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Short Communication: Novel Single Nucleotide Polymorphisms (SNPs) in the 5'UTR of Bovine Heat Shock Protein 70 ($bHSP_{70}$) Gene and its association with Service per Conception (S/C) of Pasundan cattle

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Abstract. *Said S, Putra WPB. 2018. Short Communication: Novel Single Nucleotide Polymorphisms (SNPs) in the 5'UTR of Bovine Heat Shock Protein 70 ($bHSP_{70}$) Gene and its association with Service per Conception (S/C) of Pasundan cattle. Biodiversitas 19: 1622-1625.* Heat stress in the livestock reduces reproductive traits, including of service per conception (S/C). The influence of heat stress in livestock can be handled through molecular selection. One of the candidate genes that is affecting heat stress tolerance is Bovine Heat Shock Protein 70 ($bHSP_{70}$) gene. This research was carried out to detect the single nucleotide polymorphism (SNPs) in the 5'UTR of $bHSP_{70}$ gene from 44 heads of Pasundan cows at breeding station (BPPIBT-SP Ciamis, West Java). Research showed that 18 SNPs were detected with sequencing method. The insertion mutation was detected in all animal studied and occurred at position between g.1112 and g.1113. The moderate of polymorphic informative content (PIC) were detected in two SNPs of g.1117G/A and g.1125A/C. Preliminary analysis showed that haplotype of two homozygote genotype combination from both SNPs had better of S/C value of than the others ($P<0.01$). Moreover, the average of S/C value in heterozygote genotype seems higher than homozygote genotype. However, the further research for clarification of this research results through large number of observation is important. It was concluded that two SNPs of g.1117G/A and g.1125A/C are potential as marker-assisted selection (MAS) for reproductive traits in Pasundan cows because of moderate PIC value.

Keywords: $bHSP_{70}$ gene, Pasundan cattle, SNP, PIC, S/C

INTRODUCTION

Pasundan cattle is one of Indonesian cattle that spread out at West Java Province. These cattle were chosen as Indonesian native cattle based on the decision of Ministerial Decree No: 1051/Kpts/SR.120/10/2014 (Ministry of Agriculture of the Republic of Indonesia 2014). Said et al. (2017) reported that Pasundan cattle has a combination of phenotypic characterizations from Madura (*Bos indicus*) and Bali (*Bos javanicus*). As a tropical breed, Pasundan cattle has heat tolerance traits and capable to adapt well mainly in Ciamis Regency (Putra et al. 2016). Heat stress occurs when animals are exposed to temperature beyond the upper critical level, high humidity and low air movement causing an increase in heat production in the animal body (Banks et al. 2009). In addition, reducing the heat stress effect through management improvement, e.g., close house system and fan application, are too expensive for most farmers in Indonesia. Heat stress in cattle can redux reproductive traits (Das et al. 2016), including the service per conception (S/C). High number of S/C indicates that the cow is not productive and it increases the artificial insemination (AI) cost (Mwatawala and Kifaro 2009). However, heat stress in cattle can be reduced through molecular selection. One of the candidate gene that affecting heat stress tolerance is bovine Heat Shock Protein 70 ($bHSP_{70}$) gene. Moreover, heat stress tolerance is heritable trait and can be used for genetic improvement in the future (Ravagnolo and Misztal 2000).

The $bHSP_{70}$ gene is important for producing heat shock protein 70 (HSP_{70}) that affecting heat stress (Mohanarao et al. 2014). Heat shock protein 70 is one of HSPs family that has molecular weight of 68-73 kDa (Pockley et al. 2008) with length consisted of 641 amino acids (Gade et al. 2010). The coding region of $bHSP_{70}$ gene in cattle (*Bos taurus* and *Bos indicus* and buffaloe (*Bubalus bubalis*) is similar in length 1,926 bp (Sodhi et al. 2013). The HSP_{70} is present in all cells of the body. It increases in numbers when an animal is subjected to various stressors such as heat, cold, and oxygen deprivation. In addition, the HSP_{70} is well correlated with the development of thermotolerance in many cell types (Li and Max 1989). The $bHSP_{70}$ gene can be used as a candidate gene for selection of cattle based on heat tolerance traits (Archana et al. 2017). Heat adaptability is a complex phenomenon that depends on the integrity and proper coordination of various systems like respiratory, circulatory, excretory, nervous, endocrine and enzymatic systems of animal body (Mishra and Tapan 2014).

The genetic polymorphism of $bHSP_{70}$ gene explains the difference between individuals in the stress condition. Several polymorphisms were reported in previous studies in the 5'UTR (Cai et al. 2005; Rosenkrans et al. 2010; Basirico et al. 2011; Turner et al. 2013; Gafer et al. 2015;

Ramesha et al. 2016; Oner et al. 2017), 3'UTR (Adamowicz et al. 2005; Basirico et al. 2011; Oner et al. 2017) and coding regions (Brown et al. 2010; Habib et al. 2017). In addition, the bHSP$_{70}$ gene polymorphisms were affected by reproductive traits in Deoni cattle (Ramesha et al. 2016), cellular thermotolerance traits in Tharparkar cattle (Bhat et al. 2016), peripheral blood mononuclear cells (PBMC) response in Holstein Friesian and Sahiwal cows (Basirico et al. 2011; Parmar et al. 2015), sperm motility in Qincuan and Egyptian bulls (Zhang et al. 2015; Gafer et al. 2015) and horn-fly infestation response in Brahman and Angus cows (Turner et al. 2013). There are no studies on genetic characterization of bHSP$_{70}$ gene in Pasundan cattle as well as environmental resistance of the breed.

This research was conducted to detect SNPs in the 5'UTR of bHSP$_{70}$ gene in Pasundan cows, reared at breeding station and its association with S/C. The result of this cattle can be used as early information for developing molecular selection program to improve reproductive traits in the future.

MATERIALS AND METHODS

Blood sample and DNA extraction

The blood samples were collected from 44 Pasundan cows kept at the breeding center (BPPIBT-SP Cijeungjing, Ciamis, West Java) using vacutainer containing K_2EDTA about 3 mL for each cattle through jugular veins. The DNA of blood samples were extracted using Genomic DNA Mini Kit (Geneaid Biotech Ltd., Taiwan), following the procedures instruction. The extracted DNA was appropriately labeled and stored at -20 °C for the next analysis.

PCR amplification and DNA sequencing

The PCR reaction was performed in a Mastercycler® gradient (Eppendorf, Germany) with a pair of primer according to Basirico et al. (2011), i.e., Forward: 5'-GCCAGGAAACCAGAGACAGA-3' and Reverse: 5'-CCTACGCAGGAGTAGGTGGT-3'. This primer amplified the bHSP$_{70}$ gene from nucleotide position 749 to 1287 bp (329 bp) according to GenBank: M98823.1. The PCR reagent was performed in the 30 µL total volume, comprising 7.8 µL of ddH_2O, 0.6 µL of primer forward and reverse (1 µM), 15 µL of PCR kit (Mytaq^{TM} HS Red Mix, USA), and 6 µL of DNA genome. DNA amplification was performed using Mastercycler® (Eppendorf, Germany) and performed with predenaturation temperature at 94°C for 2 minutes and following 39 cycles of denaturation at 94°C for 30 seconds, annealing at 58.2°C for 1 minutes, extension at 68°C at 1 minutes, and final extension at 68°C for 10 minutes. The 30 µL of PCR products were directly sequenced by commercial laboratory service, i.e., First BASE Laboratories Sdn. Bhd. (Malaysia) using ABI Prism 96-capillary 3730xl DNA analyzer (Applied Biosystems, USA). Sequence analysis began with a contig analysis which combines both the sequencing results (forward and reverse) to obtain a complete single sequence of each sample. The sequence obtained were aligned with the reference sequence and analyzed by using BioEdit ver 7.2.0 software (Hall 1999).

Statistical analysis

Statistical analysis for the bHSP$_{70}$ gene sequence consisted of genotype frequency, allele frequency, expected heterozygosity (H_e), observed heterozygosity (H_o), number of effective allele (n_e) and polymorphic informative content (PIC) and Hardy-Weinberg equilibrium were calculated based on Nei and Kumar (2000). Data of S/C were analyzed using general linear model (GLM) at the level of probability 0.01 to test the significance of the differences between the averages studied and based on the formula of the mathematical model:

$$Y_i = \mu + S_i + e_i$$

Where:
Y_i = the value of S/C
μ = means of S/C
S_i = effect of i^{th} SNPs
e_i = error term

RESULTS AND DISCUSSION

The bHSP$_{70}$ gene of Pasundan cattle was successfully amplified using a pair of primer (Figure 1). The result indicated that amplification fragment had good specificity and could proceed directly to sequencing analysis. The size of the PCR product was in accordance with the reference sequence. A total of 17 SNPs and one insertion/deletion (indel) were detected in the 5'UTR of bHSP$_{70}$ gene in Pasundan cattle (Tabel 1). Most of the mutation types that occurred in this study are transversion (61.11%) and followed by transition (33.33%). Ten SNPs of g.1045G/A; g.1069C/T; g.1096A/G; g.1117G/A; g.1125A/C; g.1128G/T; g.1134T/C; g.1164G/T; g.1204T/C and g.1255C/T so far are detected in many cattle breeds such as Brahman (Rosenkrans et al. 2010), native Turkish breeds and Friesian Holstein (Oner et al. 2017). The moderate PIC value (0.30<PIC<0.50) was found in two SNPs of g.1117G/A and g.1125A/C (Table 2). Oner et al. (2017) reported that SNP of g.1117G/A in Boz Irk breed has moderate PIC value (0.31), which is similar to this study. Three SNPs of g.1125A/C; g.1128G/T and g.1204T/C have a moderate PIC value in Brahman (Rosenkrans et al. 2010), Friesian Holstein and native Turkish breeds (Oner et al. 2017). In contrast, two SNPs of g.1128G/T and g.1204T/C in this study had a low PIC value (PIC<0.30). Banks et al. (2009) reported that two SNPs of g.1125A/C and g.1128G/T have significant association with calving percentage of Brahman cows with genotype AA (g.1125A/C) and GG (g.1128G/T) as the best genotype. Meanwhile, Turner et al. (2013) reported that SNP of g.1128G/T has significant association with horn-fly infestation response in Brahman and Angus cows with GG or TT as the best genotype. In addition, Genotype GT in SNP of g.1128G/T and it suggested the best genotype for

PBMC responses in Friesian Holstein (Basirico et al. 2011). Research showed that most of animal studied had GG genotype (0.93) in SNP of g.1128G/T and suggested that Pasundan cattle has potential traits for high calving percentage and horn-fly infestation resistance and important to investigate with large number of observation.

Preliminary analysis with limited number of samples (13 heads of Pasundan cows) showed that two SNPs of g.1117G/A and g.1125A/C were not affected to S/C (Table 3). However, the homozygote genotype had lower of S/C value than heterozygote genotype in both SNP. Banks et al. (2009) obtained the homozygote genotype (AA) as the best genotype for reproductive traits in Brahman cows based on SNP of g.1125A/C. This result is similar to this study. Singe nucleotide polymorphism of g.1125A/C under Hardy-Weinberg equilibrium (χ^2<5.99), revealed that the distribution of A and C alleles on this SNP were randomly distributed and no genetic drift effect (Table 2). The highest n_e value was detected in SNP of g.1125A/C (1.98) and indicated that this SNP is polymorphic. Therefore, molecular selection based on SNP of g.1125A/C in regarding improve reproductive traits of Pasundan cattle, can be performed in the future. Haplotyping based on two SNP of g1117G/A and g.1125A/C were significantly affected the S/C in the animal studied (P<0.01). In addition, animal with homozygote genotype of GG/AA (g.1117G/A) and AA/CC (g.1125A/C) has lower of S/C value than the other genotype combinations (P<0.01). The further research to investigate this finding is important regarding obtain marker-assisted selection (MAS) for reproductive traits in the future through large number of sample.

In conclusion, the 5'UTR of $bHSP_{70}$ gene of Pasundan cattle is polymorphic with 18 mutation sites. Two SNPs of g.1117G/A and g.1125A/C have moderate PIC value (PIC>0.30) and it is potentially as the marker-assisted selection (MAS) for reproductive traits of Pasundan cows. Preliminary analysis reveals that homozygote genotype animals have lower S/C value than other genotypes.

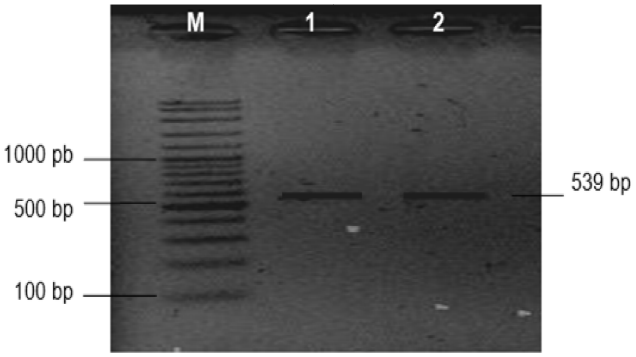

Figure 1. The amplification of bHSP gene (539 bp) in Pasundan cows separated on 1% agarose gel. M: marker (DNA ladder 100 bp); line 1-2: number of samples

Table 1. Identification of SNPs in the 5'UTR of $bHSP_{70}$ gene in Pasundan cows

Position	Nucleotide change	Mutation type	N	Frequency
862	A→T	Transversion	2	0.05
1017	C→G	Transversion	4	0.09
1019	T→G	Transversion	2	0.05
1036	C→T	Transition	1	0.02
1045	G→A	Transition	6	0.14
1050	T→C	Transition	3	0.07
1058	A→G	Transition	2	0.05
1069	C→T	Transition	6	0.14
1096	A→G	Transition	4	0.09
1112/1113	ins./del.C	Insertion	44	1.00
1117	G→A	Transition	24	0.55
1125	A→C	Transversion	10	0.23
1128	G→T	Transversion	3	0.07
1134	T→C	Transition	12	0.27
1164	G→T	Transversion	6	0.14
1204	T→C	Transition	34	0.77
1255	C→T	Transition	5	0.11
1262	C→T	Transition	3	0.07

Note: N: number of observation

Table 2. Results of statistical analysis in the SNPs at 5'UTR of $bHSP_{70}$ gene in Pasundan cows

SNP	Genotype frequency			Allele frequency		H_e	H_o	n_e	PIC	χ^2
g.862A/T	AA (0.96)	AT (0.02)	TT (0.02)	A (0.97)	T (0.03)	0.07	0.02	1.07	0.06	18.87
g.1017C/G	CC (0.91)	CG (0.09)	GG (0.00)	C (0.95)	G (0.05)	0.09	0.09	1.10	0.08	0.10*
g.1019T/G	TT (0.95)	TG (0.05)	GG (0.00)	T (0.98)	G (0.02)	0.04	0.05	1.05	0.04	0.02*
g.1036C/T	CC (0.98)	CT (0.02)	TT (0.00)	C (0.99)	T (0.01)	0.02	0.02	1.02	0.02	0.01*
g.1045G/A	GG (0.86)	GA (0.05)	AA (0.09)	G (0.89)	A (0.11)	0.20	0.05	1.25	0.18	26.38
g.1050T/C	TT (0.93)	TC (0.07)	CC (0.00)	T (0.97)	C (0.03)	0.07	0.07	1.07	0.06	0.06*
g.1058A/G	AA (0.95)	AG (0.05)	GG (0.00)	A (0.97)	G (0.03)	0.04	0.05	1.05	0.04	0.02*
g.1069C/T	CC (0.86)	CT (0.00)	TT (0.14)	C (0.86)	T (0.14)	0.24	0.00	1.31	0.21	44.00
g.1096A/G	AA (0.91)	AG (0.09)	GG (0.00)	A (0.95)	G (0.05)	0.09	0.09	1.10	0.08	0.10*
g.1117G/A	GG (0.45)	GA (0.25)	AA (0.30)	G (0.58)	A (0.42)	0.49	0.25	1.95	0.37	10.44
g.1125A/C	AA (0.34)	AC (0.43)	CC (0.23)	A (0.56)	C (0.44)	0.49	0.43	1.98	0.37	0.69*
g.1128G/T	GG (0.93)	GT (0.07)	TT (0.00)	G (0.97)	T (0.03)	0.07	0.07	1.07	0.06	0.06*
g.1134T/C	TT (0.73)	TC (0.16)	CC (0.11)	T (0.81)	C (0.19)	0.31	0.16	1.45	0.26	10.55
g.1164G/T	GG (0.86)	GT (0.14)	TT (0.00)	G (0.93)	T (0.07)	0.13	0.14	1.15	0.12	0.24*
g.1204T/C	TT (0.05)	TC (0.18)	CC (0.77)	T (0.14)	C (0.86)	0.24	0.18	1.31	0.21	2.29*
g.1255C/T	CC (0.89)	CT (0.11)	TT (0.00)	C (0.94)	T (0.06)	0.11	0.11	1.12	0.10	0.16*
g.1262C/T	CC (0.93)	CT (0.00)	TT (0.07)	C (0.93)	T (0.07)	0.13	0.00	1.15	0.12	44.00

Note: SNP: single nucleotide polymorphism; H_e: expected heterozigosity; H_o: observed heterozigosity; n_e: number of effective allele; PIC: polymorphic informative content; χ^2: chi-square value; *under Hardy-Weinberg equilibrium (χ^2<5.99)

Table 3. Preliminary analysis for investigating the effect of two SNPs with moderate PIC value to service per conception (S/C) in Pasundan cows

SNP	Genotype	N	S/C
g.1117G/A	GG	3	1.67±1.15
	AG	7	3.29±1,25
	AA	3	1.00±0.00
g.1125A/C	AA	3	1.33±0.58
	AC	7	3.14±1.46
	CC	3	1.67±1.15
Haplotype	Hom./Hom.	4	1.00±0.00^{a}
	Hom./Het.	4	2.25±0.96^{ab}
	Het./Het.	5	3.60±1.34^{b}

Note: N: number of observation; Hom.: homozygote genotype; Het.: heterozygote genotype. Means in the same column with different superscript differ significantly (P<0.01)

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