Transmissible spongiform encephalopathies (TSEs) in mammals are caused by abnormally folded prion proteins that induce conversion of the normal and noninfectious cellular form of the host prion protein (PrP^C) into an abnormal and infectious form (PrP^Sc) [21]. Bovine spongiform encephalopathy (BSE) is a TSE of cattle, which is transmitted to humans via consumption of BSE-contaminated beef and causes the development of a variant type of Creutzfeldt-Jakob disease (vCJD) [9]. Susceptibility or resistance to TSEs can be influenced by several factors of the host prion protein, such as specific amino acid polymorphisms, number of octapeptide repeats present and prion protein expression levels. Amino acid mutations in the prion protein are major factors influencing susceptibility and resistance to TSEs in humans and sheep. Susceptibility to BSE may also be enhanced by bovine PrP^C having 7 or more octapeptide repeats, though only Brown Swiss cattle have been shown to have 7 octapeptide repeats [3, 10].

In general, it is hypothesized that frequencies of insertion/deletion (indel) polymorphisms at two non-coding regions of bovine PRNP, 23indel and 12indel sites, are associated with PrP^C expression levels [23]. The first polymorphism is a 23-bp deletion (23del) within the upper region of the promoter that removes a binding site for the RP58 repressor protein, and the second is a 12-bp deletion (12del) within intron 1 that removes an SP1 transcription factor binding site. A reporter gene assay demonstrated an interaction between the two postulated transcription factors and lower expression levels of the 23-bp insertion (23ins)/12-bp insertion (12ins) allele compared with the 23del/12del allele [23]. Cattle possessing these deletions and therefore lacking binding sites for their respective regulatory elements have been reported to be more susceptible to classical BSE [12, 24], but these polymorphisms do not influence resistance to atypical BSE [2, 6]. Based on results of molecular weight analysis, atypical BSE can be divided into two subtypes: a subtype with a lower molecular mass of the unglycosylated PrP^Sc isoform (L-type) and a subtype with a higher molecular mass of the unglycosylated PrP^Sc isoform (H-type). An amino acid replacement at codon 211 (glutamic acid to lysine, E211K) encoded by bovine PRNP has been reported in a few H-type
atypical BSE cases [19, 22]. The E211K change is analogous to the human E200K amino acid replacement, which is a trigger of the development of heritable human TSE [8]. Recently, new information regarding the frequency and distribution of bovine \textit{PRNP} indel polymorphisms has been reported for Vietnamese local cattle and native Chinese cattle [17, 27, 29], and the information suggests that Asian local breed cattle have a variety of genetic divergences within the \textit{PRNP} for potential association with BSE.

In order to obtain information on \textit{PRNP} in Southeast Asian cattle and water buffalo, we investigated \textit{PRNP} polymorphisms of cattle and water buffalo in Vietnam, Indonesia and Thailand.

**MATERIALS AND METHODS**

*Genomic DNA samples from cattle and water buffalo in Vietnam, Thailand and Indonesia*: We collected liver or spleen samples from 288 cattle (\textit{Bos taurus} and \textit{B. indicus}) and 60 water buffalo (Swamp buffalo, \textit{Bubalus bubalis}) in Vietnam, Indonesia and Thailand (Table 1 and Fig. 1). Vietnamese samples were collected from nine provinces in the northern part of Vietnam at the period from July to August in 2007. Indonesian samples were collected from seven provinces in March 2007. For the Thai samples, we bought spleens of cattle and water buffalo at several grocery stores belonging to three different logistic groups in Bangkok in December 2005. All of the bovine liver and spleen samples were subjected to extraction of genomic DNA as described elsewhere [17]. The extracted DNA solutions were stored at $-30^\circ$C until use.

*PCR and sequencing analyses*: The extracted DNA solutions were used for PCR assays to determine the frequencies of polymorphisms within the 23indel, 12indel and octapeptide repeat regions of bovine \textit{PRNP}. The primers pair 23F-23R was used for genotyping 23indel, and the primers pair 12F-12R was used for genotyping 12indel in each PCR assay [2]. We designed the primer pair OetF (5’-GCAACCGTATCCACCTCAG-3’) and OetR (5’-TGGCTTACTGGGTTTGTTCC-3’) for PCR to amplify the octapeptide repeat region on the basis of the sequence data obtained from GenBank (Acc. No. AJ298878). The entire coding region of \textit{PRNP} was amplified as two fragments by PCR using the primer pairs F1-R2 and F2-R1 [2]. PCRs for the above four regions were performed with the following conditions of amplification: 2 min at 94°C followed by 13 cycles of 30 sec each at 94°C, 65°C and 72°C with stepwise lowering of the annealing temperature from 65°C to 55.4°C by the 13th cycle; and 23 cycles of 30 sec each at 94°C, 52°C and 72°C, followed by incubation at 72°C for 5 min as a final extension step. DNA sequencing was carried out on the PCR products of the entire coding region of \textit{PRNP}. Purification of the PCR products for sequencing was done using a SUPRECM™, PCR (Takara Bio Inc., Otsu, Japan). When the PCR products were heterozygous for octapeptide repeats in the coding region, the 5-repeat and the 6-repeat fragments were individually recovered by using a QIAquick Gel Extraction Kit (Qiagen, Tokyo, Japan). DNA sequencing was carried out in both directions on an ABI PRISM™ 310 Genetic Analyzer using a BigDye® Terminator v1.1 Cycle Sequencing Kit (Life Technologies Japan, Tokyo, Japan). Nucleic acid sequences were assembled and edited by using sequence alignment editing software (BioEdit version 7.0.5, http://www.mbio.ncsu.edu/bioedit/bioedit.html).

**Statistical analyses**: We categorized all Bovinae animals into 5 groups, Vietnamese cattle, Indonesian cattle, Thai cattle, Indonesian water buffaloes and Thai water buffaloes, to determine whether the Hardy-Weinberg equilibrium (HWE) was applicable to the population in each group. Then, in the 23indel and 12indel polymorphisms, haplotype frequencies

| Countries (total heads) | Sampling areasa) | Species or breedb) (subtotal heads) |
|------------------------|------------------|-------------------------------------|
| Vietnam (100)          |                  |                                     |
| Caô Bằng               |                  | Local cattle (36), Water buffalo (1) |
| Bạc Kan                |                  | Local cattle (8)                     |
| Tuyên Quang, Hòa Bình |                  | Local cattle (5, each)               |
| Lạng Sơn               |                  | Local cattle (2)                     |
| Hà Nội                 |                  | Local cattle (15)                    |
| Nghệ An                |                  | Local cattle (6)                     |
| Ninh Bình, Thanh Hóa   |                  | Local cattle (11, each)              |
| Indonesia (135)        |                  |                                     |
| Sumatra Utara          |                  | Brahman cross (6), Local cattle (14) |
| Sumatra Barat          |                  | UN (18)                              |
| Lampung                |                  | Local cattle (2), Bali cattle (1), Limousin (2), UN (13), Water buffalo (2) |
| Sulawesi Selatan       |                  | Peranakan Ongole (13), Limousin (2), Brahman (1), Simmental (4) |
| Kalimantan Selatan     |                  | Local cattle (11), Water buffalo (6) |
| Java Timur             |                  | Bali cattle (20)                     |
| Yogyakarta             |                  | Bali cattle (13), Brahman (1), Water buffalo (6) |
| Thailand (113)         |                  |                                     |
| Bangkok                |                  | Local cattle (68), Water buffalo (45) |

a) In Vietnam and Indonesia, each name of the sampling area means that of a province. Bangkok is the name of the capital city of Thailand. b) Vietnamese local cattle group consisted of Vietnamese Yellow local cattle and their cross breeds. UN means unknown cattle breed.
were derived from the genotypic data. The test of HWE and the haplotype estimation were performed by using gene analysis software, Haplovie 4.2 (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haplovie/haplovie). Differences in frequency distributions of allele, genotype and haplotype were calculated with Fisher’s exact test by using the freely available statistical software ‘EZR’ (version 1.00; Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for the free statistical soft-

Fig. 1. Sampling areas in Vietnam and Thailand (Panel A) and in Indonesia (Panel B). A: Vietnamese samples (99 cattle and 1 water buffalo) were collected from nine provinces in the northern part of Vietnam (nos. 1 to 7, Hà Nội and Ninh Bình). Numbers indicate the names of provinces. 1, Cao Bằng; 2, Bắc Kạn; 3, Tuyên Quang; 4, Hòa Bình; 5, Lang Sơn; 6, Nghệ An; and 7, Thanh Hóa. Thai samples (68 cattle and 45 water buffaloes) were bought at several grocery stores in Bangkok. B: Indonesian samples were collected from seven provinces (Sumatra Utara, Sumatra Barat, Lampung, Yogyakarta, Java Timur, Kalimantan Selatan and Sulawesi Selatan). The names of islands from which samples were collected in Indonesia are shown in italics.
ware ‘R’ (version 2.13.0; The R Foundation for Statistical Computing) [13]. For comparison of allelic and genotypic polymorphism frequencies in the 2 indel sites and haplotype frequency, the following three cattle groups and one water buffalo group were used as reference groups: UK healthy and BSE-affected Holstein Friesian cattle [12], B. indicus of five breeds [3] and Anatolian water buffalo (B. bubalis) [20]. German healthy and BSE-affected cattle [24] were used as reference groups for comparison of allelic and genotypic polymorphism frequencies among octapeptide repeat polymorphisms. Because of the variety of cattle breeds and species, we compared the frequencies of 23indel, 12indel, haplotype and octapeptide repeat polymorphisms in subgroups of Indonesian local cattle with those of the reference groups. For comparison among the subgroups of Indonesian local cattle, information for subgroups with less than 10 head of cattle was excluded, because the numbers of cattle in these subgroups were too small for statistical analyses.

RESULTS

23indel, 12indel, haplotype and octapeptide repeat polymorphisms (Table 2): The frequency distributions of alleles and genotypes in the 23indel site of Indonesian cattle and Thai cattle were significantly lower than those of all of the reference cattle groups. However, a significant difference in the frequency distribution of alleles or genotypes in the 23indel site was not found between Vietnamese cattle and the UK reference cattle groups. In the 12indel site of cattle, the frequency distributions were clustered on the ins allele and the ins/ins genotype. The frequencies of the 12del allele and del/del genotype of the cattle of all three countries were significantly lower than those in all of the reference cattle groups. Significant differences in frequencies of the 12indel site were found between Peranakan Ongole breed cattle and the UK reference groups and between Sumatra Utara breed cattle and B. indicus, respectively. The major haplotypes were 23ins-12ins in Kalimantan Selatan local cattle and Bali cattle, 23del-12ins in Peranakan Ongole breed and 23del-12del in Sumatra Utara local cattle. Haplotype 23ins-12del was minor or was not detected. The frequencies of 23del-12del were significantly lower in all cattle subgroups except Sumatra Utara local cattle than in the reference groups. The frequencies of 23ins-12ins were significantly higher in Kalimantan Selatan local cattle and Bali cattle than in the reference groups. For all of the tested subgroups, the HWE was applicable for the 12indel polymorphism, but was not applicable for the 23indel polymorphism. As for the frequency of octapeptide repeat polymorphism, only two subgroups (Kalimantan Selatan local cattle and Bali cattle) showed a significant difference from the reference groups.

Single nucleotide polymorphisms in the coding region of PRNP (Table 4): A total of 15 SNPs were detected in the coding region of PRNP in 85 cattle DNA samples selected from the three countries. All of the genotypes for octapeptide repeat polymorphism (6/6, 6/5 and 5/5) were included in the selected samples. Numbering of each nucleotide and amino acid position refers to the sequence of Bali cattle obtained in this study (GenBank accession no. AB761619). Three sites at nucleotide positions 8 (A to C), 461 (G to A) and 554 (A to G) were non-silent mutations. These SNPs corresponded to the following amino acid substitutions: lysine to threonine (K3T), serine to asparagine (S154N) and asparagine to serine (N185S). Of the remaining 12 SNPs that showed silent mutations, four sites at positions 108 (T to G), 267 (T to A), 554 (A to G) and 783 (C to T) were unique to Indonesian cattle, particularly Kalimantan Selatan local cattle and Bali cattle. These 4 SNPs have not yet been reported for domestic cattle, and nucleotide mutation at position 783 (C to T) is known only for Banteng (B. javanicus) [26]. There was no PRNP allele in our samples that exhibited E211K amino acid replacement, which is considered to be a possible cause of H-type atypical BSE.

DISCUSSION

Our results provide information on PRNP of livestock in these Southeast Asian countries. Significantly lower frequencies of the del polymorphism in the 23indel site were found in cattle in Indonesia and Thailand, and significantly lower frequencies of the del polymorphism in the 12indel site were found in cattle in all three countries. In contrast, the distributions of 23del polymorphisms in Vietnamese cattle showed a tendency to be similar to those in the UK reference cattle groups. The breeding history of Vietnamese cattle may have contributed to the distributional similarity. In Vietnam, more than 80% of dairy cattle are cross-bred between Hol-
stein Friesian cattle and local Yellow cattle and Red Sindhi, a *B. indicus* breed introduced to Vietnam at the beginning of the 20th Century [1, 28].

The frequency patterns of 2 indel polymorphisms in Indonesian cattle and Thai cattle, especially Bali cattle and Kali-mantan Selatan local cattle in Indonesia, do not conform to any of those previously reported for domestic cattle. These unique genetic backgrounds of *PRNP* probably originated from their ancestral animals. Bali cattle are generally recognized as domesticated cattle from wild Banteng. Bali cattle

Table 2. Distributions of allele and genotype frequencies for 23-bp indel, 12-bp indel and octapeptide repeat polymorphisms in *PRNP* of the bovidae animals examined in this study

| 23-bp indel | Frequencies (%) | P-value | Genotype | Frequencies (%) | P-value |
|-------------|----------------|---------|----------|----------------|---------|
| Bovine groups (heads) | ins | del | UK healthy HF | UK BSE HF | B. indicus | Anatolian WB |
| Vietnamese cattle (99) | 33 | 67 | 0.367 | 0.017 | <0.001 | <0.001 |
| Indonesian cattle (121) | 49 | 51 | <0.001 | <0.001 | <0.001 | <0.001 |
| Thai cattle (68) | 53 | 47 | <0.001 | <0.001 | <0.001 | <0.001 |
| Indonesian WB (14) | 100 | 0 | <0.001 | <0.001 | <0.001 | 0.142 |
| Thai WB (45) | 53 | 47 | <0.001 | <0.001 | <0.001 | <0.001 |
| UK healthy HF (276) | 29 | 71 | - | 0.047 | <0.001 | <0.001 |
| UK BSE HF (363) | 24 | 76 | 0.047 | - | 0.004 | <0.001 |
| B. indicus (58) | 12 | 88 | <0.001 | 0.004 | - | <0.001 |
| Anatolian WB (106) | 92 | 8 | <0.001 | <0.001 | <0.001 | - |

| 12-bp indel | Frequencies (%) | P-value | Genotype | Frequencies (%) | P-value |
|-------------|----------------|---------|----------|----------------|---------|
| Bovine groups (heads) | ins | del | UK healthy HF | UK BSE HF | B. indicus | Anatolian WB |
| Vietnamese cattle (99) | 95 | 5 | <0.001 | <0.001 | 0.017 | 0.002 |
| Indonesian cattle (121) | 79 | 21 | <0.001 | <0.001 | 0.081 | 0.084 |
| Thai cattle (68) | 88 | 13 | <0.001 | <0.001 | 1.000 | 0.749 |
| Indonesian WB (14) | 100 | 0 | <0.001 | <0.001 | 0.043 | 0.031 |
| Thai WB (45) | 84 | 16 | <0.001 | <0.001 | 0.687 | 0.725 |
| UK healthy HF (270) | 37 | 63 | - | 0.001 | <0.001 | <0.001 |
| UK BSE HF (350) | 28 | 72 | 0.001 | - | <0.001 | <0.001 |
| B. indicus (58) | 87 | 13 | <0.001 | <0.001 | - | - |
| Anatolian WB (106) | 86 | 14 | <0.001 | <0.001 | 0.867 | - |

| Haplotypes | Frequencies (%) | P-value | References |
|------------|----------------|---------|------------|
| Bovine groups (heads) | 23ins/12ins | 23del/12del | 23del/12ins | 23del/12del |
| Vietnamese cattle (99) | 52 | 0 | 63 | 5 |
| Indonesian cattle (121) | 47 | 1 | 33 | 19 |
| Thai cattle (68) | 54 | 0 | 34 | 13 |
| Indonesian WB (14) | 100 | 0 | 0 | 0 |
| Thai WB (45) | 54 | 0 | 30 | 16 |
| UK healthy HF (273) | 29 | 0 | 8 | 63 |
| UK BSE HF (353) | 24 | 0 | 4 | 72 |
| B. indicus (58) | 12 | 0 | 75 | 13 |
| Anatolian WB (106) | 86 | 8 | 1 | 5 |

| Octapeptide repeat | Frequencies (%) | P-value | References |
|--------------------|----------------|---------|------------|
| Bovine groups (heads) | 6/5 | GER healthy | GER BSE |
| Vietnamese cattle (99) | 99 | 1 | 0.015 | 0.031 |
| Indonesian cattle (121) | 84 | 16 | 0.010 | 0.008 |
| Thai cattle (68) | 99 | 1 | 0.084 | 0.075 |
| Indonesian WB (14) | 93 | 7 | 0.655 | 0.634 |
| Thai WB (45) | 82 | 18 | 0.013 | 0.012 |
| GER healthy (48) | 95 | 5 | - | 1.000 |
| GER BSE (43) | 95 | 5 | 1.000 | - |

GER, German; UK, British; HF, Holstein Friesian; WB, Water buffalo. In cases in which a significant difference was detected by Fisher’s exact test, each *P*-value is shown in bold font (P<0.001). Data for Vietnamese WB (1 head) was excluded from this table.
Table 3. Distributions of allele and genotype frequencies for 23-bp indel, 12-bp indel and octapeptide repeat polymorphisms in PRNP among subgroups of Indonesian local cattle

| Bovine subgroups (heads) | 23-bp indel | | 12-bp indel | | Octapeptide repeat |
|--------------------------|-------------|-----------------|-----------------|-----------------|
|                          | Allele Frequencies (%) | P-value against the reference groups | Allele Frequencies (%) | P-value against the reference groups | Allele Frequencies (%) | P-value against the reference groups |
|                          | ins/ del UK healthy HF | UK BSE HF | B.indicus ins/ del | UK healthy HF | UK BSE HF | B.indicus |
| Kalimantan Selatan Lc (11) | 77/23 | <0.001 <0.001 <0.001 | 97/3 | <0.001 <0.001 <0.001 |
| Peranakan Ongole (13) | 19/81 | 0.376 0.815 0.345 | 8/23 | 0.151 0.247 0.340 |
| Sumatra Utara Lc (14) | 7/93 | 0.009 0.040 0.738 | 0/14 | 0.021 0.119 0.775 |
| Bali cattle (34) | 97/3 | <0.001 <0.001 <0.001 | 94/6 | <0.001 <0.001 <0.001 |

| Haplotype | Frequencies (%) |
|--------------------------|-----------------|
|                          | P-value against the reference groups |
|                          | UK healthy HF | UK BSE HF | B.indicus |
| Kalimantan Selatan Lc (11) | 73/5 | 0.040 0.172 | 0.001 |<0.001 <0.001 |
| Peranakan Ongole (13) | 12/0 | 0.365 0.350 | 0.001 |<0.001 0.935 |
| Sumatra Utara Lc (14) | 7/0 | 0.857 0.571 | 0.001 |<0.001 <0.001 |
| Bali cattle (34) | 96/2 | 2.3 | 0.001 |<0.001 <0.001 |

| Octapeptide repeat | Allele Frequencies (%) | P-value against the reference groups |
|-------------------|-----------------|-----------------|
|                          | 6/5 GER healthy GER BSE | 6/6/5/5/5 GER healthy GER BSE |
| Kalimantan Selatan Lc (11) | 64/36 | <0.001 <0.001 | 36/55 9 | 0.001 |<0.001 |
| Peranakan Ongole (13) | 88/12 | 0.365 0.350 | 0.001 |<0.001 0.335 |
| Sumatra Utara Lc (14) | 100/0 | 0.857 0.571 | 0.001 |<0.001 0.563 |
| Bali cattle (34) | 68/32 | <0.001 <0.001 | 47/41 12 | <0.001 <0.001 |

GER, German; UK, British; Lc, Local cattle; HF, Holstein Friesian. UK healthy HF and UK BSE HF, B. indicus, GER healthy and GER BSE cattle data were from references [3, 12, 24]. In cases in which a significant difference was detected by Fisher's exact test, each P-value is shown in bold font (P<0.001).

are different from all other species of cattle as a result of difference in their origin and evolution. B. taurus (European breed) and B. indicus (Zebu breed) are known to have been divided from a common ancestor, Aurochs (Bos primigenius), more than 3 million years ago. In contrast, restriction fragment length polymorphisms of mitochondrial DNA and the sequences of mitochondrial genes for cytochrome b confirmed that B. javanicus had a different ancestor from that of both B. taurus and B. indicus [14]. Moreover, a wide gene flow from B. javanicus extends to Southeast Asian local cattle to varying degrees [18]. It is notable that the del allele frequencies of two indel polymorphisms were low in Bali cattle, and our results suggested that both 23del and 12del polymorphisms are nonexistent or very few in B. javanicus and the ancestral animal of Southeast Asian local cattle.

An association between haplotype frequency for indel polymorphisms and BSE susceptibility has been reported in some domestic cattle breeds of B. taurus [7, 12]. The haplotype 23del-12del, which is associated with BSE incidence, is common in B. taurus, while the haplotype 23ins-12ins is the major haplotype in Indonesian and Thai cattle and in water buffalo. Functional studies of the promoter region of bovine PRNP indicated that 12del results in a significantly low level of PRNP expression compared with that in the case of 12ins [23]. However, the frequency of 12del allele is higher in the population of BSE-affected cattle, because the 12del site is in strong linkage disequilibrium with the polymorphism in the 23indel site of PRNP [11, 12, 23, 29]. The 12indel polymorphism of bovine PRNP contains a putative binding region of SP1, which activates a wide range of viral and cellular genes. The 23-bp insertion leads to strong and specific binding with RP58, which represses transcription by interfering with the DNA-binding activity of SP1 [15]. Therefore, PRNP expression level is lower in the haplotype 23ins-12ins than in the haplotype 23del-12del owing to the repressive effect of the haplotype 23ins-12ins [23].

Although an association between octapeptide repeat polymorphism of bovine PRNP and typical BSE incidence has not been reported in cattle, transgenic mice expressing bovine PrPSc of which the coding region contains 7 or 10 repeats have been reported to be more susceptible to BSE inoculums [4, 5]. While most domestic cattle have 5 or 6 octapeptide repeats [3, 24, 27, 29], seven repeats were reported in at least 5% of Brown Swiss breed [25] and 4 repeats were
identified in an animal of *B. indicus × B. taurus* composite cattle and 2 domesticated cattle from wild Gaur (*Bos gaurus*) in Asia, named Mythun [26, 27]. In this study, an abnormal number of octapeptide repeats was not detected, but frequencies of the octapeptide repeat in Bali cattle and Kalimantan Selatan local cattle showed genetic differences from those of *B. taurus* and *B. indicus*.

In this study, we detected three non-silent mutations: lysine to threonine (K3T), serine to asparagine (S154N) and asparagine to serine (N185S). The mutation of K3T, which is located in a signal sequence of PrP, has been reported to be present in at least 6% of native Chinese cattle [29]. Our results suggested the probability of genetic interchange among Vietnamese local cattle, Kalimantan Selatan local cattle and native Chinese cattle. On the other hand, the mutation of S154N, which is located close to the first α-helix [16], is a very minor amino acid replacement in domestic cattle. The replacement has been observed in all alleles for a wide variety of Bovinae animals (Lesser kudu, Nilgai, Asian water buffalo, Lowland anoa, African buffalo and Forest buffalo) [26]. The mutation of N185S, which is located within the second α-helix [16], has not been reported in domestic cattle, but has been confirmed in *B. javanicus* and African buffalo [26]. There has been no report showing an association between the 3 non-silent mutations and BSE susceptibility. We detected several mutations that were reported in *B. javanicus*. The similarity between Bali cattle and *B. javanicus* in PRNP polymorphisms supports the hypothesis that Bali cattle are domesticated from *B. javanicus*, and our results suggest that the genetic diversity of *PRNP* in Indonesian local cattle is a result of gene flow from *B. javanicus*.

Our results suggest that Southeast Asian cattle and water buffaloes have low susceptibility to BSE, particularly to classical BSE. In Southeast Asian countries where crossbreeding with *B. taurus* and *B. indicus* has been increasing, selective improvement in local cattle is important as an approach to provide additional protection against BSE infection.

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**REFERENCES**

1. Ashbaugh, H. R. 2010. A descriptive survey of dairy farmers in Vinh Thinh Commune, Vietnam. pp. 9–11. In: Public Health. Ohio State University, Ohio.

2. Brunelle, B. W., Hamir, A. N., Baron, T., Biacabe, A. G., Richt, J. A., Kunkle, R. A., Cutlip, R. C., Miller, J. M. and Nicholson, E. M. 2007. Polymorphisms of the prion gene promoter region influence classical bovine spongiform encephalopathy susceptibility and are not applicable to other transmissible spongiform encephalopathies in cattle. *J. Anim. Sci. 85*: 3142–3147. [Medline] [CrossRef]

3. Brunelle, B. W., Greenlee, J. J., Seabury, C. M., Brown, C. E. 2nd and Nicholson, E. M. 2008. Frequencies of polymorphisms associated with BSE resistance differ significantly between *Bos taurus*, *Bos indicus*, and composite cattle. *BMC Vet. Res. 4*: 36. [Medline] [CrossRef]

4. Castilla, J., Gutierrez-Adan, A., Brun, A., Pintado, B., Parra, B., Ramirez, M. A., Salguero, F. J., Diaz San Segundo, F., Rabano, A., Cano, M. J. and Torres, J. M. 2004. Different behavior toward bovine spongiform encephalopathy infection of bovine prion protein transgenic mice with one extra repeat octapeptide insertion mutation. *J. Neurosci. 24*: 2156–2164. [Medline] [CrossRef]

5. Castilla, J., Gutierrez-Adan, A., Brun, A., Pintado, B., Salguero, F. J., Parra, B., Segundo, F. D., Ramirez, M. A., Rabano, A., Cano, M. J. and Torres, J. M. 2005. Transgenic mice expressing bovine PrP with a four extra repeat octapeptide insertion mutation show a spontaneous, non-transmissible, neurodegenerative dis-
17. Muramatsu, Y., Sakemi, Y., Horuchi, M., Ogawa, T., Suzuki, K., Kanameda, M., Hanh, T. T. and Tamura, Y. 2008. Frequencies of PRNP gene polymorphisms in Vietnamese dairy cattle for potential association with BSE. *Zoonoses Public Health* 55: 267–273. [Medline] [CrossRef]

18. Namikawa, T. and Widodo, W. 1978. Electrophoretic variation of hemoglobin and serum albumin in the Indonesian cattle including Bali cattle (*Bos banteng*). *Jpn. J. Zootech. Sci.* 49: 817–827.

19. Nicholson, E. M., Brunelle, B. W., Richt, J. A., Kehrli, M. E. Jr. and Greenlee, J. J. 2008. Identification of a heritable polymorphism in bovine *PRNP* associated with genetic transmissible spongiform encephalopathy: evidence of heritable BSE. *PLoS ONE* 3: e2912. [Medline] [CrossRef]

20. Oztbak, K., Ozkan, E., Soyasal, I., Paya, I. and Un, C. 2009. Detection of prion gene promoter and intron 1 indel polymorphisms in Anatolian water buffalo (*Bubalus bubalis*). *J. Anim. Breed. Genet.* 126: 463–467. [Medline] [CrossRef]

21. Prusiner, S. B. 1998. Prions. *Proc. Natl. Acad. Sci. U.S.A.* 95: 13363–13383. [Medline] [CrossRef]

22. Richt, J. A. and Hall, S. M. 2008. BSE associated with prion protein gene mutation. *PLoS Pathog.* 4: e1000156. [Medline] [CrossRef]

23. Sander, P., Hammann, H., Droegemuller, C., Kashevchik, V., Schiebel, K. and Leeb, T. 2005. Bovine prion protein gene (*PRNP*) promoter polymorphisms modulate PRNP expression and may be responsible for differences in bovine spongiform encephalopathy susceptibility. *J. Biol. Chem.* 280: 37408–37414. [Medline] [CrossRef]

24. Sander, P., Hammann, H., Pfieffer, I., Wenmeuer, W., Brenig, B., Groschup, M. H., Ziegler, U., Distl, O. and Leeb, T. 2004. Analysis of sequence variability of the bovine prion protein gene (*PRNP*) in German cattle breeds. *Neurogenetics* 5: 19–25. [Medline] [CrossRef]

25. Schläpf, I., Saibekova, N., Gaillard, C. and Dolf, G. 1999. A new allelic variant in the bovine prion protein gene (*PRNP*) coding region. *Anim. Genet.* 30: 386–387. [Medline] [CrossRef]

26. Seabury, C. M., Honeycutt, R. L., Rooney, A. P., Halbert, N. D. and Derr, J. N. 2004. Prion protein gene (*PRNP*) variants and evidence for strong purifying selection in functionally important regions of bovine exon 3. *Proc. Natl. Acad. Sci. U.S.A.* 101: 15142–15147. [Medline] [CrossRef]

27. Shimogiri, T., Msalya, G., Myint, S. L., Okamoto, S., Kawabe, K., Tanaka, K., Mannen, H., Minezawa, M., Namikawa, T., Amano, T., Yamamoto, Y. and Maeda, Y. 2010. Allele distributions and frequencies of the six prion protein gene (*PRNP*) polymorphisms in Asian native cattle, Japanese breeds, and myt-hun (*Bos frontalis*). *Biochem. Genet.* 48: 829–839. [Medline] [CrossRef]

28. Stanton, E. and Stanton, S. 2011. 8.5 Dairy cattle breeds. pp. 29–30. In: Vietnam Livestock Genetics: A Review of the Market and Opportunities for Canadian Livestock Genetics Exporters, Agriculture and Agri-Food Canada.

29. Zhao, H., Wang, X. Y., Zou, W. and Zhang, Y. P. 2010. Prion protein gene (*PRNP*) polymorphisms in native Chinese cattle. *Genome* 53: 138–145. [Medline] [CrossRef]