ABSTRAK

Identifikasi Molekuler *Haemoproteus columbae* pada Burung Merpati (*Columba livia*) di Provinsi Daerah Istimewa Yogyakarta (DIY) menggunakan Standar PCR dan *Nested* PCR

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Haemoproteus columbae (*H. columbae*) merupakan salah satu spesies parasit darah, *avian hemosporidian,* yang paling sering dilaporkan dan telah terdistribusi secara meluas di seluruh dunia. Prevalensi *Haemoproteus sp.* tercatat cukup tinggi, namun gejala klinis yang ditunjukkan tidak spesifik. Morfologi beberapa spesies dari genus *Haemoproteus* sulit dibedakan melalui pemeriksaan apus darah. Analisis molekuler diperlukan untuk meneguhkan identitas spesies *H. columbae.* Gen sitokrom b dari DNA mitokondria (*cytb* mtDNA) telah banyak digunakan untuk meneliti hubungan spesies dari genus dan famili yang sama.

Penelitian ini bertujuan untuk mengetahui sekuen nukleotida dari gen *cytb H. columbae* pada burung merpati asal Daerah Istimewa Yogyakarta (DIY). Darah diekstrasi menggunakan *GeneJET Genomic DNA Purification Kit*. Amplifikasi gen *cytb* dilakukan menggunakan teknik *Polymerase Chain Reaction* (PCR). Primer yang terlibat pada standar PCR adalah HamNF1 dan HaemNR3, sedangkan primer pada *nested* PCR berupa Haem1NFL, Haem1NR, Haem2F, dan Haem2R2. Produk PCR disekuensing dan dianalisis menggunakan program MEGA XI. Pohon filogenetik dikerjakan dengan metode *Negihbor-Joining* dengan nilai *Bootstrap* 1000.

Produk PCR yang dihasilkan dari seluruh sampel *H. columbae* berukuran 525 bp. *Haemoproteus columbae* Kota Yogyakarta, Kabupaten Sleman, Bantul, Gunung Kidul, dan Kulon Progo memiliki kemiripan dengan *H. columbae* South Africa, ditunjukkan dengan jarak genetik sebesar 0%-1%. Teknik *nested* PCR meningkatkan konsentrasi DNA target yang rendah karena *nested* PCR melibatkan dua kali reaksi amplifikasi dengan dua pasang primer spesifik pada gen target.

Kata Kunci: *H. columbae*, *cytb*, esktraksi DNA, *Polymerase Chain Reaction* (PCR), standar PCR, *nested* PCR, sekuensing

ABSTRACT

Molecular Identification of *Haemoproteus columbae* in Pigeons (*Columba livia*) from Special Region of Yogyakarta (DIY) using Standard PCR and Nested PCR

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Haemoproteus columbae (*H. columbae*) is one of the most frequently reported species of blood parasite, *avian hemosporidian*, and has been widely distributed throughout the world. The prevalence of *Haemoproteus sp*. recorded high enough, but the clinical symptoms shown are not specific. The morphology of several species of the *Haemoproteus* genus is difficult to distinguish by blood smear examination. Molecular analysis is needed to confirm the species identity of *H. columbae*. The cytochrome b gene from mitochondrial DNA (cytb mtDNA) has been widely used to investigate the relationship between species of the same genus and family.

This study aims to determine the nucleotide sequence of the cytb *H. columbae* gene in pigeons from the Special Region of Yogyakarta (DIY). Blood was extracted using the GeneJET Genomic DNA Purification Kit. The amplification of the cytb gene was carried out using the Polymerase Chain Reaction (PCR) technique. The primers involved in standard PCR were HamNF1 and HaemNR3, while primers in nested PCR were Haem1NFL, Haem1NR, Haem2F, and Haem2R2. The PCR products were sequenced and analyzed using the MEGA XI program. The phylogenetic tree was carried out using the Negihbor-Joining method with a Bootstrap value of 1000.

The PCR product produced from all *H. columbae* samples was 525 bp in size. *Haemoproteus columbae* Yogyakarta City, Sleman Regency, Bantul, Gunung Kidul, and Kulon Progo have similarities with *H. columbae* South Africa, indicated by genetic distance of 0%-1%. The nested PCR technique increases the concentration of low target DNA because nested PCR involves two amplification reactions with two pairs of primers specific to the target gene.

Keywords: *H. columbae*, cytb, DNA extraction, Polymerase Chain Reaction (PCR), standard PCR, nested PCR, sequencing