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IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF BOVINE HERPERVIRUSES (BoHV) DNA TERMINASE PARTIAL GENE IN ACEH CATTLE

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ABSTRACT

Bovine Herpesvirus (BoHV) is a member of Herpesviridae family caused infectious bovine rhinotracheitis (IBR) among cattles, resulting in economic loss in cattle industry. BoHV-1 infection in cows is closely related to abortion, respiratory infection, reduction of milk production, infertility, and low birth weight. The aims of this study were to identify and characterize the molecular of BoHV-1 and other types of BoHV and other Herpesviridae family using PCR to amplify DNA terminase gene. Four out of 210 nasal swab samples were positive for herpes virus based on DNA terminase gene detection. Further characterization of these positive samples showed 99-100% identities to BoHV-1 and BoHV-6 sequences. Genetic distance between genera of BoHV-1 and BoHV-6 was 0.518 and within genera was 0.001 and 0.044. According to phylogenetic tree analysis of DNA terminase gene, 3 samples were clustered with BoHV-1 in the genus of Varicellovirus; meanwhile one sample was clustered with BoHV-6 in Macavirus genus. This study provides scientific information on molecular characteristics of Herpesviridae family, especially BoHV-1 in Aceh province, Indonesia.

Key words: BoHV, characterization, DNA terminase

ABSTRAK

Bovine Herpesvirus (BoHV) adalah anggota keluarga Herpesviridae, merupakan virus yang berperan sebagai patogen pada ternak sapi dan menyebabkan Infectious Bovine Rhinotreacheitis (IBR) yang membawa kerugian ekonomi pada industri peternakan. Infeksi BoHV-1 pada sapi sangat berkaitan dengan kejadian abortus, penyakit pernafasan, penurunan produksi susu, infertilitas, dan penurunan bobot lahir. Tujuan penelitian ini difokuskan pada identifikasi dan karakterisasi molekuler terhadap BoHV-1 dan tipe lainnya, serta kemungkinan adanya anggota keluarga Herpesviridae lainnya dengan menggunakan konsensus PCR dalam mengamplifikasi gen DNA terminase. Empat dari 210 sampel usapan hidung yang di PCR menunjukkan hasil positif terhadap herpesvirus pada gen DNA terminase. Karakterisasi lebih lanjut terhadap urutan sampel menunjukkan hasil identitas 99-100% terhadap urutan referensi BoHV-1 dan BoHV-6. Jarak genetik between genera BoHV-1 dan BoHV-6 adalah 0,518 dan within genera adalah 0,001dan 0,044. Berdasarkan analisis pohon filogenetik gen DNA terminase menunjukkan bahwa urutan yang dianalisis berkelompok menjadi 2 genus, yaitu genus Varicellovirus yang identik terhadap BoHV-1 dan genus Macavirus yang identik terhadap BoHV-6. Hasil penelitian ini memberikan informasi ilmiah tentang karakteristik molekuler anggota keluarga Herpesviridae khususnya BoHVyang beredar di Indonesia secara umum dan khususnya di sentra peternakan yang ada di provinsi Aceh.

Kata kunci: BoHV, karakterisasi, DNA terminase

INTRODUCTION

Bovine Herpesvirus (BoHV) is a member of Herpesviridae family. The virus causes disease in cattle and infected cattles may suffer from abortion, low milk production, respiratory symptoms, low birth weight of neonates, and infertility (Muylkens *et al*., 2007; Yildirim *et al*., 2011). BoHV specieses that are known as pathogen in cattle are BoHV-1, BoHV-2, BoHV-4, BoHV-5, and BoHV-6 (Campos *et al*., 2014; Gagnon *et al*., 2017; Aslan *et al*., 2015).

Several diseases caused by Bovine Herpesvirus-1 (BoHV-1) are infectious bovine rhinotrachaetis (IBR), infectious pustular vulvovaginalis (IPV), and infectious pustular balanoposthitis (IPB) (Ackermann dan Engels, 2006; Muylkens *et al*., 2007). An investigation in the first and second trimester of abortion among cattle revealed that BoHV-1 was the caused of abortion (Yildirim *et al*., 2011). An analysis model was used in dairy farms to see the impact of BoHV-1 infection on milk production. The result showed a reduction in milk production on average 0.92 kg/day/head (van Schaik *et al*., 1999). Due to the challenges and losses caused by BoHV, it is important to control diseases caused by BoHV, including surveillance and serological and/or molecular identification of the virus. This is in accordance to the Minister of Agriculture Decree No.4026/kpts/OT.140/ 4/2013 (2013), on diagnosis of strategical infectious animal diseases and exotic diseases stated that IBR is one of the 22 diseases that must be controlled and mitigated by the central government, provincial government, and local district/municipality government in accordance to their authority. Moreover, IBR is one of disease that must be eradicated from breeding and artificial insemination centers in Indonesia.

Disease Investigation Center (DIC) in Indonesia has routinely performed serological test for BoHV-1 and discovered high seropositive, about 66.65% (BVet Medan, 2014) and 51.7% (BVet Bukittinggi, 2014)

using virus neutralization test in cattle farms and breeding centers. BoHV-1 detection in nasal swab and semen from cattles without any clinical signs in Bandung, Bogor, and Pasuruan using nested PCR, showed positive result in 3.68% (14/381) for cattles in Bandung and Bogor, and 16.67% (4/24) positive result for cattles in Pasuruan (Saepulloh *et al*., 2008).

The BoHV tipe which is found in Indonesia based on molecular characteristic of glycoprotein D (gD) gene is Bovine Herpesvirus-1.1 (BoHV-1.1) (Saepulloh *et al*., 2009). Currently, there is no official report of the existence of other BoHV types (BoHV-2, BoHV-3, BoHV-4, BoHV-5, dan BoHV-6) in Indonesia, neither from serological test nor agent identification. This study aimed to detect the presence of BoHV-1 and other herpes virus family among local Aceh cattles, to determine molecular characterization of DNA terminase gene partially on detected herpes virus family, and also to analyze and to identify genetic relationship between the detected viruses.

MATERIALS AND METHODS

Nasal swab samples of this study were archive samples collected from Aceh cattle in Indrapuri district, Aceh Besar Regency. The samples were kept in a 4° C and transported to laboratory for storage at -40° C until further analysis. Total samples in this study were 210 samples.

This study was conducted in the Virology Laboratory of Medan Diseases Investigation Center and Biotechnology Laboratory of The Primate Research Center, Institute for Research and Community Services, Bogor Agricultural University (PSSP LPPM-IPB).

Molecular Detection Using Degenerate Primer

DNA was extracted from nasal swab samples using QIAamp DNA Blood mini Kit (Qiagen, Hilden, Germany) according to the guideline from manufacturer. The concentration and purity of DNA were measured and then the sample was stored at -40° C or used directly for PCR.

PCR amplification was carried out using nested PCR method and degenerate primer according to PREDICT protocol (Anthony *et al*., 2013). In this study two sets of primers were used (Table 1) to amplify the DNA terminase target gene. Amplification process was carried out using PCR machine (VeritiThermal Cycler, Applied Biosystem, USA) referring to van Devanter *et al*. (1996) and Chmielewicz *et al*. (2001). PCR product was analyzed on 1.8 % agarose gel electrophoresis containing 1 µg/ml ethidium bromide (EtBr). Electrophoresis result was further visualized in GelDoc using Quantity One program (BioRad).

Sequencing and Molecular Analysis

Positive samples based on PCR result were sequenced. Sequencing result was analyzed using MEGA 6.0 (Molecular Evolutionary Genetics Analysis 6) (Tamura *et al*., 2013) and aligned using ClustalW to determine the variance in nucleotide sequence. The alignment result was further analyzed for identity tracing (% identity) using Basic Local Alignment Search Tool (BLAST) (McGinnis and Madden, 2004) and BioEdit program (Hall, 2011). Geneious program was used to translate the nucleotide sequences into amino acid. MEGA 6.0 software was used to determine genetic distance and to construct the phylogenetic tree (Tamura *et al*., 2013).

RESULTS AND DISSCUSSION

DNATerminase Gene Amplification

DNA terminase gene amplification resulted positive band around 416 bp using TS-TERM-708s and TS-TERM-708as primers (Figure 1). The result was similar to Chmielewicz *et al*. (2001), whereby herpes virus detection using degenerate primer for DNA terminase gene as the target would yield an amplicon 419 bp. The different amplicon length between our result and the reference was probably caused by species difference. The analyzed sample sequence was identical to BoHV-1 whereas the reference sequence was Alcelaphine herpesvirus 1 (AlHV-1). Chmielewicz *et al*. (2001) also detected possibly novel gamma herpes virus from goats which yielded an amplicon with several base pairs different. Similar detection was carried out by Kleiboeker *et al*. (2002), in deers suspected of Malignant Catarrhal Fever (MCF) due to Deer herpesvirus. Detection using degenerate primer also produced an amplicon with several base pairs different**.**

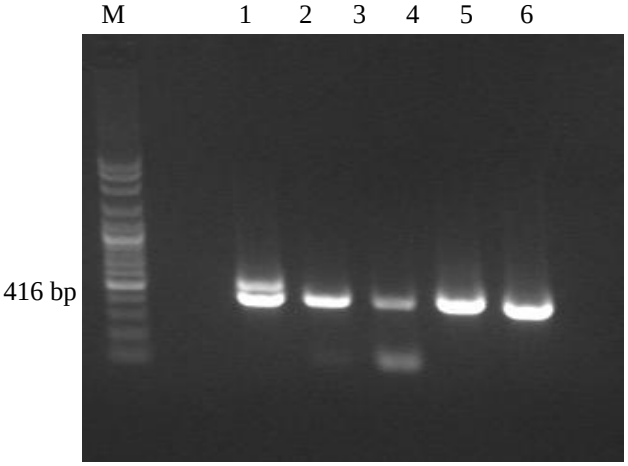

Figure 1. DNA amplification of DNA *Terminase* gene using *degenerate* primer. Positive results showed amplicon in 416 base pairs. M= 100 bp DNA Marker, 1= DIC 1.1, 2= DIC 1.2, 3= DIC 1.6, 4= DIC 1.7, 5= Positive control, 6= Non Template

Herpes Virus Identification based on DNA Terminase Sequence

The detection and identification of DNA terminase revealed a similarity between subfamilies of Alpha herpesvirinae and Gamma herpesvirinae. After further analysis on genus level, Gamma herpesvirinae subfamily was found to be similar in 1 sample (DIC1.6) only to 1 genus, namely Macavirus which includes BoHV-6 species. This is only detected in one sequence sample specifically DIC1.6. Alpha herpesvirinae subfamily was similar to only 1 genus, namely Varicellovirus, which includes BoHV-1 species, detected in 3 sample sequences, namely DIC 1.1, DIC 1.2 and DIC 1.7 (Tabel 2). The similarity between species was analysed using BLAST with the highest similarity value compared to other species. Two species of herpes virus, BoHV-1 and BoHV-6, was detected from DNA terminase gene. This indicates that DNA terminase is a conserved area and could be used to detect herpes virus at family level. This was in line with Przech *et al*. (2003), who found that DNA terminase gene is a conserved area which is used by the virus during viral genomic cleavage dan packaging. Several researches have used DNA terminase gene to detect herpes virus existence in animals (Kurobe *et al*., 2008; Kleiboeker *et al*., 2002; Chmielewicz *et al*., 2001).

DNATerminase Matrix Identity

Results of matrix identity analysis of DNA terminase gene sequence showed similarity in identity value using Bioedit and BLAST software. The identity value of the samples was 99-100% towards the reference viral sequence. The obtained value was the highest identity percentage compared to the reference viral sequence in GenBank database with query cover value 100% (Table 3). This result is consistent with Saepulloh *et al*. (2009), who stated that the matrix identity of BoHV-1 isolates in Indonesia was 98.8-100 % compared to Cooper strain Bovine herpesvirus 1. The result indicated that the sample sequence analyzed was identical to the reference.

Genetic Distance and Phylogenetic Construction of DNA Terminase Gene

The genetic distance between genera Varicellovirus and Macavirus on DNA terminase target gene was 0.518. Large difference between genera indicates different genetic characteristic, which could be seen on phylogenetic tree. The phylogenetic tree of the samples formed two different genera cluster. The first cluster was members of Alpha herpesvirinae subfamily which consisted of DIC1.1, DIC1.2, and DIC1.7. The second cluster was Gamma herpesvirinae subfamily which consisted of DIC 1.6 sequence sample.

Table 1. Degenerate PCR primer of DNA Terminase target gene

Primer	Primer sequence	PCR product size
TS-TERM-707s[a]	5′-TTGTGGACGAGRSIMAYTTYAT-3′	519 bp
TS-TERM-707as[a]	5′-ACAGCCACGCCNGTICCIGAIGC-3′	
TS-TERM-708s[b]	5′-GCAAGATCATNTTYRTITCITC-3′	419 bp
TS-TERM-708as[b]	5′-TGTTGGTCGTRWAIGCIGGRT-3′	

Source: van Devanter *et al*. (1996), Chmielewicz *et al*. (2001); [a]Primer for first phase PCR ; [b]Primer for second phase PCR

Table 2. Result of DNA terminase gene identification

No	Sample	PCR	BLAST		
			Sub family	Genus	Spesies
1	DIC1.1	Positive	Alpha herpesvirinae	Varicellovirus	BoHV-1
2	DIC1.2	Positive	Alpha herpesvirinae	Varicellovirus	BoHV-1
3	DIC1.6	Positive	Gamma herpesvirinae	Macavirus	BoHV-6
4	DIC1.7	Positive	Alpha herpesvirinae	Varicellovirus	BoHV-1

Table 3. Matrix identity of sample and reference virus on DNA terminase target gene

DNA terminase target gene		% Identity		No Aaccess
Sample	Virus Reference	Bioedit	BLAST	
DIC1.1, DIC1.2, DIC1.7	Bovine herpesvirus 1 Cooper strain	100	100	KU198480.1
	Bovine herpesvirus type 1.2 SP1777strain	100	100	KM258883.1
	Bovine herpesvirus type 1.2 SM023strain	100	100	KM258882.1
	Bovine herpesvirus type 1.2 K22strain	100	100	KM258880.1
	Bovine herpesvirus type 1.1 NVSL isolate	100	100	JX898220.1
	Bovine herpesvirus type 1.1	100	100	AJ004801.1
	Bovine herpesvirus type 1 Cooper	100	100	Z48053.1
	Bovine herpesvirus type 1.2 B589strain	99.7	99	KM258881.1
	Bovine alpha herpesvirus 1 216 II isolate	99.4	99	KY215944.1
DIC1.6	Bovine herpesvirus 6 Pennsylvania 47	99.4	99	KJ705001.1

The first cluster (DIC1.1, DIC1.2, and DIC1.7) was included in genus Varicellovirus and had 100% identity value to BoHV-1 strain Cooper. Based on the phylogenetic tree analysis, the first cluster was closely related to another BoHV-1 variant from genus Varicellovirus, namely BoHV type 1.2 strain SP1777, BoHV type 1.2 strain SM023, BoHV type 1.2 strain K22, BoHV type 1.1 strain NVS, with identity value of 98-100%. Some analysis on genetic characterization showed 98-100% identity to BoHV-1 variants from Varicellovirus genus (Saepulloh *et al*., 2009; Majumder *et al*., 2013; Patil *et al*., 2016).

The second cluster (DIC 1.6) belongs to genus Macavirus in the Gamma herpesvirinae subfamily with 99% identity value as BoHV-6. The phylogenetic tree showed that DIC1.6 is closely related to other Macavirus members, namely Caprine gammaherpesvirus-2 (CprHV-2) with 70.30% identity value, Ovine gammaherpesvirus-2 (OvHV-2) with 70% identity value, and Alcelaphine gammaherpesvirus-1 (AlHV-1) and Alcelaphine gammaherpesvirus-2 (AlHV-2) with 70% and 69.1% identity value, respectively. Previous genetic analysis also found that OvHV-2 and AlHV-1 had 70% and 69% identity value, respectively, towards presumptive BoHV-6 samples (Rovnak *et al*., 1998).

Within genera distance of Macavirus and Varicellovirus were 0.044 and 0.001, respectively (Table 4). The small genetic distance of Varicellovirus indicated that its members is genetically more stable compared to members of Macavirus. Previous study on diversity of virus Suid herpesvirus-1 (SuHV-1) evolution stated that smaller within genera genetic distance is more stable compared to other groups with larger value (Fonseca *et al*., 2016).

DNA terminase phylogenetic tree was constructed by comparing 5 other genus as the outgroup, namely genus Radhinovirus and Lymphocryptovirus of Gamma herpesvirinae subfamily, genus Roseolovirus and Cytomegalovirus of Beta herpesvirinae subfamily, and genus Simplexvirus of Alpha herpesvirinae subfamily. The outgroups were clustered based on each genus characteristic and described the relationship between our samples and some reference of herpes virus (Figure 2).

Table 4. Genetic distance within genera and between genera on DNA terminase target gene

DNA terminase target gene		Genus	Between genera		Within genera
No	Subfamily		1	2	
1	Alpha herpesvirinae	Varicellovirus	ID		0.001
2	Gamma herpesvirinae	Macavirus	0.518	ID	0.044

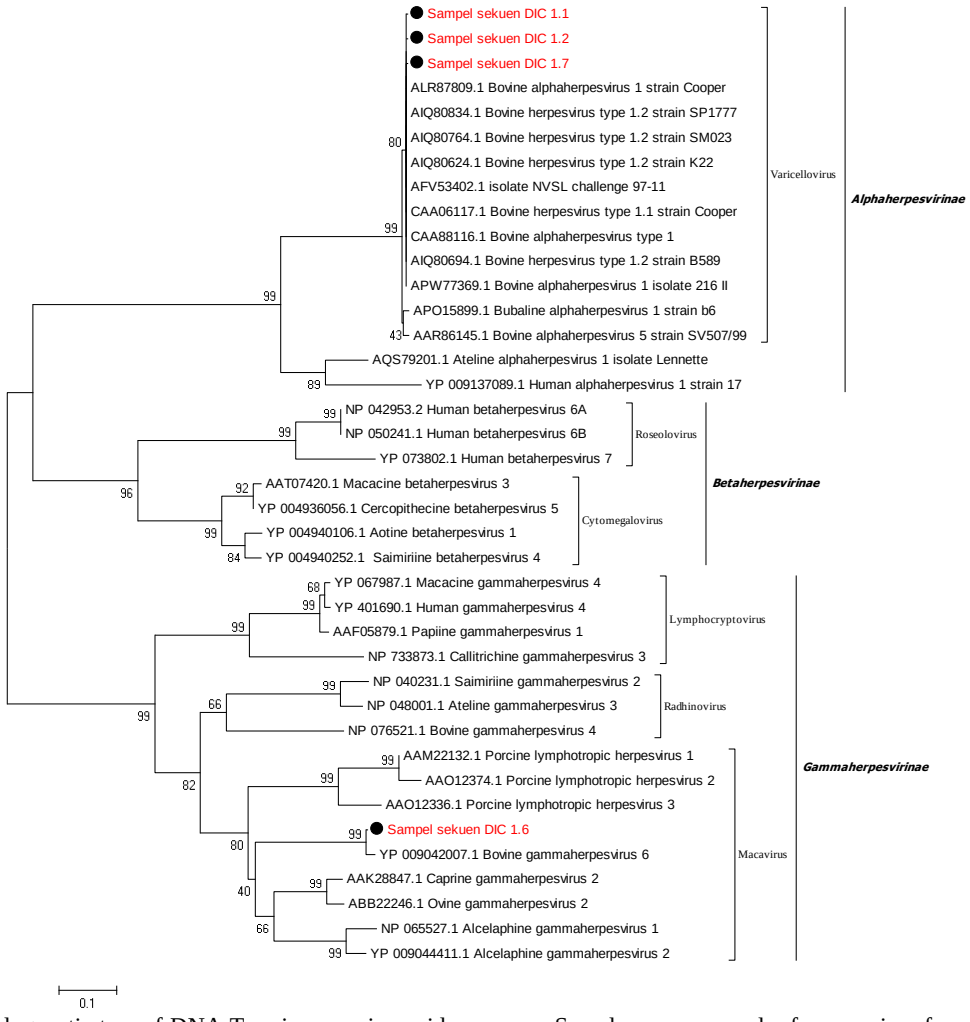

Figure 2. Phylogenetic tree of DNA Terminase amino acid sequence. Sample sequence and reference virus from Herpesviridae family was analyzed using MEGA 6 software using Neighbor Joining (NJ) method and equal input model with boostrap value 2000×. Black dot (●) indicates the analysed sampel sequence

CONCLUSION

Molecular detection using degenerate primer could be used to detect multispecies of Herpesviridae from nasal swab samples of local Aceh cattle. Molecular characterization on partial DNA terminase gene sample showed identical sequence to BoHV-1 and BoHV-6. This information can be used as preliminary data for further isolation and characterization, as well as early caution toward potential emerging diseases.

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