry (heavy worm infection), particularly in chickens and turkeys, which It inhabits the small intestine, and can be occasionally seen in commercial eggs (Griffiths, 1978). It is damage the intestinal mucosa of chickens leading to blood loss, secondary infection and occasionally the obstruction of small intestines due to high worm burden (Malatji *et al*., 2019). Heavy infection is the major cause of reduced egg production in poultry husbandry (Reid and Carmon, 1958; Soulsby, 1982; Jacobs *et al*., 2003) and still produces economic losses in modern poultry production system (Fioretti *et al*., 2005; Garedaghi, 2011). The parasite has a direct life cycle and poultry is infected by ingestion of embryonated eggs containing the second larval stage (Fioretti *et al*., 2005). Transport hosts such as earthworms are thought to play a role in transmission of parasite, and hence free range birds tend to have a higher risk of infection (Ramadan and Znada, 1992; Anderson, 2000). It is common for infection of older birds (Ramadan and Znada, 1992; Anderson, 2000), but the greatest degree of damage is often found in birds under 12 weeks of age (Jacobs *et al*., 2003). Infected chickens are suffer from slow growth and decreased weight gain and usually causes severe damage to the intestines due to migration of the worms in the tissue phase of the intestinal mucosa layer, which affects the proliferation of goblet cells in the duodenums (Prastowo and Ariyadi, 2019). There is no protective maternal immunity against *A. galli* infection (Rahimian *et al*., 2017). The objective of the study is conducted to identify for the first time *Ascaridia galli* by molecular diagnosis in local breed chickens (*Gallus gallus domesticus*) at Baghdad city.

Materials and Methods

Samples collection

Fifty two fecal samples (about 5-10 g) were collected from the adult local breed chickens (*Gallus gallus domesticus*) from the different local markets at Baghdad city.

DNA extraction from stool

G-spin DNA extraction kit (Intron Biotechnology, cat.no. 17045) was used for eggs DNA extraction of *Ascaridia galli*.

Primers of *Ascaridia galli*

The sequence information was used and specific primers of *Ascaridia galli* gene were designed (*Ascaridia galli* 18S small subunit ribosomal RNA gene, partial sequence) in conserved regions sequence giving an amplicon with a length of 760 bp and investigated by IDT (Integrated DNA Technology Company, Canada) and the optimal condition has identified for initial denaturation and annealing, also changed the concentration for DNA template between 1.5-2µl (Table 1).

Table 1 : The specific primers *Ascaridia galli* (design primers).

Primers	Sequence	Tm (ºC)	GC (%)	Product size
Forward	5'- AAGGAAGGCAGCAGGCGCG - 3'	60.5	57.9	760 base pair
Reverse	5'- CGTGTTGAGTCAAATTAAGCCGC - 3'	60.2	47.8	

Maxime PCR Pre Mix kit (i-Taq) 20µlrxn (Cat. No. 25025) was used for PCR product and for gene diagnosis used Taq PCR PreMix and the optimum conditions of gene detection initial denaturation (95ºC; 3 min; 1 cycle), denaturation (95ºC; 45 sec; 35 cycle), annealing (64ºC; 45 sec; 35 cycles), extension 1 (72ºC; 45 sec; 35 cycles) and extension 2 (72ºC; 10 min; 1 cycle) with gradient annealing 52, 54, 56, 58, 60 and 62.

Agarose gel electrophoresis of DNA

Electrophoresis has been done to determine DNA pieces (visualization of the PCR product) after the process of extraction or to detect the result of the interaction of PCR during the presence of the standard DNA to distinguish the bundle size of the outcome of the interaction of PCR on the agarose gel (2%) with Red safe nucleic acid stain (Cat. No. 21141) and used standard markers(100bp DNA ladder) Kapa

Universal DNA ladder (cat # KK6302) and visualized under gel UV Transmission (Vilberlourmat –France) for determining the size and quantity of stranded DNA on agarose gel.

Results

Conventional PCR was employed for molecular detection of *Ascaridia galli* in local domesticated chickens (*Gallus gallus domesticus*) at Baghdad city by amplification of 18S ribosomal RNA gene which showed a clear band at 760 bp and the total infection rate was 17.30% (9/52). (Table 1; Figure, 1).

Table 1 : The total infection rate of *Ascaridia galli* (eggs) in local breed chickens (*Gallus gallus domesticus*) at Baghdad city.

No. of samples examined	Positive	Percentage (%)
52	9	17.30

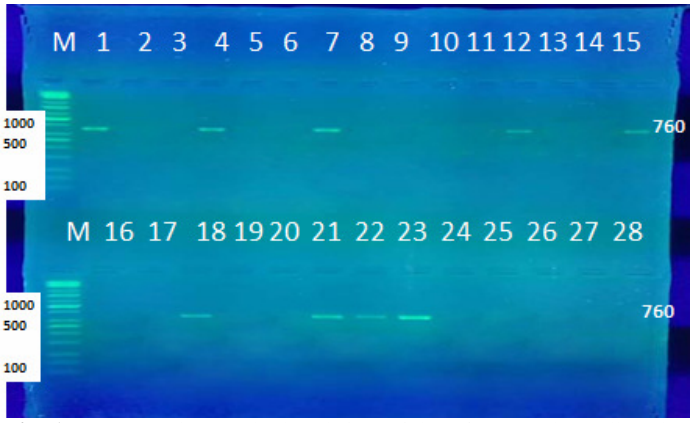

Fig. 1 : PCR product at the band size 760bp of *Ascaridia galli* . The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder (100bp) and the lanes 1, 4, 7, 12, 15, 18, 21, 22 and 23 a positive results.

The positive results were recorded in the GenBank under the accession number MK271771.1, with an identity 99 – 100 % (18S ribosomal RNA gene) with UAS, Japan, Brazil, and China isolates (Table, 2, Fig. 2).

Table 2 : *Ascaridia galli* isolates with NCBI sequence ID: EF180058.1

SOURCE	Identities	Expect	Score	Sequence ID	Range of nucleotide	Nucleotide	Location	Type of substitution	No.
Ascaridia galli 18S ribosomal RNA gene	99%	0.0	1342	ID: EF180058.1	375 to 1131	G>C	477	Trinsvertion	1
						G>C	532	Trinsvertion	
						G>C	633	Trinsvertion	
						T>G	648	Trinsvertion	
						T>G	773	Trinsvertion	
Ascaridia galli 18S ribosomal RNA gene	99%	0.0	1330	ID: EF180058.1	375 to 1131	G>C	477	Trinsvertion	2
						G>C	532	Trinsvertion	
						G>C	633	Trinsvertion	
						T>G	648	Trinsvertion	
						T>G	773	Trinsvertion	
						A>G	1102	Transition	
						T>A	1120	Trinsvertion	
Ascaridia galli 18S ribosomal RNA gene	99%	0.0	1242	ID: EF180058.1	387 to 1087	G>C	477	Trinsvertion	3
						G>C	532	Trinsvertion	
						G>C	633	Trinsvertion	
						T>G	648	Trinsvertion	
						T>G	773	Trinsvertion	
Ascaridia galli 18S ribosomal RNA gene	99%	0.0	1241	ID: EF180058.1	394 to 1093	G>C	532	Trinsvertion	4
						T>G	648	Trinsvertion	
						T>G	773	Trinsvertion	
						T>C	1090	Transition	
Ascaridia galli 18S ribosomal RNA gene	99%	0.0	1254	ID: EF180058.1	394 to 1093	G>C	532	Trinsvertion	5
						T>G	648	Trinsvertion	
Ascaridia galli 18S ribosomal RNA gene	99%	0.0	1330	ID: EF180058.1	375 to 1131	G>C	477	Trinsvertion	6
						G>C	532	Trinsvertion	
						G>C	633	Trinsvertion	
						T>G	648	Trinsvertion	
						T>G	773	Trinsvertion	
						A>G	1102	Transition	
						T>A	1120	Trinsvertion	
Ascaridia galli 18S ribosomal RNA gene	99%	0.0	1254	ID: EF180058.1	394 to 1093	G>C	532	Trinsvertion	7
						T>G	648	Trinsvertion	
Ascaridia galli 18S ribosomal RNA gene	99%	0.0	1254	ID: EF180058.1	394 to 1093	G>C	532	Trinsvertion	8
						T>G	648	Trinsvertion	
Ascaridia galli 18S ribosomal RNA gene	99%	0.0	1342	ID: EF180058.1	375 to 1131	G>C	477	Trinsvertion	9
						G>C	532	Trinsvertion	
						G>C	633	Trinsvertion	
						T>G	648	Trinsvertion	
						T>G	773	Trinsvertion	

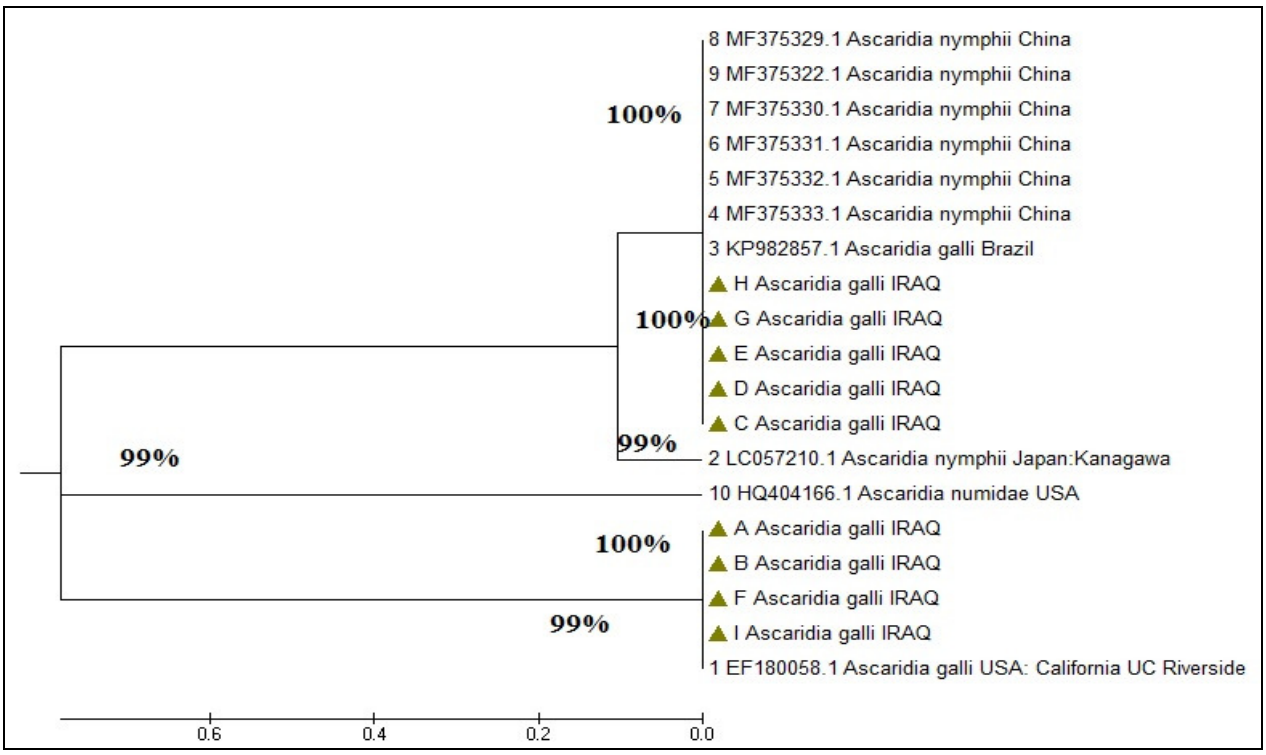

Fig. 2 : Phylogenetic tree of *Ascaridia galli* isolates and eother isolates of the world.

Discussion

Ascaridia galli is the most common nematode and has a worldwide distribution (Permin *et al*., 1997; Ashenafi and Eshetu, 2004; Martin-Pacho *et al*., 2005; Rabbi *et al*., 2006; Abdelqader *et al*., 2008). The present result was showed that the prevalence of *Ascaridia galli* 17.30% in the adult local breed chickens (*Gallus gallus domesticus*) in Baghdad city, that was close or differ from the previous studies that estimate the prevalence or incidence of the parasite before; in Iran refers that the prevalence varies from 10-44% among the native and industrialized poultry at different areas (Eslami, 2006), Ackert (1927) estimate the incidence of parasite 49%, Chand (1967) found 60% in India, Dzido (1973) recorded 28% in Poland , Pal and Ahmed (1985) found an infection rate 26.70% in Pakistan, and in Germany it was 66.6% (Kaufmann and Gauly, 2009). An explanation of the high infection rate of the parasite referred before that parasite eggs are detected in fecal samples in the late stage of worm infection levels and the environmental contamination with are often already high (Tarbiat, 2018), also the parasite has a direct life cycle (Fioretti *et al*., 2005) and the histotrophic phase is a normal part of the parasite life cycle (Herd and McNaught, 1975), which larvae rarely penetrate deep in to intestinal tissue (Ackert, 1923), on the same hand, the spread of transport hosts such as earthworms and free range birds increase the risk of infection of this parasite (Ramadan and Znada, 1992; Anderson, 2000). In addition, It is a common parasite of older birds (Ramadan and Znada, 1992; Anderson, 2000), the infection rate was higher in deep litter system in the cage system (Hemalatha *et al*., 1987), and in the floor and free-range systems, the risk of infection is known to be very high (Permin *et al*., 1997). Also the environmental factors (temperature, relative humidity, pH and oxygen) influencing the fate of *A. galli* eggs within the environment and in domesticated animals, parasite burden has become overwhelming because parasite dispersal, infective stages accumulate in the soil as a result of confinement of animals in pastures and/or pens and the prevalence of parasite infections varies with climatic conditions and husbandry conditions. In free range and litter-based housing systems indoors where the bedding material and available pastures gives an increase in fecal contact (Tarbiat , 2010). Bhalerao (1935) listed before four species *A. lineata, A. gall, A. granulosa* and *A. compar* as parasite of domestic fowl in India and Baylist (1936) considered two of them, viz., *A. lineata* and *A. granulosa* as the synonyms of *A. galli* and the development of reliable methods such as genetic provides powerful tools for dynamic diagnosis of nematodes in poultry and other livestock, so the used of PCR for diagnosis of *A. galli* such as using mtDNA (Katakam *et al*., 2010) and other *Ascaris* (Nejsum *et al*., 2008).

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