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. meleagrisidis in turkey, H. nettionis in ducks and geese and H. columbae in pigeons and doves (Samour, 2008). It has 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 examined under light microscope in higher magnification with Giemsa stain 10% (Samour, 2008); The slides were kept in -20ºC and used for conventional PCR diagnosis (28 samples).

DNA extraction from 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 ddH2O 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 ddH2O water to reach a final volume 100 µl, was investigated by IDT (Integrated DNA Technologies company, Canada) (Table -1).

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.

Sequence alignment was performed for 7 isolates by 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.

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

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5’-3’ )</th>
<th>Template strand</th>
<th>Length</th>
<th>Tm</th>
<th>GC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward</td>
<td>CTGCACGAAAAGGTGTAACGA</td>
<td>Plus</td>
<td>20</td>
<td>58.51</td>
<td>50.00</td>
</tr>
<tr>
<td>Reverse</td>
<td>CCGAGGTGCCAAACCTTTTC</td>
<td>Minus</td>
<td>20</td>
<td>59.69</td>
<td>55.00</td>
</tr>
<tr>
<td>Product length</td>
<td>523</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Molecular diagnosis of *Haemoproteus columbae* in local domestic pigeons (*Columba livia domestica*) in Baghdad city

**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*).

<table>
<thead>
<tr>
<th>No. of Samples Examined</th>
<th>Positive</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>14</td>
<td>20.00</td>
</tr>
</tbody>
</table>

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

**Infection rate according to sex**

Table 3 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.

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of samples examined</th>
<th>Positive</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>35</td>
<td>5</td>
<td>14.28</td>
</tr>
<tr>
<td>Females</td>
<td>35</td>
<td>9</td>
<td>25.71</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>14</td>
<td>20.00</td>
</tr>
</tbody>
</table>

χ² = 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 transversion) of nucleotide locations of the local isolates with isolates of NCBI ID: EU327518.1 (Table 4).

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

<table>
<thead>
<tr>
<th>Source</th>
<th>Identities</th>
<th>Expect</th>
<th>Score</th>
<th>Sequence ID</th>
<th>Nucleotide Location</th>
<th>Type of substitution</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoproteus sp. large subunit ribosomal RNA gene</td>
<td>99%</td>
<td>0.0</td>
<td>740</td>
<td>ID: EU327518.1</td>
<td>T&gt;C 191</td>
<td>Transition</td>
<td>1</td>
</tr>
<tr>
<td>Haemoproteus sp. large subunit ribosomal RNA gene</td>
<td>99%</td>
<td>0.0</td>
<td>731</td>
<td>ID: EU327518.1</td>
<td>T&gt;C 191</td>
<td>Transition</td>
<td>2</td>
</tr>
<tr>
<td>Haemoproteus sp. large subunit ribosomal RNA gene</td>
<td>99%</td>
<td>0.0</td>
<td>763</td>
<td>ID: EU327518.1</td>
<td>C&gt;G 115</td>
<td>Transversion</td>
<td>3</td>
</tr>
<tr>
<td>Haemoproteus sp. large subunit ribosomal RNA gene</td>
<td>99%</td>
<td>0.0</td>
<td>715</td>
<td>ID: EU327518.1</td>
<td>T&gt;C 474</td>
<td>Transition</td>
<td>4</td>
</tr>
<tr>
<td>Haemoproteus sp. large subunit ribosomal RNA gene</td>
<td>99%</td>
<td>0.0</td>
<td>699</td>
<td>ID: EU327518.1</td>
<td>T&gt;A 282</td>
<td>Transversion</td>
<td>5</td>
</tr>
<tr>
<td>Haemoproteus sp. large subunit ribosomal RNA gene</td>
<td>99%</td>
<td>0.0</td>
<td>683</td>
<td>ID: EU327518.1</td>
<td>T&gt;A 282</td>
<td>Transversion</td>
<td>6</td>
</tr>
<tr>
<td>Haemoproteus sp. large subunit ribosomal RNA gene</td>
<td>99%</td>
<td>0.0</td>
<td>643</td>
<td>ID: EU327518.1</td>
<td>T&gt;A 250</td>
<td>Transversion</td>
<td>7</td>
</tr>
</tbody>
</table>

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.

Acknowledgement

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Ethics

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

References


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