INTISARI

Wabah penyakit *avian influenza* (AI) di Indonesia, diketahui menyebabkan kematian unggas di sejumlah peternakan ayam petelur komersial di Jawa Barat dan Jawa Tengah, sejak pertengahan tahun 2003. Wabah tersebut disebabkan oleh virus *avian influenza* (VAI) subtipe H5N1. Perkembangan penyakit penyakit AI sejak tahun 2003 sampai 2008 menunjukkan adanya variasi gejala klinis dan patologis tertentu. Penyakit AI masih terjadi secara sporadis, tidak saja di peternakan yang belum menerapkan vaksinasi AI tetapi juga di peternakan yang sudah melakukan vaksinasi. Penelitian ini merupakan studi retrospektif untuk dapat mengetahui dinamika molekuler gen HA pada marka patogenisitas molekuler, tapak perlekatan reseptor (TPR), tapak antigenik, dan domain fusigenik, virus AI subtipe H5N1 yang diisolasi dari berbagai spesies unggas asal Pulau Jawa dan Lampung selama periode tahun 2003 sampai 2008. Isolat tersebut diperoleh dari kasus dengan variasi gejala klinis dan patologis serta mempunyai riwayat vaksinasi dan tanpa vaksinasi AI. Isolasi dan propagasi VAI dilakukan dengan menanam virus tersebut pada telur ayam berembrio (TAB) *specific pathogen free* (SPF) umur 9-11 hari. Pertumbuhan virus dalam cairan alantois diidentifikasi dengan uji serologis dan *reverse transcriptase polymerase chain reaction* (RT-PCR) pada fragmen gen H5 dan N1. Karakterisasi marka patogenisitas molekuler dan TPR dilakukan dengan mengamplifikasi fragmen gen HA yang terdapat *cleavage site* (CS) dan *receptor binding domain*. Hasil RT-PCR gen HA VAI pada fragmen CS dan TPR selanjutnya disekuensing. Urutan nukleotida hasil sekuensing dianalisis menggunakan program Mega 4.0, yang meliputi *multiple aligment, deductive amino acid prediction, philogenetic tree and pair wise distance calculation.* Hasil penelitian ini menunjukkan bahwa semua VAI yang diisolasi dari berbagai spesies unggas sejak tahun 2003 sampai 2008 dapat diidentifikasi sebagai VAI subtipe H5N1. Analisis sekuens marka patogenisitas molekuler teridentifikasi lima motif susunan asam amino basa multipel yang dapat diklasifikasikan sebagai motif HPAI. Asam amino tertentu pada marka patogenisitas tersebut teramati adanya mutasi dan delesi. Virus AI dalam penelitian ini masih memiliki susunan asam amino yang bersifat lestari pada domain fusigenik. Asam amino TPR yang teridentifikasi menunjukkan kecenderungan berikatan dengan reseptor unggas, SA ά 2,3 galaktosa. Analisis tapak antigenik dapat diketahui mutasi asam amino pada tapak antigenik tertentu. Analisis filogenetik mengindikasikan bahwa tidak terdapat pengelompokan virus berdasarkan spesies unggas dan sebaran geografis tertentu. Secara garis besar VAI membentuk dua klaster virus yang berdasarkan pada tahun isolasi, yaitu klaster tahun 2003 sampai 2005 dan tahun 2006 sampai 2008. Analisis jarak genetik fragmen gen HA *cleavage site* dan TPR menunjukkan peningkatan substitusi nukleotida yang teramati pada virus yang diisolasi pada tahun 2003 dibandingkan dengan virus yang diisolasi pada tahun 2008. Hasil analisis *Chi-square* menunjukkan bahwa variasi karakter lesi dan status vaksinasi tidak menunjukkan hubungan dengan kejadian mutasi pada fragmen gen HA VAI subtipe H5N1 yang diteliti. Data yang diperoleh dalam penelitian ini menunjukkan bahwa VAI subtipe H5N1 terus berevolusi.

Kata kunci: *avian influenza*, haemaglutinin, *cleavage site*, tapak perlekatan reseptor

ABSTRACT

Outbreak of avian Influenza (AI) in Indonesia has been reported since the mid of 2003, affected to layer commercial farm in Province of Central and West Java. Clinical sign observation since in the early outbreak in the year 2003 to 2008 indicated some variation of symptoms of AI virus infection. Moreover, the AI infections are still existing in the field sporadically, both in farms with and without AI vaccination practices. This study was a retrospective study which was designed to molecularly characterize of hemagglutinin (HA) gene of AI virus, particularly in the fragment of cleavage site (CS), fusion site (FS), receptor binding site (RBS), and antigenic sites (AS). Avian influenza viruses isolated from various poultry since 2003 until 2008 outbreak in Indonesia, which exhibited variation of symptoms and were obtained from farm with and without AI vaccination practices. Isolation and propagation were done on the chicken embryonated egg specific pathogen free. For virus detection and subtyping, serological identification and reverse transcriptase polymerase chain reaction (RT-PCR) method of H5 and N1 gene fragment was performed. Further characterizations were amplification of CS, FS, RBS, and AS domain of HA gene. All of RT-PCR HA gene positive products were sequenced to determine the nucleotide composition at the targeted fragment. Obtained sequences were analyzed by program Mega 4.0 versions, included multiple alignment, deductive amino acid prediction, phylogenetic tree and pair wise distance calculation. The result showed that all AIV isolates were identified as H5N1 subtype. Sequence analysis of CS area determined five motives of multiple basic amino acids which were classified as highly pathogenic avian influenza virus. The features of the motive were observed have some mutations and deletions. Multiple alignment of FS domain of all viruses isolated since 2003 to 2008 showed a conserved domain. Further characterizations on amino acid responsible for RBS indicated a binding preference to avian like receptor, sialic acid α 2, 3 Gal. Antigenic sites analysis determined some mutations of amino acid in certain antigenic sites. Phylogenetic study showed that clustering of AIV did not based on species of bird or geographic origin. Generally, AIV created two clusters of viruses based on years of AIV isolation. Viruses isolated at the beginning of outbreak in the year 2003, 2004, and 2005 tended to cluster which was different from that of isolated from 2006 to 2008. Pair wise distance calculation demonstrated the increase of amino acids substitution were observed at the AIV isolated in the year 2003 to 2008. Chi-square analyses indicated that the lesion type character and vaccination status were not associated with the mutation of HA gene of AIV H5N1 subtype, at CS area, FS, RBS and AS which were studied. Our finding demonstrated that the AIV H5N1 subtype in this study showed molecular dynamics of HA gene and suggests that the AIV H5N1 subtype is evolving continuously.

Key words: avian influenza, haemagglutinin, cleavage site, receptor binding site