

PENGARUH SIFAT DEPENDENSI NAD DENGAN TINGKAT VIRULENSI, UJI SENSITIVITAS TERHADAP ANTIBIOTIK SERTA HUBUNGAN KEKERABATAN BERDASARKAN SEKUEN GEN HEMAGGLUTININ DARI *Avibacterium paragallinarum* ISOLAT AYAM PETELUR DI DAERAH ISTIMEWA YOGYAKARTA

INTISARI

Infectious coryza (snot) adalah penyakit pernafasan bagian atas pada unggas yang disebabkan oleh *Avibacterium paragallinarum*, yang bersifat membutuhkan *nicotinamide adenine dinucleotide* (NAD-dependen) atau tidak (NAD-independen) dan dibagi menjadi serotipe A, B dan C. Penelitian ini bertujuan untuk mengetahui sifat NAD, penentuan serotipe, sensitivitas *A. paragallinarum* terhadap antibiotik, pengaruh sifat dependensi NAD terhadap tingkat virulensi *A. paragallinarum* serta mengetahui kedekatan genetik berdasarkan gen hemagglutinin (*hagA*) dengan isolat referen Page. Sampel penelitian sebanyak 30 ekor ayam petelur yang menunjukkan gejala snot (kebengkakan wajah dan adanya leleran nasal berbau busuk) diperoleh dari peternakan di Sleman (10 ekor), Gunung Kidul (6 ekor), Bantul (6 ekor) dan Kulon Progo (8 ekor). Tahapan pertama penelitian yaitu swab nasal ayam dikultur pada media plat agar coklat kemudian dilakukan isolasi dan identifikasi dengan uji biokimiawi, pengamatan gejala klinis dan perubahan patologi pada sampel. Uji koloni satelit untuk mengetahui sifat dependensi terhadap NAD dilakukan pada media plat agar darah dan dikultur silang dengan *Staphylococcus aureus*. Penentuan serotipe isolat *A. paragallinarum* dengan uji hemagglutinasinya (HI). Sebanyak 21 dari 30 sampel teridentifikasi *A. paragallinarum*. Hasil uji koloni satelit teridentifikasi delapan sampel NAD-dependen dan 13 sampel NAD-independen. Seluruh sampel merupakan serotipe A. Tahapan kedua yaitu uji patogenisitas menggunakan 30 ekor ayam petelur betina strain Lohmann berumur 5 minggu yang dibagi menjadi 3 kelompok (kontrol, NAD-dependen dan NAD-independen), diinfeksi antigen konsentrasi 6×10^8 CFU/ml (McFarland No. 2) sebanyak 0,2 ml/ekor, pengamatan gejala klinis selama 10 hari dilanjutkan nekropsis, isolasi ulang dan pemeriksaan patologis pada sinus infraorbitalis, kulit fasial dan trakea. Semua ayam coba dari dua kelompok yang diinfeksi *A. paragallinarum* menunjukkan gejala klinis snot yang tidak sama. Tidak ada perbedaan yang bermakna pada skor gejala klinis ($p > 0,05$), perubahan makroskopik ($p > 0,05$) dan mikroskopik ($p > 0,05$) antara kelompok NAD-dependen dengan kelompok NAD-independen. Tahapan ketiga yaitu uji sensitivitas *A. paragallinarum* terhadap antibiotik dan uji PCR untuk deteksi gen *hagA* dilanjutkan sekuensing. *Avibacterium paragallinarum* bersifat sensitif terhadap ampisilin dan amoksisilin (100%) serta kloramfenikol (90,48%). Resisten terhadap streptomisin (80,95%) dan doksisisiklin (71,43%). Bersifat intermediet terhadap enrofloksasin (80,95%). Hasil analisis filogenetik sekuen gen *hagA* menunjukkan delapan sampel berada dalam satu kluster dan memiliki jarak yang dekat dengan *A. paragallinarum* isolat referen Page A, isolat Kume serotipe A-1, A-3 dan C-3 serta isolat serotipe A dari Cina dan Korea Selatan.

Kata kunci: *Avibacterium paragallinarum*, NAD, uji sensitivitas antibiotik, uji patogenisitas, *hagA*

THE RELATIONSHIP OF NAD-DEPENDENCE WITH VIRULENCE, ANTIMICROBIAL SUSCEPTIBILITY TEST AND PHYLOGENETIC RELATIONSHIP BASED ON HEMAGGLUTININ GENES OF *Avibacterium paragallinarum* FROM LAYER ISOLATES IN THE SPECIAL REGION OF YOGYAKARTA

ABSTRACT

Infectious coryza (snot) is an upper respiratory-tract disease in birds caused by *Avibacterium paragallinarum*, which can either be nicotinamide adenine dinucleotide dependent (NAD-dependent) or not (NAD-independent), and can be classified into 3 serotypes (A, B, and C). This study aimed to identify the characteristics of NAD, serotype, the sensitivity of *A. paragallinarum* to antibiotics, the impact of NAD-dependence towards the virulence of *A. paragallinarum*, and phylogenetic relationship based on the hemagglutinin gene (*hagA*) with isolate reference Page. This study utilized samples of 30 layers with symptoms of snot (swollen face with odorous nasal discharge) from various poultry farms in Sleman (10 layers), Gunung Kidul (6 layers), Bantul (6 layers), and Kulon Progo (8 layers). In the first stage of the study, the nasal discharge from the chickens was cultured in a chocolate agar plate before isolated and identified through biochemical testing, clinical symptoms and pathological changes of the samples were observed. Satellite colony testing was conducted on the blood agar plate and was cross-cultured using *Staphylococcus aureus*. Serotyping of *A. paragallinarum* was conducted through hemagglutination inhibition (HI) test. 21 of the 30 samples were identified as *A. paragallinarum*, with 8 samples being NAD-dependent and 13 samples being NAD-independent, and 21 samples being serotype A. The second stage, namely pathogenicity test, utilized 30 layers of *Lohmann strain* aged 5 weeks that were divided into 3 groups, (control, NAD-dependent, and NAD-independent). The chickens were infected with concentrate antigen 6×10^8 CFU/ml (McFarland No. 2) with the dose of 0.2 ml/bird, and clinical symptoms were observed for 10 days before the implementation of necropsy, reisolation, and pathological examination of the infraorbital sinus, facial skin, and trachea. There was no significant difference in the clinical symptom score, macroscopic change, and microscopic change ($p > 0.05$) between the NAD-dependent and NAD-independent groups. The third stage tested the sensitivity of *A. paragallinarum* to antibiotics, PCR test for testing the *hagA* gene, followed by sequencing. *Avibacterium paragallinarum* was discovered to be sensitive to ampicillin and amoxicillin (100%) and chloramphenicol (90.48%); resistant to streptomycin (80.95%) and doxycycline (71.43%); while having intermediate sensitivity to enrofloxacin (80.95%). The result of phylogenetic sequencing of *hagA* gene shown that eight of the samples belong to one cluster and one branch with the *A. paragallinarum* reference isolate Page A, isolate Kume A-1, A-3, C-3 and serotype A from China and South Korea.

Keywords: *Avibacterium paragallinarum*, NAD, antimicrobial susceptibility test, pathogenicity test, *hagA*