Identifikasi spesies parasit malaria menggunakan dna barcode COI cytochrome c oxidase subunit I dan molekul darc duffy antigen receptor for chemokines serta DBP duffy binding protein yang berperan dalam zoonosis malaria = Identification of malaria parasite species through dna barcode coi cytochrome C oxidase subunit I and molecules of darc duffy antigen receptor for chemokines and DBP duffy binding protein playing role in zoonotic malaria

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Abstrak

ABSTRAK
Latar belakang: Zoonosis malaria telah menjadi perhatian komunitas kesehatan dunia setelah adanya laporan kasus di Sarawak pada tahun 2004. Penyakit ini disebabkan oleh parasit malaria satwa primata Plasmodium knowlesi dengan inang alami Macaca fascicularis dan M. nemestrina. Baku emas diagnosis parasit malaria masih berdasarkan pada identifikasi mikroskopik. Selain membutuhkan keahlian yang tinggi, teknik ini terkadang sulit menentukan spesies parasit bila terjadi infeksi campuran dan parasitemia yang sangat rendah. Belakangan diusulkan DNA barcoding, suatu metode identifikasi menggunakan penanda gen sitokrom c oksidase subunit I COI DNA mitokondria untuk spesiasi. Penelitian yang dilakukan bertujuan untuk mengembangkan metode identifikasi spesies parasit menggunakan gen COI sebagai penanda molekul dan mengungkap dasar molekul transmisi zoonosis parasit malaria dengan mempelajari peran gen penyandi protein DARC Duffy Antigen Receptor for Chemokines dan DBP Duffy Binding Protein yang berhubungan dengan invasi sel darah merah.Metode: Verifikasi potensi barcode COI sebagai penanda identifikasi spesies parasit malaria dilakukan dengan studi in-silico, sedangkan validasi penggunaan barcode COI dilakukan dengan analisis sensitivitas dan spesifisitas. Teknologi molekuler PCR-Sequencing dilakukan untuk mengaplikasikan barcode COI pada penapisan parasit malaria di populasi manusia dan satwa primata, serta identifikasi variasi genetik gen penyandi protein DARC dan DBP terutama pada daerah pengikatan ligan parasit dan reseptor inang.Hasil: Studi in-silico menunjukkan bahwa DNA barcoding berpotensi sebagai penanda identifikasi parasit malaria. Primer yang dirancang mengamplifikasi daerah COI sepanjang 670 pb berhasil mengidentifikasi parasit malaria dengan sensitivitas 1 ndash; 3 parasit/ l. Pada penapisan parasit malaria di populasi manusia di Kalimantan Tengah ditemukan 3,34 78/2309 kasus malaria, di mana dua diantaranya adalah kasus malaria knowlesi, yang secara statistik berbeda bermakna bila dibandingkan dengan mikroskopik 2,82 dan 18S rRNA 1,82 . Pada daerah yang sama, penapisan parasit malaria di populasi satwa primata, ditemukan 52,01 168/323 sampel orangutan dan 23,25 10/43 sampel monyet Macaca positif malaria. Spesies parasit yang ditemukan pada orangutan adalah P. species tipe parasit ovale, P. species tipe vivax-cynomolgi, P. species tipe vivax-hylobati dan P. species tipe malariae-inui, sedangkan pada monyet Macaca meliputi P. knowlesi, P. coatneyi, P. inui, juga P. spesies tipe malariae-inui, spesies parasit yang sama ditemukan di orangutan. Studi ini juga menemukan keanekaragaman genetik pada gen penyandi protein Duffy Antigen Receptor for Chemokines manusia maupun satwa primata dan Duffy Binding Protein parasit malaria yang memainkan peran penting dalam invasi parasit malaria.Kesimpulan: Barcode COI dapat secara spesifik dan sensitif mengidentifikasi spesies parasit malaria dan dapat

diaplikasikan sebagai alat identifikasi zoonosis malaria. Terdapat variasi genetik gen penyandi protein Duffy Antigen Receptor for Chemokines dan Duffy Binding Protein yang berhubungan dengan invasi sel darah merah.
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ABSTRACT
Background Zoonotic case of malaria had just come to the attention of public health communities after the Sarawak study in 2004. Zoonotic malaria is caused by Plasmodium knowlesi, primarily a simian malaria parasite in wild long tail macaque Macaca fascicularis and pig tail macaque M. nemestrina as the reservoir hosts. The diagnosis of malaria parasites has mainly relied on the microscopic examination. However, this method is labor intensive, requires an experienced microscopist and difficult in identifying mixed infections in very low parasitemia cases. Recently, DNA barcoding system, which is based on the PCR amplification of a short and highly conserved region of mitochondrial cytochrome c oxidase sub unit I COI has shown to be an invaluable tool for diagnosing and differentiating the species of wide range of organisms. This study was aimed to develop identification tools of malaria parasite by using mtDNA COI gene as a molecular marker and reveal the molecular basis of zoonotic malaria by identifying the genetic variation of protein coding gene of DARC Duffy Antigen Receptor for Chemokines and DBP Duffy Binding Protein that are related to receptor ligand interaction in red blood cell invasion.Methods In silico study was carried out for verifying the potential of DNA barcoding based on the mtDNA COI gene sequence as a marker identification. Sensitivity and specificity analyses were carried out to validate the use of DNA barcoding for medical diagnosis of parasitic infection. Molecular technology of PCR Sequencing was carried out for screening malaria parasit in human and non human primate population and identifying the genetic variation within protein coding gene of DARC and DBP. Results We have initiated a study to explore the use of DNA barcoding for malaria parasite diagnosis through in silico study. We have thus designed primers spanning a 670 bp fragment of the 5 rsquo region of COI gene that could detect parasite isolates as low as 1 3 parasite per l. DNA barcode was used to detect malaria parasite in human population in Central Kalimantan. Of the 2309 subjects, 78 3.34 subjects were malaria positive of which two samples were determined as P. knowlesi infection. The detection rate of COI barcode was significantly higher as compared to microscopic 2.82 and 18S rRNA 1.82 analyses. Of the 366 non human primate samples that include 323 orangutan and 43 macaque 168 orangutan were found to be positive for either P. species ovale type, P. species vivax cynomolgi type, P. species vivax hylobati type and P. species malariae inui type. In macaque, 10 samples were positive for P. knowlesi, P. coatneyi, P. inui and P. species malariae inui type similar to that found in orangutan. The study has also found genetic variation in both human and non human primates Duffy Antigen Receptor for Chemokines and malaria parasite Duffy Binding Protein.Conclusions The study showed that mtDNA COI can be used to diagnose malaria parasites at very low parasitemia level and applied as a diagnosis tool for identification of zoonotic malaria. There is genetic variation in both human and non human primates Duffy Antigen Receptor for Chemokines and malaria parasite Duffy Binding Protein as major determinants for the invasion of malaria parasite.