First Record of Avian Plasmodium DNA from Mosquitoes Collected in the Yaeyama Archipelago, Southwestern Border of Japan

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ABSTRACT. We studied the prevalence of avian Plasmodium in 509 mosquitoes from 9 species collected from the Ishigaki and Iriomote islands in the Yaeyama Archipelago, located southwest from the mainland of Japan. Two identical avian Plasmodium lineages were detected from Culex (Culiciomyia) nigropunctatus. Detected lineages were phylogenetically classified into different clade to avian Plasmodium lineages from birds and mosquitoes in the mainland of Japan but identical to a lineage detected from a resident bird, White-breasted Waterken (Amaurornis phoenicuarius). This is the first detection of avian Plasmodium DNA from mosquitoes in the Yaeyama Archipelago and suggested that resident birds might have been infected with an avian Plasmodium lineage specific to the studied area and C. nigropunctatus could be the candidate vector mosquito species.

KEY WORDS: Avian Plasmodium, Culex (Culiciomyia) nigropunctatus, Japan, mosquito, Yaeyama Archipelago.

NOTE Parasitology

Avian Plasmodium spp. are blood protozoa that cause the vector-borne disease of bird malaria on a worldwide scale and are transmitted mainly by mosquitoes of the genera Aedes, Anopheles, Culex and Culiseta [20]. Previous studies in Japan reported that several species of wild and captive birds were infected with various strains of avian Plasmodium [10–12], and some mosquito species were reported as their vectors [2–7]. Elucidating the prevalence and transmission pathways of avian Plasmodium may provide important information for evaluating the risk of parasite infections in the study areas [3, 4]. In the mainland of Japan, the prevalence of avian malaria in birds and mosquitoes has been gradually demonstrated [3–7, 10, 12]; however, the situation in the southwestern border area of Japan remains unknown, except in Minami-Daito Island [2] (Fig. 1).

The Yaeyama Archipelago, including the Ishigaki, Iriomote and Yonaguni Islands, is a part of the Nansei Archipelago in Japan (Fig. 1). Because migratory and stray birds from the Eurasian Continent and areas further south of Taiwan and the Philippines have been observed in the Yaeyama Archipelago [15, 25], such birds could be potential transporters of pathogens. Therefore, these islands bordering mainland Japan might also be regarded as the invasion pathway of immigrant infectious etiological agents of Japanese birds. The introduction of the unusual pathogen (P. relictum) and its vector (Culex quinquefasciatus) resulted in the elimination or serious damage to native wild birds in the Hawaii Islands [22]. Hence, the current prevalence of pathogens in host animals and vectors should be demonstrated to monitor the invasion of new pathogens.

Avian malaria infection of a resident bird, the White-breasted Waterken, was reported in the Yaeyama Archipelago (personal communication); however, possible vector species remain unknown. In this study, to estimate the occurrence of avian Plasmodium transmission in the Yaeyama Archipelago, where many endemic mosquito species are distributed [17], we investigated the prevalence of Plasmodium in mosquitoes in the Ishigaki and Iriomote islands of this archipelago to the far southwest of Japan.

Mosquito samples were collected in the Yaeyama Archipelago of Japan (Fig. 1). In Ishigaki Island (24.20’N, 124.09’E), which geographically belongs to the Yaeyama Archipelago of Japan (Fig. 1), mosquitoes were collected by 2 different methods, sweeping nets and CDC traps, from July 7 to 8, 2008. The sweep net collections were made every 20–30 min in a shaded vegetated area. CDC traps without light but baited with 1 kg of dry ice were used to collect host-seeking mosquitoes. Twenty dry-ice traps were distributed in areas with shade trees and operated from the evening to the early morning. Avian malaria DNA was only detected from one mosquito sample in Ishigaki Island; therefore, we analyzed additional mosquito samples that were collected in Iriomote Island (24.17’N, 123.51’E), which also geographically belongs to the Yaeyama Archipelago of Japan (Fig. 1), to investigate the distribution of ectothermic animal-biting mosquito species by using black-light traps and CDC traps equipped with a portable CD player playing frog calls [9, 19], from April 3 to 7, 2008.

Mosquitoes were identified and divided into species according to Tanaka et al. [17] and Toma and Miyagi [18].

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After identification, all collected mosquitoes were kept at –20°C until processed. Female mosquitoes (n=1–5) of the same species were pooled and DNA was extracted with a REDExtract-N-Amp Tissue polymerase chain reaction (PCR) kit (Sigma-Aldrich, St. Louis, MO) as described previously [2]. Avian malaria parasite DNA was amplified by nested PCR for the partial cyt b gene of the avian malaria mitochondrial genome as described previously [2, 14]. In brief, after DNA extraction, we used DW2 and DW4 primers for the 1st PCR, and APFN and APRN primers for the second. PCR products were subsequently sequenced in both directions using a BigDye terminator mix (Applied Biosystems, Foster City, CA). Phylogenetic analysis of the amplified 470-bp sequences was performed by the neighbor-joining method with the PAUP program (http://paup.csit.fsu.edu/) using the Kimura 2-parameter model to estimate evolutionary distances and 1,000 times bootstrap resampling to assess tree topology.

To estimate the infection rate of the examined mosquitoes, the minimum infection rate (MIR) of each mosquito species was calculated as described previously [23]. MIR has been utilized to estimate an infection rate on the assumption that at least one individual of the pooled sample group is infected. The formula for MIR is as follows: MIR = (number of PCR-positive samples/number of collected specimens) × 1,000.

A total of 509 mosquitoes of the following 9 species were collected: Ae. riversi (n=13); Armigeres subalbatus (n=20); C. bitaeniorynchus (n=36); C. (Culiciomyia) nigropunctatus (n=17); Mansonia crassipes (n=127); M. ochracea (n=1); Orthopodomyia anopheloides (n=32); Uranotaenia macfarlanei (n=205); U. ohamai (n=58); and all collected mosquitoes were classified as unfed according to World Health Organization criteria [24] (Table 1).

Of the DNA samples from C. nigropunctatus, 2 of 118 tested positive for avian malaria by PCR, while the remaining 8 species were all negative (Table 1). One of two avian malaria-positive samples was collected in Ishigaki Island and the other was collected in Iriomote Island (Table 1). The MIR of the avian malaria parasite per 1,000 for C. nigropunctatus was calculated as 117.6 for the Yaeyama Archipelago (2/17) and 90.9 for Iriomote Island (1/11), while for Ishigaki Island, the MIR of parasite per 1,000 for C. nigropunctatus was calculated as 166.7 (1/6), (Table 1). These results are similar to the MIR of avian Plasmodium in C. sasai (MIR: 142.8), the same subgenus mosquito of Culiciomyia as C. nigropunctatus as reported in previous study of Japan [5].

The DNA sequences detected from the 2 positive samples were identical to each other (Fig. 2) and were classified into an apparently different cluster to the avian Plasmodium lineages previously found in birds and mosquitoes in other
gested that 

that this bird species could be the host of the avian 

Ishigaki and Iriomote islands (Fig. 2). Our results suggest

could bite White-breasted Waterken and be

remains unknown whether 

which is

were reported to feed on some bird species and to be posi-

cycles might be complicated.

The present findings indicate probable contact between infected birds and mosquitoes; however, our results are not sufficient to demonstrate the whole transmission cycle of avian Plasmodium in the Yaeyama Archipelago. Further organized sampling and analysis might be able to demonstrate the host birds and vector mosquitoes of avian Plasmodium in these border islands of Japan and might provide essential information for evaluating the risk of parasite infection and for identifying new pathogens of mosquito-borne disease in birds in Japan.

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Table 1. Collected mosquitoes and number of Plasmodium-positive DNA samples in Ishigaki and Iriomote Islands by polymerase chain reaction

<table>
<thead>
<tr>
<th>Study area</th>
<th>Mosquito species</th>
<th>Collected mosquitoes</th>
<th>DNA samples</th>
<th>Positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ishigaki</td>
<td>Ae. riversi</td>
<td>13</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ar. subalbatus</td>
<td>20</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>C. bitaeniorhynchus</td>
<td>24</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>C. nigropunctatus</td>
<td>6</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>M. crassipes</td>
<td>127</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>M. ochracea</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>O. anopheloides</td>
<td>32</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>223</td>
<td>59</td>
<td>1</td>
</tr>
<tr>
<td>Iriomote</td>
<td>C. bitaeniorhynchus</td>
<td>12</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>C. nigropunctatus</td>
<td>11</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>U. macfarlanei</td>
<td>205</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>U. ohamaei</td>
<td>58</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>286</td>
<td>59</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>509</td>
<td>118</td>
<td>2</td>
</tr>
</tbody>
</table>

areas of Japan. This study provides the first account of the detection of avian Plasmodium DNA from C. nigropunctatus. The detected lineages were also identical to the avian Plasmodium (AB601445) found in 2 wild White-breasted Waterken (Amaurornis phoenicurus) birds distributed in the Ishigaki and Iriomote islands (Fig. 2). Our results suggest that this bird species could be the host of the avian Plasmodium detected in C. nigropunctatus. A previous study suggested that C. nigropunctatus feeds on birds [1], but there is no evidence on feeding on White-breasted Waterken. Furthermore, as this study did not investigate the occurrence of oocyst or sporozoite of Plasmodium in C. nigropunctatus, it remains unknown whether C. nigropunctatus has the vector competence of avian Plasmodium. However, C. sasai which is Culicomyia mosquito same as C. nigropunctatus, were reported to feed on some bird species and to be positive for avian Plasmodium DNA [5]. Therefore, C. nigropunctatus could bite White-breasted Waterken and be probable vector species of avian Plasmodium. Two of the other mosquito species collected in this study, C. bitaeniorhynchus and Ar. subalbatus, have been shown to feed on birds [16] and therefore could be vectors of avian Plasmodium [20], but they tested negative for parasite DNA amplification in the present study. Over 50 species of mosquitoes, including those reported as vectors of avian Plasmodium [2, 20], have been recorded in the studied area [9, 17]; however, we have no available samples to be investigated, except for the 9 studied species in the present study.

The White-breasted Waterken, a possible host bird, was reported as a resident species of the Yaeyama Archipelago [15], and C. nigropunctatus, the probable vector mosquito, was often collected in the bottom layers of the island forests (personal communication). These results suggest that the overlap of habitats of the host bird and the vector will provide an opportunity for contact.

Previously, we observed avian malaria-positive mosquitoes in the Minami Daito Island, in the subtropical zone of Japan (Fig. 1), during multiple periods throughout the year; March–July, September and December [2]. In the present study, avian Plasmodium DNA was detected from mosquitoes collected in April and July, suggesting that avian Plasmodium infection occurred concordant with the detection periods of Minami Daito Island. In addition to the climate condition and a variety of mosquito species in the studied sites, Minami Daito Island (Fig. 1) is also used as a resting and wintering site for migratory and/or stray birds travelling from various directions [15, 25]. Therefore, compared to lineages found in the mainland, in the temperate areas of Japan, more variable Plasmodium lineages may be distributed in the Yaeyama Archipelago, and the transmission cycles might be complicated.
Fig. 2. Phylogenetic status of avian Plasmodium lineages detected from mosquito species collected in the Ishigaki and Iriomote islands of Japan. The phylogenetic relationships among the amplified 470-bp sequences of the cyt b gene were found in mosquitoes or birds by using the neighbor-joining method. Numbers in branches indicate bootstrap values of 1,000 replicates. The mosquito and bird silhouettes indicate avian Plasmodium lineages from mosquitoes and birds, respectively. Species names with a gray background indicated lineages found in the Nansei Archipelago of Japan. Avian malaria lineages detected in this study are indicated in the tree within boxes and by a mosquito silhouette within a box. Superscript arabic numerals of accession numbers indicate the following host mosquito species of avian Plasmodium detected in previous studies in Japan [2–7]: 1) Aedes albopictus; 2) Culex (Culiciomyia) sasai; 3) Lt. fuscatus; 4) Lt. vorax; 5) C. pipiens pallens; 6) C. pipiens quinquefasciatus; 7) Coquillettidia sp.. Known avian malaria species, including Plasmodium cathemerium (AY377128), P. circumflex (AF495576) [13], P. elongatum (DQ659588), P. gallinaceum (AB250690), P. juxtanucleare (AB250415), P. nucleophilum (AF254962), P. relictum (AY099041) [21], Haemoproteus lanii (DQ630012), H. sylvae (AY099040), and other avian malaria lineages found recently in birds (AB601432 to AB601437, AB601438, AB601440, AB601441, AB601445) and mosquitoes (AB308044 to AB308048, AB308051, AB308052, AB458849 to AB458851, AB474377, AB474378, AB542064) of Japan were obtained from GenBank for comparison. Leucocytozoon sabrazesi (AB299369) was used as the outgroup in the tree.
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