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MOLECULAR DIAGNOSIS OF *HAEMOPROTEUS COLUMBAE* IN LOCAL DOMESTIC PIGEONS (*COLUMBA LIVIA DOMESTICA*) IN BAGHDAD CITY

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Abstract

The aim of the present study was estimate the prevalence and molecular detection of *Haemoproteus columbae* in Baghdad city by using 70 (35 males and 35 females) of local domesticated pigeons (*Columba livia domestica*). The result revealed a total infection rate was 20%, which divided into 14.28 (5/35) in males and 25.71% (9/35) in females and the species was documented by PCR and sequencing was *H. columbae*. In conclusion, we think it is the first molecular diagnostic study of *H. columbae* of local domesticated pigeons in Baghdad city.

Keywords : *Haemoproteus columbae*, *Columba livia*, Molecular diagnosis, PCR.

Introduction

The genus *Haemoproteus* includes a large number of intracellular protozoan parasites of birds distributed all over the world. It is the most common blood parasite of birds and has been reported from 67% of total bird species (Burry-Caines and Bennett, 1992). There are about 128 species of *Haemoproteus* (Bennett *et al*., 1994), which are host specific and can be divided into five distinct morphological forms (Bennett and Peirce, 1988). Few species are known to be pathogenic, *H. meleagridis* in turkey, *H. nettionis* in ducks and geese and *H. columbae* in pigeons and doves (Samour, 2008). It have been determined that *H. columbae* is the most common blood parasite of pigeons and the infection rate may be as high as 75%, and it is in ranging from 6 to 86% (Samani *et al*., 2013). Due to the an important of the parasite and there is molecular diagnosis in our knowledge this study was designed.

Materials and Methods

Pigeons

Seventy local domestic pigeons (*Columba livia domestica*) were purchased from the local markets in Baghdad city during the period 1/1/2018 to 1 /1/2019. The pigeons were brought to the parasitology laboratory, College Veterinary Medicine, University of Baghdad for parasitic laboratory examination.

Blood samples collection

About 1ml of ulnar vein wing blood samples of seventy local domestic pigeons were collected (Al-Daraji *et al*., 2008) in a sterile tube with anticoagulant ethylene diamine tetra acetic acid (EDTA), which divided into two parts, the first part about 0,25 ml for thin blood smears and stained with Giemsa stain 10% (Samour, 2008); The slides were examined under light microscope in higher magnification (40X and 100X) for the detection parasite (Zajac and Conboy *et al*., 2012). The parasites identification was done according to Soulsby (1982); Urquhart *et al*. (1996). The second part about 0,75 ml was kept in -20ºC and used for conventional PCR diagnosis (28 samples).

DNA extractionfrom Blood

G-spin DNA extraction kit (intron biotechnology/ Korea cat.no. 17045) was used for DNA extraction from the blood samples according to the manufacturer′s procedure and extracted DNA was stored at -20ºC for genomic analysis.

The primers used in the interaction

Lyophilized primers were dissolved in the free ddH_2O to give a final concentration of 100 pmol/µl as stock solution and keep a stock at -20 to prepare 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free ddH_2O water to reach a final volume 100 µl, was investigated by IDT (Integrated DNA Technologies company, Canada) (Table -1)

Table 1 : The specific primers *Haemoproteus* of large subunit ribosomal RNA gene.

Primers	Sequence (5'->3')	Template strand	Length	Tm	GC%
Forward	CTGCACGAAAGGTGTAACGA	Plus	20	58.51	50.00
Reverse	CCGAGGTGCCAAACCTTTTC	Minus	20	59.69	55.00
Product length	523				

Maxime PCR PreMix kit (i-Taq) 20µlrxn (Cat. No. 25025) was used for PCR product. PCR amplification was carried out in 25 µl reaction mixtures containing 5µl Taq PCR PreMix, 1µl of each primer, 1.5µl of DNAs and 16.5µl D.W.

PCR was performed as follows : initial denaturation at 94ºC for 4 min followed by 35 cycles consisting of denaturation-2 at 94°C for 30 sec, annealing at 60°C for 1 min, extension-1 at 72°C for 1.20 min and final extension-2 at 72°C for 5 min (Multi Gene Opti Max Gradient Thermal Cycler/USA).

Sequencing and Sequence Alignment

The PCR products were separated by electrophoresis (CBS, Scientific/USA) on a 2% agarose gel and they were visualized by exposure to ultra violate light 302 nm (Vilberlourmat / France) staining. A 100 bp DNA ladder (Intron/ Korea) was used as a size reference for PCR assay.

Sequencing of gene was performed for 7 isolates by national instrumentation center for environmental management (nicem) online at (http://nicem.snu.ac.kr/main /?en_skin= index. Html), biotechnology lab, machine is DNA sequencer 3730 XL, Applied Bio system), Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm.nih.gov) and Bio Edit program.

Results

Infection rate

The total infection rate of *Haemoproteus columbae* in local domestic pigeons (*Columba livia domestica*) was 20.00% (14/70) of the staining blood smears by using Giemsa stain. (Table 2 and Figure 1).

Table 2 : Total infection rate of *Haemoproteus columbae* in local domestic pigeons (*Columba livia* domestica).

No. of Samples Examined	Positive	Percentage (%)
70	14	20.00

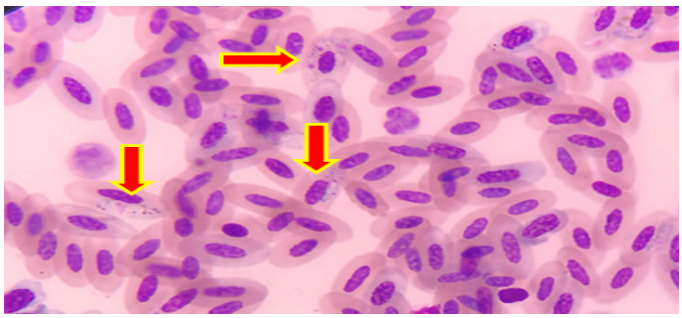

Fig. 1 : *Haemoproteus columbae* (red arrow) in blood smear stained by Giemsa stain (100X)

Infection rate according to sex

Table 3 was showed a higher infection rate of *Haemoproteus columbae* in female pigeons 25.71% (9/35), than male pigeons 14.28% (5/35) with significance (P< 0.01) difference.

Table 3 : Total infection rate of *Haemoproteus columbae* in local domestic pigeons (*Columba livia* domestica) according to sex.

Sex	No. of samples examined	Positive	Percentage (%)
Males	35	5	14.28
Females	35	9	25.71
Total	70	14	20.00
χ^2	13.88*		

*P< 0.01

Figure (2) was show the gel Electrophoresis of the PCR product of *H. columbae* with 523 bp, and the type of substitution (Transition and transvertion) of nucleotide locations of the local isolates with isolates of NCBI ID: EU327518.1 (Table 4).

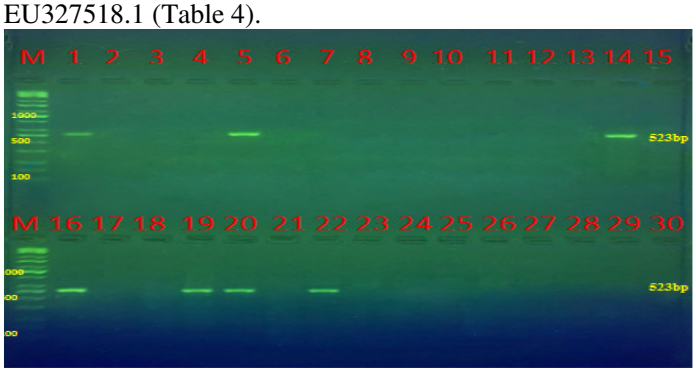

Fig. 2 : PCR products the band size was 523 bp, electrophoresis in 2 % agarose at 5 volt/cm^2 1x TBE for 1:45 hours. The lanes 1,5,14,16,19,20 and 22 a positive results .M: DNA ladder (100)

Table 4 : The type of substitution (Transition and transvertion) of nucleotide locations of the local isolates of *Haemoproteus columbae* with isolates of NCBI ID: EU327518.1.

Source	Identities	Expect	Score	Sequence ID	Nucleotide	Location	Type of substitution	No. of samples
Haemoproteus sp. large subunit ribosomal RNA gene	99%	0.0	740	ID: EU327518.1	T>C	191	Transition	1
					A>G	389	Transition	
Haemoproteus sp. large subunit ribosomal RNA gene	99%	0.0	731	ID: EU327518.1	T>C	191	Transition	2
					G>C	328	Transvertion	
					A>G	389	Transition	
					A>G	465	Transition	
Haemoproteus sp. large subunit ribosomal RNA gene	99%	0.0	763	ID: EU327518.1	C>G	115	Transvertion	3
					G>C	307	Transvertion	
					T>C	474	Transition	
Haemoproteus sp. large subunit ribosomal RNA gene	99%	0.0	715	ID: EU327518.1	C>G	114	Transvertion	4
					T>C	295	Transition	
					G>C	307	Transvertion	
					T>C	474	Transition	
Haemoproteus sp. large subunit ribosomal RNA gene	99%	0.0	699	ID: EU327518.1	T>A	282	Transvertion	5
					T>A	379	Transvertion	
Haemoproteus sp. large subunit ribosomal RNA gene	99%	0.0	683	ID: EU327518.1	A>G	242	Transition	6
					T>A	282	Transvertion	
					T>A	379	Transvertion	
Haemoproteus sp. large subunit ribosomal RNA gene	99%	0.0	643	ID: EU327518.1	T>A	250	Transvertion	7
					T>C	260	Transition	
					T>A	282	Transvertion	
					G>C	330	Transvertion	
					T>A	379	Transvertion	
					A>G	395	Transition	
					A>G	425	Transition	
					T>C	436	Transition	

Seven isolates were identified by NCBI accession numbers (MN 072337; MN 072338; MN 071241; MN 071242; MN 072339; MN 071240 and MN 071243) and version numbers (MN 072337.1; MN 072338.1; MN 071241.1; MN 071242.1; MN 072339.1; MN 071240.1 and MN 071243.1) to build a phylogenetic tree for *H. columbae* with 96 - 99% compatible of USA isolates as shown in Figure (3).

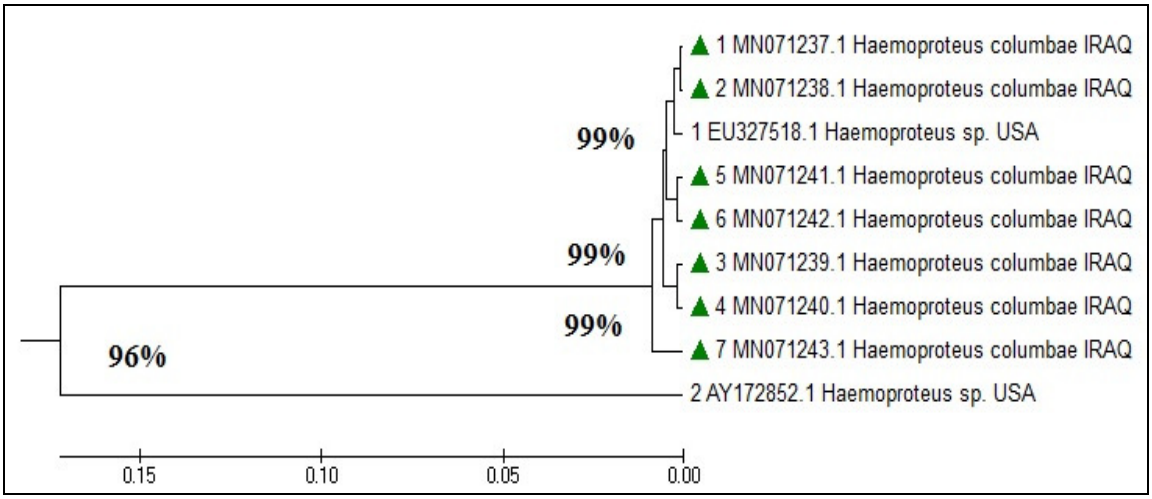

Fig. 3 : Phylogenetic tree of *Haemoproteus columbae* and other isolates in the world.

Discussion

The total infection rate of *Haemoproteus columbae* in the present study was 20.00%, that may be agree or disagree with the previous studies ,which had determined that the most common blood parasite of pigeons; 28% were found by Beadell *et al*. (2004); in total of 3059 birds samples it was found in 31.4% (Fernandez-Davila and Phalen, 2013), Hussein and Abdelrahim (2016) were recorded a high prevalence (57.2%) in 103 pigeons were captured from different localities of Qena Governorate, Egypt, or the infection rate may be as high as 75% (Samani *et al*., 2013), in different areas of Mymensingh district of Bangladesh was 20% (Dey *et al*., 2010), it was 21% in Iran, with the highest infection rate was observed in autumn (44%), while the lowest infection rate (12%) was recorded in spring (Senlik *et al*., 2005). Incidence and parasitaemia of *H. columbae* in pigeon was studied in different localities of Jammu, India for a period from April to September 2010 using thin blood smear examination, of the 150 pigeons (wild: 70, domestic: 80), 92 (61.33 %) were found to be infected, and the domestic pigeon showed higher infection rate (74.28 %) than the wild pigeons (50 %). (Borkataki *et al*., 2015). Interestingly in the present study males were found to have a lower infection rate than females with significant difference, that disagree with Clayton and Moore (1997) who referred that males were more prone to infection that could be due to sex-associated immunologic variations. The agreement or disagreement with the previous studies may be due to the parasite species that can exploit host diversity or abundance are likely to be highly successful in an environment saturated with hosts under favorable environmental conditions for parasite life cycle development (Johnson *et al*., 2013 and Kamiya *et al*., 2014). On the same way, elevational migration of avian haemosporidian parasites from hosts may be capable of transmitting great distances to new ecological habitats, but only provided the vectors necessary to complete the parasite life cycle and a better understanding of vector abundance and diversity will be an important step in the understanding of the evolution and distribution of this parasite (Harrigan *et al*., 2014). The occurrence and incidence of *Haemoproteus columbae* among domestic pigeons requires constant monitoring in order to detect and prevent potential outbreaks with control of the parasite vector.

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Ethics

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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