PENDEKATAN DIAGNOSIS IMUNOLOGIS DAN MOLEKULER *AVIAN INFLUENZA VIRUS* DAN *NEWCASTLE DISEASE VIRUS* PADA KASUS LAPANGAN

ABSTRAK

Unggas tidak hanya dapat terinfeksi oleh satu jenis virus, tetapi dapat juga terinfeksi oleh lebih dari satu jenis virus. Virus *avian influenza* dan *Newcastle disease* adalah dua patogen penting pada unggas yang dapat menyebabkan wabah berulang dan seringkali menimbulkan gejala klinis dan lesi patologis yang serupa pada unggas dengan morbiditas dan mortalitas tinggi sehingga menyebabkan kerugian ekonomis yang besar pada industri perunggasan. Penelitian ini bertujuan untuk mendeteksi dan membedakan virus patogenik pada ayam petelur komersial dengan gejala klinis serupa, terutama tortikolis dan paralisis kaki serta lesi patologis anatomis berupa foki nekrotik hemoragis pada saluran pencernaan dengan uji imunologis imunohistokimia *streptavidin biotin* (IHK SB) dan uji molekuler *simplex reverse transcriptase polymerase chain reaction* (sRT-PCR). Sampel diambil dari kasus-kasus penyakit pada unggas di peternakan ayam petelur komersial. Ayam petelur tersebut menunjukkan gejala klinis tortikolis dan paralisis kaki, serta lesi hemoragis pada paru-paru dan/atau sistem pencernaan yang diduga terinfeksi AIV dan NDV. Setelah unggas dinekropsi, paru-paru, dan saluran pencernaan diuji IHK SB, sedangkan untuk uji sRT-PCR, selain jaringan patologis, juga digunakan sera. Pada penelitian ini digunakan *kit* komersial (Roche) untuk ekstraksi RNA AIV dan larutan Trizol untuk ekstraksi RNA NDV. Primer spesifik untuk amplifikasi gen nukleoprotein (NP) AIV (552 bp) dan gen *conserved region* NDV (320 bp). Hasil IHK SB diamati dengan *microscope digital camera system* dan analisis hasil sRT-PCR dengan elektroforesis gel agarose 1,5%. Hasil penelitian ini membuktikan, bahwa dengan pewarnaan IHK SB, antigen AIV dapat dideteksi pada paru-paru, tetapi tidak pada saluran pencernaan, sedangkan, NDV ada pada saluran pencernaan, tetapi tidak pada paru-paru. Uji sRT-PCR menghasilkan amplikon 552 bp untuk gen NP AIV (serum) dan amplikon 320 bp untuk gen *conserved region* NDV (saluran pencernaan). Berdasarkan hasil penelitian ini disimpulkan, bahwa IHK SB dan sRT-PCR dapat diaplikasikan untuk peneguhan diagnosis AIV dan NDV dan terbukti, bahwa pada ayam petelur komersial dengan gejala klinis tortikolis dan paralisis kaki, serta lesi patologis hemoragis pada saluran pencernaan terinfeksi kedua macam virus tersebut.

Kata kunci: AIV, NDV, IHK SB, sRT-PCR, sera

IMMUNOLOGICAL AND MOLECULAR DIAGNOSTIC APPROACH OF *AVIAN INFLUENZA VIRUS* AND *NEWCASTLE DISEASE VIRUS* IN THE FIELD CASES

ABSTRACT

Poultry can be infected not only by one type of virus, but also by more than one type of virus. *Avian influenza virus* (AIV) and *Newcastle disease virus* (NDV) are the two important pathogens in poultry that can cause recurrent outbreaks and often lead to clinical symptoms and pathological lesions similar to that of poultry with high morbidity and mortality causing significant economic losses to the poultry industry. This study aims to detect and differentiate pathogenic viruses in commercial laying chickens with similar clinical symptoms, especially torticolis and curled toe paralysis, and anatomic pathological lesions, such as foci necrotic hemorrhages in the digestive tract by applying streptavidin-biotin immunohistochemical immunological test (IHC SB) and molecular test of simplex reverse transcriptase polymerase chain reaction (sRT-PCR). Samples (chickens) were taken from the cases of the disease in poultry in several commercial poultry farms. The layer chickens showed clinical symptoms of torticolis and curled toe paralysis, and hemorrhagic lesions in the lungs and / or digestive system suspected of being infected AIV and NDV. After being necropsied, then lungs, and gastrointestinal tract were tested IHC SB, whereas for the sRT-PCR test, in addition to pathological tissue, also used sera. In the present study, the commercial kit (Roche) for AIV RNA extractions and Trizol solution for RNA NDV extractions, and specific primers for amplification of the nucleoprotein (NP) gene of AIV (552 bp) and the conserved region gene of NDV (320 bp). The IHC SB results were examined with a digital microscope camera system and the results the sRT-PCR were analyzed with agarose gel electrophoresis 1.5%. The results of the present study indicated by applying the IHC SB, AIV antigen can be detected in the lungs, but not in the digestive tract, whereas, NDV was detected in the digestive tract, but not in the lungs. The sRT-PCR test produces 552 bp amplicons and amplicon of 320 bp for AIV NP gene (sera) and the conserved region gene of NDV (digestive tract), respectively. It was concluded, that the IHC SB and sRT-PCR can be applied for confirmation of the diagnosis of AIV and NDV and also indicated that the commercial laying with clinical symptoms of torticolis and curled toe paralysis, as well as pathological hemorrhagic lesions in the gastrointestinal tract could be infected by both viruses.

Key word: AIV, NDV, IHC SB, sRT-PCR, sera