Pengembangan Diagnosa Cepat Antraks Berbasis *Polymerase Chain Reaction* (PCR)

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Intisari

Antraks merupakan Penyakit Hewan Menular Strategis (PHMS) prioritas berdasarkan Keputusan Menteri Pertanian Nomer 237/KPTS/PK.400/M/3/2019. Deteksi Antraks di Indonesia selama ini dilakukan dengan uji kultur bakteri, *Polymerase Chain Reaction* (PCR), dan identifikasi morfologi dengan *polychrome methylene blue* (PMB). Uji cepat untuk deteksi Antraks memiliki beberapa kelemahan serta sensitivitas dan spesifisitas pengujian untuk diagnosis Antraks di seluruh Balai Veteriner dan Balai Besar Veteriner di Indonesia belum pernah dilakukan. Penelitian ini bertujuan: 1) mengembangkan metode uji molekuler untuk pengujian cepat Antraks (*singleplex* PCR) berbasis sekuen isolat lokal *Bacillus anthracis*, 2) menghitung sensitivitas dan spesifisitas *singleplex* PCR berbasis sekuen isolat lokal *Bacillus anthracis*, *multiplex* PCR metode Ramisse, dan identifikasi morfologi dengan PMB. Satu pasang primer dirancang menggunakan *software primer3* sesuai dengan sekuen *Bacillus anthracis accession number*: ABLB01000067.1, CP010853.1, LBFE01000 009.1, dan CP0095 43.1 dari *GenBank*. Empat puluh empat sampel terdiri dari 22 isolat lokal *Bacillus anthracis*, 11 *Bacillus cereus* ATCC 11778 dan 11 *Bacillus subtilis* ATCC 6633 mendapatkan perlakuan antara lain isolat *spike* ke tanah, isolat *spike* ke darah sapi dan darah kambing. Sampel diuji menggunakan *singleplex* PCR, *multiplex* PCR, dan identifikasi morfologi dengan PMB. Akurasi masing-masing uji dianalisis dengan sensitifitas dan spesifisitas pengujian. Hasil penelitian menunjukkan *singleplex* PCR berbasis sekuen isolat lokal *Bacillus anthracis* dapat digunakan untuk pengujian cepat Antraks. Uji sensitivitas dan spesifisitas *singleplex* PCR, *multiplex* PCR, dan identifikasi morfologi dengan PMB pada sampel isolat lokal *Bacillus anthracis* diperoleh nilai masing-masing, sebesar 100%. Hasil uji sensitivitas dan spesifisitas *singleplex* PCR dan *multiplex* PCR pada sampel isolat lokal *spike* ke tanah diperoleh nilai masing-masing, sebesar 100%. Hasil uji sensitivitas dan spesifisitas identifikasi morfologi dengan PMB pada sampel isolat lokal, spike ke darah sapi dan darah kambing diperoleh nilai sebesar 100%. Berdasarkan hasil penelitian dapat disimpulkan bahwa: 1) pengujian *singleplex* PCR berbasis sekuen isolat lokal *Bacillus anthracis* dapat digunakan untuk pengujian cepat Antraks, 2) *singleplex* PCR berbasis sekuen isolat lokal *Bacillus anthracis*, *multiplex* PCR metode Ramisse, dan identifikasi morfologi dengan PMB memiliki akurasi yang sangat tinggi untuk pengujian Antraks.

Kata kunci: Antraks, PCR, *Polychrome methylene blue*, Sensitivitas, Spesifisitas

Development of Rapid Test for Anthrax Diagnose Based on Polymerase Chain Reaction (PCR)

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Abstract

Anthrax is a strategic infectious animal disease based on the Decree of The Ministry of Agriculture number 237/KPTS/PK.400/M/3/2019. *Bacillus anthracis* is the aetiological agent of Anthrax, an infectious bacterial disease that affects animals and occasionally humans worldwide (zoonosis). Bacterial culture, polymerase chain reaction (PCR), and blood smears stained with aged Polychrome Methylene Blue (PMB) are used to identifying the bacterium in Indonesia. A fast diagnosis of Anthrax can be made in the field with blood smears stained with PMB, these methods have significant technical drawbacks and sensitivity and specificity PCR testing and morphological identification with PMB for Anthrax diagnose in all Disease Investigation Centers in Indonesia have never been conducted. This study aimed to developed a molecular method for Anthrax rapid based on the *Bacillus anthracis* local isolate used for singleplex PCR testing, calculating the sensitivity and specificity of singleplex PCR based on the *Bacillus anthracis* local isolate, calculating the sensitivity and specificity of multiplex PCR Ramisse's method, and calculating the sensitivity and specificity of morphological identification with PMB. One pair of primers is designed using primer3 software by following the *Bacillus anthracis* accession number sequence: ABLB010000 67.1, CP010853.1, LBFE01000 009.1 and CP0095 43.1 from GenBank. Forty-four samples consisted of 22 *Bacillus anthracis* local isolates, 11 *Bacillus cereus* ATCC 11778, and 11 *Bacillus subtilis* ATCC 6633 received several treatments including spike isolates to the soil, spike isolates into cow's blood and goat's blood. Both pure and treated isolates were tested using singleplex PCR, multiplex PCR, and morphological identification with PMB. The test results are tabulated and a 2x2 table is made. The acuration of each tests is used for analize the sensitivity and specificity. The results showed that singleplex PCR based on the *Bacillus anthracis* local isolate can be used for Anthrax rapid test; singleplex PCR based on the *Bacillus anthracis* local isolate, multiplex PCR Ramisse's method, and morphological identification with PMB in isolate had a sensitivity and specificity value of each test was 100%. The sensitivity and specificity test results of singleplex PCR and multiplex PCR on spike isolate samples to the soil obtained value of each test was 100%. The results of sensitivity and specificity of morphological identification with PMB on spike isolate samples into cow's blood and goat's blood obtained value of each test was 100%. It was concluded that singleplex PCR based on the *Bacillus anthracis* local isolate can be used for Anthrax rapid test, singleplex PCR based on the *Bacillus anthracis* local isolate, multiplex PCR Ramisse's method and morphological identification with PMB has very high acuration.

Keywords: Anthrax, PCR, Polychrome methylene blue, Sensitivity, Specificity