Detection of *Haemoproteus columbae* in Iranian Pigeons Using PCR

Abbas Doosti, Reza Ahmadi, Zahra Mohammadalipour, and Ali Zohoor

**Abstract**—*Haemoproteus* is a genus of protozoa that occurs greatly in avian populations, usually found in the peripheral blood of hosts from anywhere in the world. It is usually non-pathogenic and in pigeons only causes disease when they are stressed. The aim of this study was to determine the prevalence of *Haemoproteus columbae* in Iranian pigeons by a molecular technique (PCR). A study was done on 220 pigeons from September 2012 to April 2013 in the southwest of Iran. Blood samples were obtained from wing vein. This present study showed that the prevalence rate of *Haemoproteus columbae* was 23.18% (51/120). Considering the lack information about the prevalence of pigeon blood parasites in the southwestern of Iran, more precise molecular surveys seem to be necessary regarding the presence of *Haemoproteus columbae* parasite in Iranian pigeons.

**Keywords**— cyt B gene, *H. columbae*, Iran, PCR.

I. INTRODUCTION

Hemospororins, infect bloodsucking dipterans and some different types of vertebrates. *Haemoproteus* is the largest genus of avian haemosporidian parasites. Over 140 morphologically distinct parasites have been described and classified in two subgenera, *Haemoproteus* and Parahaemoproteus [1]. *Haemoproteus* is a genus of protozoa that is parasitic on birds, reptiles and amphibians. These protozoans parasitize many birds throughout the world and in some bird flocks, between 50–100% of the individuals are infected with these haemospororin blood parasites [2]-[3]. Feral and domestic pigeons are correlated with human habitation, often occupying and befouling buildings where people work and live [4]. Pigeons are distributed everywhere on earth and domesticated for hundreds of years. Pigeons have been used for a long time as a meat resource, laboratory animals, pets or cultural and religious symbols. In Iran, pigeons are kept mainly for pet and showing as fancy pigeons [5]. Also the role of these birds in the transfer of diseases to humans and domestic animals, especially in connection with intensive poultry production, has been well documented. Pigeons may be infected with many species of parasites, including *Hemoprodida* of the genera *Plasmodium*, *Haemoproteus*, *Haemogregarinidae*, *Leucocytozoon* of the genus *Hepatozoon* and *piroplasmids* of the genus *Babesia* [6], some of which are pathogenic to humans [4]-[7]. In columbids seven species of *Haemoproteus* were described: *H. columbae*, *H. sacharovi*, *H. macallumi*, *H. melopeliae*, *H. turtur*, *H. perise* and *H. palumbis* [8].

*H. columbae* is transmitted by Hypoboscidae flies and culicoides mosquitoes. *H. columbae* is widely dispersed in the world, especially in warm and temperate climates. The gamonts of *H. columbae* develop from tiny forms to elongated, crescent-shaped forms, which partially surround the nucleus of the host red blood cells.

The occurrence of *H. columbae* has been studied all over the world. There is very limited number of studies on pigeon blood parasites in Iran [5]-[9]. Thus, the aim of this study was to determine the frequency of *H. columbae* in Iranian pigeons using PCR.

II. MATERIALS AND METHODS

A. Blood Samples Collection

The study was carried out from September 2012 to April 2013, involving 220 free living pigeons from five localities including shahrekord, farsan, kiyar, borujen and lordegan in Chaharmahal Va Bakhtiari province, Iran. Age and weight of all pigeons were recorded. Their body weights ranged from 130 to 230g and their ages were between 6 months to 3 years. All the birds were kept in isomanagerial and nutritional regimen, allowing the water and feed at a temperature of 28°C. Blood samples were obtained from wing vein and thin blood smears were prepared from them. Blood samples were stored in EDTA and kept in refrigerator.

B. Molecular Analysis

Genomic DNA was extracted from 3 ml blood samples using a DNA extraction Kit (Qiagen, Germany), according to the manufacturer's procedure. Extracted DNA was stored at -20°C. For genetic analysis, PCR technique was used to amplify a segment of the parasite cyt b gene.

The primers of PCR reaction for detection of *H. columbae* were as follow, *H. clom-F*: 5'-TTA GAT ACA TGC ATG CAA CTG GTG-3' and *H. clom-R*: 5'-TAG TAA TAA CAG TTG CAC CCC AG-3'. The primers were designed in this study with accession number EU254553. The size of its applicant was 207 base pairs (bp).

PCR amplification was carried out in 25 μl reaction mixtures containing 1 μl DNA template, 1 μl of each primer, 1 μl MgCl, 0.5 μl of dNTPs and 0.25 μl Taq DNA polymerase.

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PCR was performed as follows: initial denaturation at 94°C for 5 min followed by 30 cycles consisting of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 5 min. PCR products were separated by electrophoresis on 1% agarose gels and were visualized under UV light after staining with ethidium bromide and images were obtained by UVI doc gel documentation systems (UK). A 100 bp DNA ladder (Fermentas, Germany) was used as a size reference for PCR assay.

Sterile water and Haemoproteus spp strain ATCC 19698 were used as negative and positive controls, respectively.

III. RESULTS

From 220 blood samples studied in this research 51 (23.18%) cases were identified to be positive for H. columbae infection. Analysis of PCR products for the presence of H. columbae on 1% agarose gel revealed 207 bp fragments (Fig. 1).

Also, 220 pigeons from five locations were divided in to 4 groups where the highest prevalence of H. columbae belonging to the age group of 2-3 year (33.75%). The results showed a high prevalence of H. columbae infection in pigeon in Chaharmahal Va Bakhtiari province. Comprehensive data is shown in Tables 1 and 2.

IV. DISCUSSION

Pigeons can carry ticks, fleas and other parasites [10]-[11]. Blood sucking insects including, louse flies (Hippoboscidae), tabanid flies (Tabanidae) and biting midges (Culicoides) are Haemoproteus transferred infections [12]-[13]. Few species of Haemoproteus are reported to cause clinical disease, including Haemoproteus nettioni, Haemoproteus meleagris and Haemoproteus columbae, in geese and turkeys, ducks, pigeons and doves, respectively [14]-[15]. Studies have shown, loss of body weight, diarrhea, anemia, anorexia, dehydration and finally death could be symptoms of infection with these parasites [5]. The pathogenicity of H. columbae is usually low but an acute form of the infection has been reported in pigeon nestling, in which heavy mortality has been recorded [16].

Aragato in 1908 suggested that only in completely healthy birds an infection by Haemoproteus columbae could be initiated, and pigeons that once had been infected appeared to be relatively immune to disease [17] Studies, to date, have determined that the infection rate ranging from 6% to 86% [9]. This is the first study to compare, the prevalence and intensity rates of parasites among pigeon species in the Chaharmahal Va Bakhtiari province, southwestern part of Iran.

In the current study the frequency of H. columbae was detected in 23.18% of pigeon blood samples by PCR technique while in a research by Gicik et al. no blood parasites were found [18]. These findings further support the idea of a high frequency of this microorganism in pigeon in southwestern of Iran.

Also, Sinlik et al. and Raharimanga et al. have reported that 21% and 19.9% birds were infected with H. columbae in Bursa region and Madagascar, respectively [19]-[20].

Furthermore Mush et al., in Botswana reported that 82% of the pigeons were infected, besides Orjakja and Nweze in Nigeria reported 37.5% pigeons were infected with H. columbae [7]-[4]-[21]. moreover Youssefi et al determined the prevalence of H. columbae from pigeon blood samples in three different locations (Babol, Lahijan and Sirouz Koh) in Iran to be overall 30% [9]. In conclusion, further investigation of prevalence of Haemoproteus columbae in order to adopt control strategies and eradication programs such as vaccination to prevent and reduce economic loss of H. columbae infection in Iranian pigeons it seems to be necessary.

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**Fig. 1** PCR amplification products with 207bp fragments: A. 100bp DNA marker, B. positive control, C. negative control, D-G. Samples from infected and healthy pigeons.

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**Table I**

<table>
<thead>
<tr>
<th>Age</th>
<th>Total number</th>
<th>Positive (%)</th>
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<tbody>
<tr>
<td>6 month to 1 year</td>
<td>44</td>
<td>5 (11.4)</td>
</tr>
<tr>
<td>1 – 1.5 year</td>
<td>50</td>
<td>8 (16)</td>
</tr>
<tr>
<td>1.5 – 2 year</td>
<td>46</td>
<td>11 (23.9)</td>
</tr>
<tr>
<td>2 – 3 year</td>
<td>80</td>
<td>27 (33.7)</td>
</tr>
<tr>
<td>Total</td>
<td>220</td>
<td>51 (23.2)</td>
</tr>
</tbody>
</table>

**Table II**

<table>
<thead>
<tr>
<th>Locality</th>
<th>No. of pigeons</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shahrekord</td>
<td>52</td>
<td>12 (27)</td>
</tr>
<tr>
<td>Farsan</td>
<td>30</td>
<td>9 (30)</td>
</tr>
<tr>
<td>Kiyar</td>
<td>30</td>
<td>6 (20)</td>
</tr>
<tr>
<td>Boroujen</td>
<td>43</td>
<td>10 (23)</td>
</tr>
<tr>
<td>Lordegan</td>
<td>24</td>
<td>5 (21)</td>
</tr>
<tr>
<td>Koohrang</td>
<td>41</td>
<td>9 (22)</td>
</tr>
<tr>
<td>Total</td>
<td>220</td>
<td>51 (23)</td>
</tr>
</tbody>
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