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High Prevalence Level of Avian Malaria in the Wild Population of the Java Sparrow

Tingginya Tingkat Prevalensi Malaria Burung pada Populasi Liar Gelatik Jawa

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Java sparrow (*Padda oryzativa*) is an endemic bird to Java and Bali. It used to be a very common bird, but due to over exploitation the bird has declined and been classified as Vulnerable (BirdLife International, 2001). In Indonesia bird-keeping is a popular pastime, with deep cultural roots (Jepson and Ladle, 2005). It is widely assumed that the hobby negatively affects wild populations of common as well as threatened birds (Jepson and Ladle, 2005; Nash, 1994), such as Java sparrow.

Other factors have been suggested as threats, i.e. destruction and habitat loss, intensive used of pesticide, and competition with Eurasian Tree sparrow (Balen, 1997; BirdLife International, 2001). So far, however, only the last factor has been studied systematically. These studies found that competition between Java sparrow with Eurasian Tree sparrow were not consistently present. Evidence from Sukawati (Bali), Sukabumi (West Java) and Malang (East Java) clearly suggests the occurrence of competition between the two species (Muchtar and Nurwatha, 2001), but not in Prambanan temples complex (Fanny *et al*., 2006) and Malang (*pers. obs*.) in which the two species were co-existing on the use of nest sites. Therefore further studies on these aspects are suggested to get more comprehensive understanding on the mechanism of the decline of the vulnerable Java sparrow.

Spread of virulent pathogen can have devastating demographic effects and significant impacts on the overall fitness of surviving individuals. For example, introduction of avian malaria and pox virus to Hawaiian Islands (Dobson and May, 1986). A study on blood parasite prevalence in forest bird in South-east Asia found that over 50% of the examined bird species were parasitized by more than one species (Paperna *et al*., 2005). However, little has been known about the level of the prevalence in non-forest bird in this area. This paper reports the finding of prevalence level of avian malaria in the wild population of Java sparrow using the molecular analysis.

Sample

In total, 38 DNA samples were used to assess the prevalence level of avian malaria in the Java sparrow. As comparison two common finches species, i.e. Chesnut Munia (*Lonchura ferruginosa*) and White-headed Munia (*Lonchura maja*), were also assessed, 15 samples respectively.

Molecular Analysis

A nested-PCR assay developed by Hellgren *et al*., (2004) was used which enable to detect in parallel three common genera blood parasites (*Haemaproteus, Plasmodium*, and *Leucocytozoon*) and involves two steps PCR. Firstly, to amplify the cytocrome b of these three genera. PCR included ~50 ng of total DNA, 1.25 mM of each deoxynucleoside triphosphate, 1.5 mM $MgCl_2$, 0.6mM of each primer, and 0.5 units Tag DNA polymerase. The primers used were HaemNFI (5'– CATA TATTAAGAGAAITATGGAG–3') and Haem NR3 (5'– ATAGAAAGATAAGAAATACC ATTC–3').

The product of this PCR was used as template for the second PCR step, respectively 1 μl for *Haemoproteus* spp.- *Plamodium* spp. and for *Leucocytozoon* spp. The primers used to amplify the former parasites were HaemF (5'–ATGGTGCTTTCGATATATGCATG – 3') and HaemR2 (5'– GCATTATCTGGATGTGA

TAATGGT–3') (Bensch *et al*., 2000). Meanwhile the primers for the latter were HaemFL (5'– ATGGTGTTTTAGATACTTAC ATT – 3') and HaemR2L (5'– CATTATCTGG ATGAGATAATGGIGC – 3') (Hellgren *et al*., 2004). These PCR was run separately in 25µl with the same proportion of reagents as in the first PCR reactions. The thermal condition of the PCR was 94 C for 30 sec, 50 C for 30 sec, and 72 C for 45 sec. The samples were incubated before cyclic reaction at 94 C for 3 min and after cyclic reaction at 72 C for 10 min; 20 cycles for the first PCR and 30 cycles for the second one. To ensure consistency of the result 15 of the samples were run three times.

Final PCR products were visualized with electrophoresis, by loading 5 µl of the products and 2 µl of loading dye (Bromophenol Blue) onto a 2% agrose gel. Ethidium bromide (EtBr) was included onto the gels to visualise the DNA. Gels were run in x1 TBE buffer at 45 MA for approximately 25 minutes.

The PCR assay positively detected 11 out of 38 samples (28.95%) for the *Haemaproteus-Plasmodium* parasites, but none for *Lyucocytozoon* in the Java sparrow blood. Meanwhile, both the blood parasites were not detected in either the Chesnut munia (*Lonchura ferruginosa*) nor in the White-headed munia (*Lonchura maja*). The repeatability test consistently produced the same results.

The positive samples then were selected for sequencing either using primer HaemF (for *Haemoproteus* spp.- *Plamodium* spp.). Double strand PCR products were purified by ethanol precipitation or spin column purification (Ultra Clean Tm, MO BIO Inc), prior to cycle sequenced using DYEnamic ET Dye Terminator Kit (MegaBACE). Sequencing products were purified and screened using MegaBACE™ DNA Analysis Systems.

About 450 base pair region was consistently generated from all positive samples sequenced. Searching for similar sequences through NCBI's database (http:// www.ncbi.nlm.nih.gov/blast/) revealed that both Haemoproteus and Plasmodium infected the Java sparrow's blood. The prevalence of infection of the former parasite (23.68%) was higher compared to those of the latter (5.26%).

The sequences of *Haemoproteus* resulting from this study has 97-99% similarity with the published sequences of the same genus in the NCBI's database; and slightly smaller (92-96%) similarity for those of *Plasmodium*. These findings suggest new haplotypes of avian malaria specific to the Java sparrow, consisting of 3 haplotypes of *Haemoproteus* (h.1-3) and 2 haplotypes of *Plasmodium* (h.4-5) (Table 1).

This study found that the prevalence of avian malaria infection in the java sparrow was very high compare to those of the other two common indonesian finches species assayed in this study, and also higher compare to those of the forest bird in java (Paperna *et al*., 2005). Using blood smear method, the latter study found that among 27 bird species of 152 birds assayed the prevalence of infection were between 4.3 - 17% and 0 - 0.4%, respectively for haemoproteus and plasmodiumrespectively, depending on the habitat types. Birds living in lowland forests of java seem to be more susceptible to infection (Paperna *et al*., 2005).

This finding suggests that the java sparrow was more prone to parasite infection, i.e. Avian malaria, compared to the more common finch species, but similar to other lowland forest birds in java. This factor could be another potential threat to bring to extinction of species. However, the impact of this infection to the demography of the Java sparrow is so far unknown. A control experiment may need to be set up to reveal this impact.

Table 1. Haplotypes of avian malaria found in the blood of the Java sparrow (*h:* haplotype; *n*: number of samples).

h.	Sequence	n
1	CTCTTAACCTTTTCCACTTTATTTTAACATTTTTTCTTCCTTATGATACTCCCACTC	5
2	CTTATAACTTCTTACACTTTATTTTAACATTTTCTATCCCTTATGATAATCTCATTT	3
3	TTTTTTTTTATAAACTCATTATAATTCTGACTCTCAATTTAAGCATATTCATTATAT	1
4	TCTTCTATTATTTTTATTACCATTCTCTGACATTCAATCTAAGCAAATTTTTTTTAT	1

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