

KARAKTERISASI BIOTIPE DAN GENOM UTUH VIRUS *BOVINE* *VIRAL DIARRHEA* KOLEKSI BALAI BESAR VETERINER WATES-YOGYAKARTA TAHUN 2013 - 2018

Intisari

Ketersediaan sapi lokal di Indonesia hingga saat ini belum mencukupi kebutuhan nasional. Gangguan produktivitas dan reproduktivitas merupakan permasalahan. Penyakit yang berkaitan dengan hal tersebut adalah *Bovine Viral Diarrhea* (BVD). Virus BVD subgenotipe -1a, -1b, -1c, dan -1d telah teridentifikasi di Indonesia, karakterisasinya secara genom utuh belum dilakukan. Tujuan penelitian adalah isolasi, identifikasi biotipe dan genotipe virus BVD dari serum sapi, analisis karakter molekular genom secara utuh. Serum sapi koleksi BBVet Wates tahun 2013-2018 yang telah diuji ELISA antigen Erns (n=88) digunakan dalam penelitian. Penelitian dilaksanakan di laboratorium Virologi-Serologi dan Bioteknologi BBVet Wates. Sampel serum yang telah diuji antigen Erns, diuji antibodi p80 BVDV kemudian diisolasi menggunakan biak sel MDBK, dan diidentifikasi dengan imunositokimia. Skrining genotipe virus BVD digunakan *multiplex* nPCR untuk amplifikasi parsial regio NS5B. Cairan biak sel (n=23) dan serum (n=21) yang memiliki nilai C_T *realtime* RT-PCR ≤ 32 dilakukan PCR genomik *two step*. Sekuensing dengan teknik NGS dilakukan terhadap 21 sampel yang memenuhi persyaratan. Hasil sekuensing dianalisis menggunakan program CLC *Genomic Workbench* 9.0 dan MEGA-X. Empat puluh delapan sampel teridentifikasi genotipe BVDV-1 dan 14 isolat virus BVD yang terisolasi memiliki biotipe *noncytopathic*. Pada penelitian ini, berdasarkan hasil analisis filogenetik regio 5'UTR-NPro-C-Erns-E1-E2-NS2-NS3-NS4A-NS4B-NS5A-NS5B-3'UTR disimpulkan 2 subgenotipe, BVDV-1a (13%) dan BVDV-1c (74%) serta adanya rekombinasi BVDV-1a dan -1b (13%). Isolat V7_BVDV-1/Indonesia-CJ-Bms/04151167-16/2015 berhasil disekuen pada seluruh regio genom virus kecuali p7. Berdasarkan hasil analisis disimpulkan perubahan asam amino berupa substitusi R146K pada regio NPro, I68L, T70Q dan P74S pada sisi antigenik dan C130G pada residu sistein regio E2, K213R dan K214N pada GAG-*binding site* serta variasi 12 residu asam amino epitop linier pada regio Erns.

Kata Kunci: biotipe, BVDV, *antigen capture* ELISA, RT-PCR, sekuensing genom utuh

CHARACTERIZATION OF BIOTYPE AND WHOLE GENOM BOVINE VIRAL DIARRHEA VIRUS FROM DISEASE INVESTIGATION CENTRE-WATES COLLECTION, 2013-2018

Abstract

The supplies of local cattles in Indonesia still cannot meet the national demand. A disorder of herd productivity and reproductive performances is one of the problems. The BVDV has spread widely across the globe including in Indonesia. Subgenotype of BVDV-1a, -1b, -1c, and -1d has been identified being circulating in Indonesia, nevertheless the characterization of BVDV as a whole genome has never been done. The study aimed to isolate and identify biotype, to screen genotype, and to analyze genetic molecular character of whole genome and the amino acid sequence of BVDV genome as well. In the present study, eighty-eight cattles sera of the collection of Disease Investigation Center (DIC), Wates, Yogyakarta between 2013-2018 were tested using antigen capture ELISA (ACE). The sera samples were analyzed using ACE to detect antibody anti-p80 BVDV. Then, the gp53 BVDV was detected by using MDBK cell *in vitro* and immunocytochemistry. The screening test of genotype BVDV was conducted through multiplex nested-PCR which used specific primer amplifying the partial region of NS5B. Samples of cell culture (n = 23) and serum (n = 21) that have C_T value of realtime RT-PCR ≤ 32 were performed two step genomic PCR. The NGS technique was carried out on 21 samples that had requirements. The results of the genomic sequencing were then analyzed by using CLC Genomic Workbench 9.0 program and MEGA X. Forty-eight samples were identified as genotype BVDV-1 in which 14 isolates of those BVDV-1 were isolated as non-cytopathic biotype. Based on phylogenetic analysis of the region 5'UTR-NPro-C-Erns-E1-E2-NS2-NS3-NS4A-NS4B-NS5A-NS5B-3'UTR, there were two subgeno-types, BVDV-1a (13%) and BVDV-1c (74%), and recombination of both (13%). Isolate V7_BVDV-1/Indonesia-CJ-Bms/04151167-16/2015 was successfully se- quenced in all regions of the viral genome except p7. There were some mutations, such as substitution of R146K in NPro region, I68L, T70Q and P74S on the antigenic site while C130G in the cysteine residue in the E2 region, K213R and K214N in the GAG-binding site and also variations in 12 amino acid residues of epitope linear in the Erns region.

Keyword: Biotype, BVDV, antigen capture ELISA, RT-PCR, whole genome sequencing