

Pengembangan kit ko-aglutinasi untuk deteksi virus *Newcastle disease* pada ayam (*Gallus-gallus* sp.) dibandingkan dengan uji molekuler dan imunohistokimia

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INTISARI

Newcastle Disease (ND) merupakan penyakit akibat infeksi *Newcastle Disease Virus* (NDV) yang menyerang ayam. *Newcastle Disease* menyebabkan kerugian ekonomi pada industri perunggasan. Diagnosa secara konvensional mampu mengidentifikasi penyakit ND namun diperlukan keahlian, alat khusus, biaya mahal dan waktu yang relatif lama. Kit-koaglutinasi merupakan metode diagnostik yang cepat, akurat, murah dan mudah digunakan. Penelitian ini bertujuan untuk membuat *rapid diagnostic test* NDV dengan kit ko-aglutinasi. Dua kelinci jantan *New Zealand White* (*Oryctolagus cuniculus*) berumur 7 bulan dengan berat badan $\pm 2,5$ kg divaksinasi dengan vaksin *live* NDV selama 4 minggu berturut-turut dengan dosis bertingkat 0,5 cc, 1,00 cc, 2,00 cc dan 3,00 cc secara intraperitoneal. Serum kelinci dipanen pada minggu ke enam kemudian diinaktivasi selama 30 menit pada suhu 56°C. Serum yang terkumpul dipresipitasi dengan amonium sulfat, kemudian didialisa tiga kali setiap 12 jam untuk dibuat sebagai antibodi primer dan bahan kit-koaglutinasi yang direaksikan dengan protein A *Staphylococcus aureus* untuk dibuat kit ko-aglutinasi terhadap NDV. Sampel organ lien, otak dan feses dari 31 ekor ayam dengan gejala terinfeksi NDV dari peternakan masing-masing digerus dan dicampur dengan PBS pH 7,2 kemudian disentrifus 8000 rpm selama 10 menit dan diambil supernatannya. Pengujian sampel dengan mencampurkan kit ko-aglutinasi 50 μ l dengan 50 μ l supernatan gerusan organ atau feses. Hasil pengujian kit ko-aglutinasi NDV pada 31 ayam menunjukkan 19 sampel positif ND ditunjukkan dengan adanya aglutinasi seperti butiran pasir, sedangkan 12 sampel negatif dengan tidak terbentuknya aglutinasi. Hasil kit ko-aglutinasi yang dikonfirmasi oleh RT-PCR menunjukkan hasil yang sesuai dengan hasil positif pada *band* ukuran 565 bp. Organ otak, lien, pulmo, proventrikulus dan duodenum diamati perubahan histopatologinya dengan pewarnaan HE dan terlihat perubahan patognomonik NDV yaitu *perivascular cuffing*, pneumonia hemoragi, splenitis, enteritis nekrotik hemoragi dan proventrikulitis hemoragi nekrotik. Berdasarkan pengamatan organ dengan pewarnaan imunohistokimia menunjukkan hasil positif dengan warna coklat tua sebagai reaksi antigen-antibodi pada jaringan dari 19 sampel yang positif dengan uji kit ko-aglutinasi dan RT-PCR. Hasil isolasi virus NDV, uji hemaglutinasi dan hemaglutinasi inhibisi juga menunjukkan 19 sampel positif ND. Kit ko-aglutinasi efektif untuk mendeteksi penyakit ND pada ayam dengan nilai diagnostik sensitivitas dan spesifisitas yang tinggi sebesar 100%.

Kata kunci: *Newcastle Disease Virus*, kit ko-aglutinasi, antibodi primer, imunohistokimia, RT-PCR

Development of co-agglutination kit for detection of Newcastle disease virus in chickens (*Gallus-gallus* sp.) compared with molecular test and immunohistochemistry

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ABSTRACT

Newcastle Disease (ND) is a disease caused by Newcastle Disease Virus (NDV) infection that affecting chickens. Newcastle Disease causes economic losses in the poultry industry. Conventional diagnosis is able to identify ND disease but requires expertise, special tools, expensive costs and relatively long time. Co-agglutination kit are fast, accurate, inexpensive and easy to use diagnostic methods. This study aims to make a rapid diagnostic test of NDV with a kit co-agglutination. Two New Zealand White male rabbits (*Oryctolagus cuniculus*) aged 7 months and weighed $\pm 2,5$ kg were vaccinated with live NDV vaccine for 4 consecutive weeks with multilevel doses of 0.5 cc, 1.00 cc, 2.00 cc and 3.00 cc intraperitoneally. Rabbit serum is harvested at the sixth week and then inactivated for 30 minute at 56°C. The collected serum was precipitated with ammonium sulfate, then dialyzed three times every 12 hours to be made as primary antibodies and co-agglutination kit materials reacted with protein A *Staphylococcus aureus* to make a co-agglutination kit against NDV. Samples of spleen, brain and feces from 31 chickens with symptoms of NDV infection from farms were crushed and mixed with PBS pH 7.2 then centrifuge 8000 rpm in 10 minutes and supernatants were taken. Sample testing by mixing a 50 μ l co-agglutination kit with 50 μ l supernatant of organ or feces scouring. The results of testing the NDV co-agglutination kit on 31 chickens showed 19 positive ND samples were shown by the presence of agglutination such as grains of sand, while 12 negative samples with no agglutination formed. The results of the co-agglutination kit confirmed by RT-PCR showed results that match the positive results on the band size of 565 bp. Brain, spleen, pulmo, proventriculus and duodenum were observed for histopathological changes with HE staining and visible pathognomonic changes in NDV such as perivascular cuffing, haemorrhagic pneumonia, splenitis, necrotic haemorrhage enteritis and necrotic haemorrhage proventriculitis. Based on observation of organs by immunohistochemistry staining showed positive results with a dark brown color as an antigen-antibody reaction in tissue from 19 positive samples with co-agglutination kits and RT-PCR test. The results of NDV virus isolation, hemmagglutination and hemmagglutination inhibition tests also showed 19 positive ND samples. Co-agglutination kits are effective for detecting ND disease in chickens with high sensitivity and specificity diagnostic value that is 100%.

Key words: Newcastle Disease Virus, co-agglutination kit, primar antibody, immunohistochemistry, RT-PCR