

DETERMINASI MORFOLOGI MORFOMETRI DAN KARAKTERISASI MOLEKULER *Eimeria zuernii* DI PULAU JAWA

Oleh
Fathur Rohman Haryadi

Abstrak

Eimeria zuernii merupakan parasit saluran pencernaan ternak yang bersifat patogen serta dapat berdampak terhadap penurunan produktivitas pada inang, kerugian ekonomi, bahkan berujung kematian. Deteksi agen melalui metode konvensional memiliki keterbatasan karena kesulitan membedakan morfologi antar spesies. Pengembangan metode diagnosis diperlukan untuk meningkatkan sensitivitas dan spesifitas. Penelitian ini bertujuan untuk mengkaji *Eimeria zuernii* melalui uji morfologi morfometri, uji dan karakterisasi molekuler berdasar marka *Internal Transcribed Spacer-1* (ITS-1). Sampel feses segar sapi potong sebanyak 387 dikoleksi dari beberapa daerah di pulau Jawa (Bandung, Kebumen, Gunung Kidul, Magetan, Bojonegoro, Malang, Bondowoso), dikerjakan secara bertahap melalui metode konvensional (flotasi gula jenuh) dan molekuler. Hasil pengamatan menggunakan mikroskop optilab menunjukkan morfologi berbentuk sub sferis, dinding tipis, tanpa mikropil, berwarna kuning dan keputihan. *Scanning Electron Microscopy* (SEM) menunjukkan morfologi berbentuk bulat (sub sferis), tidak memiliki mikropil, tidak terdapat papila, permukaan halus (sedikit bergelombang). Morfometri oosista terdiri dari panjang (P), lebar (L) dan *shape index* (SI), diukur dengan *software Image Raster 3*. Hasil morfometri sampel penelitian menunjukkan variasi dengan rentang P 11,43-45,14 μm , L 10,72-45,3 μm dan SI 0,81-1,44. Deteksi molekuler menggunakan teknik *nested Polymerase Chain Reaction* (nPCR) dengan dua pasang primer (*outer* dan *inner*). Elektroforesis sampel positif hasil PCR diperoleh ampikon berukuran 344 bp. Analisis hasil sekuensing menggunakan *Basic Local Alignment Search Tool* (BLAST) dan *software Molecular Evolutionary Genetics Analysis* (MEGA) versi 12, menunjukkan bahwa sekuens sampel penelitian dengan sekuens referensi memiliki homologi $\geq 93,10\%$ dan *percent identity* 99,95%. Terjadi variasi perubahan nukleotida seperti transisi, transversi, delesi dan insersi. Rentang jarak genetik sampel penelitian dengan referensi dalam kisaran 0,00-1,23. Konstruksi pohon filogenetik dengan *Maximum likelihood* (ML) menunjukkan sampel penelitian dari tiap daerah membentuk sub kluster tersendiri (mengelompok) dan masih dalam satu nenek moyang dengan sekuens referensi. Penelitian dapat disimpulkan, determinasi *Eimeria zuernii* dapat dideteksi secara konvensional atau molekuler dengan marka ITS-1. Setiap uji bersifat komplementer terhadap uji lain dalam mendukung diagnosa yang tepat dan akurat.

Kata kunci : *Eimeria zuernii*, feses, molekuler, morfologi, morfometri



MORPHOLOGICAL DETERMINATION OF MORPHOMETRIC AND MOLECULAR CHARACTERIZATION OF *Eimeria zuernii* IN JAVA ISLAND

Oleh
Fathur Rohman Haryadi

Abstract

Eimeria zuernii is a pathogenic parasite of the digestive tract of livestock that can result in decreased productivity in the host, economic losses, and even death. Agent detection through conventional methods has limitations due to the difficulty of distinguishing morphology between species. Development of diagnostic methods is needed to increase sensitivity and specificity. This study aims to examine *Eimeria zuernii* through morphometric morphology tests, molecular tests and characterization based on Internal Transcribed Spacer-1 (ITS-1) markers. Fresh feces samples of 387 beef cattle were collected from several areas in Java (Bandung, Kebumen, Gunung Kidul, Magetan, Bojonegoro, Malang, Bondowoso), processed in stages using conventional (saturated sugar flotation) and molecular methods. The results of observations using an optilab microscope showed a sub-spherical morphology, thin walls, without micropyle, yellow and pale in color. Scanning Electron Microscopy (SEM) shows a spherical (sub-spherical) morphology, no micropyle, no papillae, smooth surface (slightly wavy). The oocyst morphometry consists of length (P), width (L) and shape index (SI), measured using Image Raster 3 software. The morphometric results of the research samples showed variations with a range of P 11.43-45.14 μm , L 10.72-45.3 μm and SI 0.81-1.44. Molecular detection using the nested Polymerase Chain Reaction (nPCR) technique with two pairs of primers (outer and inner). Electrophoresis of positive PCR samples obtained an amplicon measuring 344 bp. Analysis of sequencing results using the Basic Local Alignment Search Tool (BLAST) and Molecular Evolutionary Genetics Analysis (MEGA) software version 12, showed that the research sample sequences with reference sequences had homology $\geq 93.10\%$ and percent identity 99.95%. There are variations in nucleotide changes such as transitions, transversions, deletions and insertions. The genetic distance range of the research sample with the reference is in the range of 0.00-1.23. Phylogenetic tree construction with Maximum Likelihood (ML) shows that research samples from each region form their own sub-clusters (grouped) and are still in the same ancestor with the reference sequence. The study concluded that the determination of *Eimeria zuernii* can be detected conventionally or molecularly with the ITS-1 marker. Each test is complementary to other tests in supporting a precise and accurate diagnosis.

Keywords: *Eimeria zuernii*, feces, molecular, morphology, morphometry