

## KARAKTERISASI STAPHYLOCOCCUS DAN HAPTOGLOBIN SEBAGAI DASAR PENGEMBANGAN DIAGNOSTIK MASTITIS PADA KAMBING PERANAKAN ETAWA

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### INTISARI

Mastitis subklinis mempunyai dampak kerugian ekonomi yang cukup tinggi, yaitu penurunan kuantitas produksi susu secara drastis, dan diikuti dengan penurunan kualitas susu. Metode diagnosa mastitis yang sering digunakan adalah dengan uji penghitungan *Somatic Cell Count* (SCC) dan *California Mastitis Test* (CMT), namun memiliki kelemahan menghasilkan hasil positif maupun negatif palsu. Haptoglobin adalah protein fase akut yang konsentrasinya meningkat dengan cepat ketika terjadi infeksi, sehingga dapat digunakan untuk sarana deteksi bakterial mastitis subklinis pada kambing PE. Penelitian ini bertujuan untuk mengembangkan sarana deteksi *Staphylococcal* mastitis subklinis pada kambing PE berdasar biosensor haptoglobin.

Penelitian dilakukan melalui tiga tahap. Penelitian pada tahap pertama, dilakukan isolasi *staphylococcus* pada 105 sampel susu yang dikoleksi dari 58 ekor kambing PE dari sentra peternakan di wilayah DIY. Identifikasi dan karakterisasi fenotipik *staphylococcus* ditentukan berdasarkan pewarnaan Gram, *mannitol salt agar* (MSA), katalase, dan uji *Voges-Proskauer* (VP). Identifikasi genotipik ditentukan berdasarkan deteksi keberadaan gen 23SrRNA, *nuc* dan *coa* menggunakan primer spesifik untuk *Staphylococcus aureus* dengan *polymerase chain reaction* (PCR) dan dikonfirmasi menggunakan metode *sequencing* untuk membedakan *Staphylococcus aureus* dengan *staphylococcus* yang lain. Pada penelitian tahap kedua, dilakukan karakterisasi molekular haptoglobin dari sel somatik susu kambing PE berdasarkan *sodium dodecyl sulphate polyacrilamid gel electrophoresis* (SDS-PAGE), dan *one step RT-PCR*. Penelitian tahap ketiga, dilakukan uji sensitivitas dan spesifitas haptoglobin sebagai biomarker deteksi mastitis subklinis melalui uji serologis *enzyme linkage immunosorbent assay* (ELISA). Haptoglobin yang telah diisolasi dan dipurifikasi, digunakan untuk memproduksi antibodi poliklonal anti-haptoglobin pada mencit *balb/c*. Produksi haptoglobin dan anti-haptoglobin digunakan sebagai sarana deteksi 59 sampel susu kambing PE di lapangan melalui uji ELISA *antigen capture*, dengan membandingkan uji CMT dan SCC serta konfirmasi keberadaan patogen dengan PCR. Akurasi hasil uji biosensor haptoglobin ditentukan melalui uji sensitifitas dan spesifitas, serta uji *Cohen's Kappa*.

Dari hasil penelitian diketahui bahwa dari 105 sampel susu kambing PE terdapat 26 isolat secara fenotipik teridentifikasi sebagai *Staphylococcus aureus*. Berdasarkan identifikasi molekular dari 26 isolat, terdapat 9 isolat positif terdeteksi gen

23S rRNA, gen *nuc* dan *coa* yang mengindikasikan sebagai spesies spesifik *Staphylococcus aureus*. Haptoglobin (Hp) dapat diidentifikasi dari sel somatik susu kambing PE mastitis klinis maupun subklinis dengan berat molekul 18 kDa dan 32 kDa. Berdasarkan hasil *one step* RT-PCR dapat diidentifikasi mRNA haptoglobin dari sel somatik susu kambing PE yang mengalami mastitis klinis maupun subklinis dengan ukuran 174 bp. Hasil uji terhadap 59 sampel susu kambing PE di lapangan dengan uji SCC, CMT dan biosensor haptoglobin diperoleh hasil sebagai berikut: Nilai terendah haptoglobin dengan konsentrasi  $104,19 \pm 18,83$  mg/mL, diperoleh pada susu dengan uji CMT skor 0 dengan jumlah SCC  $0,67 \pm 0,23 \times 10^6$  sel/mL. Nilai haptoglobin tertinggi dengan konsentrasi  $686,66 \pm 140,70$  mg/mL, diperoleh pada susu dengan uji CMT skor 3 dengan jumlah SCC  $5,12 \pm 2,06^6$  sel/mL. Bakteri *Staphylococci* memegang peranan penting dalam meningkatkan konsentrasi haptoglobin, berdasarkan konfirmasi PCR terdapat 52,54% *Coagulase Negative Staphylococcus* (CNS) dan sebanyak 30,51% *Staphylococcus aureus*. Berdasarkan hasil uji sensitifitas dan spesifitas, biosensor haptoglobin lebih spesifik dibandingkan SCC dalam mendeteksi *staphylococcal mastitis* subklinis sebesar 73% dengan sensitivitas 79%. Berdasarkan persentase nilai koefisien Kappa (*Cohen's Kappa*), kombinasi penggunaan biosensor haptoglobin dan uji CMT memiliki angka kepercayaan tertinggi (85%). Dalam melakukan deteksi mastitis subklinis pada kambing PE, CMT dapat digunakan sebagai uji skrining mastitis, dilanjutkan uji biosensor haptoglobin untuk menentukan *staphylococcal mastitis* subklinis secara lebih tepat.

Kata kunci: Mastitis subklinis, *Staphylococcus aureus*, *Coagulase Negative Staphylococcus* (CNS), kambing PE, haptoglobin

## CHARACTERIZATION OF STAPHYLOCOCCUS AND HAPTOGLOBIN AS BASIC DEVELOPMENT DIAGNOSTIC MASTITIS OF ETTAWA CROSSBRED GOATS

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### ABSTRACT

Subclinical mastitis causes economic losses, due to decrease in the quantity and quality of milk production. Diagnosis of subclinical mastitis cases routinely used *Somatic cell count* (SCC) test and *California mastitis test* (CMT), but both of these diagnostic methods have low accuracy due to possible false positive and negative results. Haptoglobin is an acute phase protein that will rapidly increase during inflammatory process, therefore haptoglobin could be used as a marker for detection of bacterial subclinical mastitis in PE goat. The aim of this study was to developed detection of *Staphylococcal* subclinical mastitis based on haptoglobin biosensor

This study was conducted through three steps. The first step of study was to isolate *Staphylococcus* from 105 milk samples of 58 PE goats in DIY. Phenotypic Identification and characterization were performed based on the Gram staining, *Mannitol salt agar* (MSA), catalase, and *Voges-Proskauer* (VP) tests. Genotypic identification were determined for detection of 23SrRNA, *nuc* and *coa* genes by using species specific primers for *Staphylococcus aureus* with *polymerase chain reaction* (PCR) and confirmed with *sequencing* method to differentiate *Staphylococcus aureus* with another strain *Staphylococcus*. The second step of study were molecular characterization haptoglobin isolated from milk somatic cell based on *sodium dodecyl sulphate polyacrilamid gel electrophoresis* (SDS-PAGE), and *one-step* RT-PCR. The Third step was designed to analyse the sensitivity and specificity tests of haptoglobin as biomarker for subclinical mastitis detection with serological test by *enzyme linkage immunosorbent assay* (ELISA). Purified haptoglobin was used to produce anti-haptoglobin polyclonal antibodies in *balb / c* mice. Haptoglobin and anti-haptoglobin were used for detecting 59 samples of goat milk of PE in the field through ELISA antigen capture test, by comparing CMT and SCC test and confirmation of pathogen with PCR. The accuracy of the haptoglobin biosensor was determined by the sensitivity and specificity test, as well as the *Cohen's Kappa test*.

Of the 105 milk samples collected from 58 PE goats, there were 26 isolates confirmed phenotypically as *Staphylococcus aureus* however, based on the molecular characterization there were 9 isolates positive for 23SrRNA gene, *nuc* and *coa* genes, indicated specific species of *Staphylococcus aureus*. Haptoglobin were identified from milk somatic cell PE goat both clinical mastitis and subclinical mastitis with molecular weight of about 18 kDa and 32 kDa. Based on One Step RT-PCR, the mRNA

haptoglobin were identified with a target size of 174 bp. A total of 59 samples of field milk PE goats were tested by using anti-haptoglobin ELISA antigen capture as well as CMT and SCC test. The lowest haptoglobin concentration ( $104,19 \pm 18.83$  mg/mL) was detected in the group of milk goat with CMT score 0, and SCC  $0,67 \pm 0.23 \times 10^6$  cells/mL. The highest haptoglobin concentration ( $686,66 \pm 140$  mg/mL) was detected in the group with CMT score 3 and SCC  $5,12 \pm 2.06 \times 10^6$  cells/mL. *Staphylococci* play a role in increasing the concentration of haptoglobin, based on the PCR confirmation of their presence of 52.54% *Coagulase Negative Staphylococcus* (CNS) and 30.51% *Staphylococcus aureus*. According to the sensitivity and specificity tests, haptoglobin biosensors were more specific than SCC for detecting *Staphylococcal* subclinical mastitis with the specificity value of 73% and sensitivity value 79%. Based on the percentage of coefficient value Kappa (*Cohen's Kappa*), the combination of haptoglobin biosensor and CMT test has the highest confidence value of 85%. From this study can be concluded that for detecting subclinical mastitis in PE goats, CMT can be used as a mastitis screening test, followed by haptoglobin biosensor assays to determine the *Staphylococcal* subclinical mastitis more precisely.

Keywords : Subclinical mastitis, *Staphylococcus aureus*, *Coagulase Negative Staphylococcus* (CNS), haptoglobin, Ettawa crossbred goat