The natural resistance associated macrophage protein 1 (Nramp1) has been reported to confer resistance or susceptibility to Mycobacterium bovis, Salmonella typhimurium, and Leishmania donovani in the mouse, Mus musculus [13]. A Gly and Asp substitution at position 169 of the mouse Nramp protein is invariably associated with the resistant and susceptible phenotypes, respectively. Furthermore, these pathogens cause losses in the livestock industry worldwide and are transmissible to the human population despite the widespread application of antimicrobials and vaccination. Bovine (Bos taurus) NRAMP1 cDNA has been cloned and was found to have an 86.9% sequence identity with mouse Nramp1 [4]. The NRAMP1 genes of other ruminants have high sequence identity with the bovine NRAMP1 gene. For example, the NRAMP1 genes of the bison (Bison bison), water buffalo (Bubalus arnee bubalis), sheep (Ovis aries) and red deer (Cervus elaphus) [3, 5–7, 9] were reported to have 99.1%, 98.4%, 97.0% and 96.7% sequence identity with the bovine NRAMP1 gene, respectively. It was reported in cattle that a microsatellite polymorphism in the 3′ untranslated region affected the expression of the NRAMP1 gene and also affected on Brucella abortus replication [1], but it was not associated with M. bovis infection [2]. The present study aimed to detect polymorphisms in the NRAMP1 gene from different cattle and buffalo breeds.

Genomic DNAs from five breeds of cattle and four breeds of buffalo were used in the study. Sequencing showed two nucleotide substitutions found in intron 4, three in exon V, and ten in intron 5. An amino acid substitution was observed at nucleotide position 1202 in exon V of the Japanese black, Angus, Philippine and Bangladesh swamp-type buffaloes which coded for Thr, while the Korean cattle, Holstein, African N’dama, Indonesian swamp-type buffalo and the Bangladesh river-type buffalo had Ile. All the breeds of cattle and buffaloes tested in this study coded for Gly at the position in exon VI which corresponds to the same amino acid of the murine Nramp1-resistant phenotype at position 169. The phylogenetic relationship among the different breeds showed a cluster comprised mainly of cattle and another mainly of buffaloes.

NOTE Immunology

Sequence Analysis of the NRAMP1 Genes from Different Bovine and Buffalo Breeds

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ABSTRACT. The natural resistance associated macrophage protein 1 (Nramp1) has been reported to confer resistance or susceptibility to Mycobacterium bovis, Salmonella typhimurium, and Leishmania donovani in the mouse, Mus musculus. A Gly and Asp substitution at position 169 of the mouse Nramp protein is invariably associated with the resistant and susceptible phenotypes, respectively. The present study aimed to detect polymorphisms in the NRAMP1 gene from different cattle and buffalo breeds. Genomic DNAs from five breeds of cattle and four breeds of buffalo were used in the study. Sequencing showed two nucleotide substitutions found in intron 4, three in exon V, and ten in intron 5. An amino acid substitution was observed at nucleotide position 1202 in exon V of the Japanese black, Angus, Philippine and Bangladesh swamp-type buffaloes which coded for Thr, while the Korean cattle, Holstein, African N’dama, Indonesian swamp-type buffalo and the Bangladesh river-type buffalo had Ile. All the breeds of cattle and buffaloes tested in this study coded for Gly at the position in exon VI which corresponds to the same amino acid of the murine Nramp1-resistant phenotype at position 169. The phylogenetic relationship among the different breeds showed a cluster comprised mainly of cattle and another one mainly of buffaloes.

KEY WORDS: bovine, NRAMP1, nucleotide sequence.
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Japan. The phylogenetic analyses were done using the neighbor-joining (NJ) method [11]. Alignment of multiple sequences was done using the CLUSTAL X program [12].

The 781-bp region between positions 1071 and 1852 of the NRAMP1 gene covers the area between intron 4 and intron 6. Since the region of the murine Nramp1 which is a match for exon VI of the bovine NRAMP1 is related with the amino acid substitution causing the resistant or susceptible phenotypes, it would be interesting to determine the sequence of exon VI and the adjacent region of the NRAMP1 gene. As shown in Table 1, two nucleotide substitutions were found in intron 4, three in exon V, and ten in intron 5. There were no nucleotide substitutions found in exon VI or in intron 6. An amino acid substitution was observed at nucleotide position 1202 only in exon V. Japanese black, Angus, Philippine swamp-type buffalo and River-type buffalo coded for Thr (ACT) at this position, while the Korean cattle, Holstein, African N’dama, Indonesian swamp-type buffalo and the Bangladesh river-type buffalo had Ile (ATT). We will study in the future whether this amino acid exchange might influence the resistivity against bacterial infection. It was reported in the mouse that the resistant Nramp1 genotype carries Gly at position 169 in exon VI but the sensitive type has Asp [8]. All the breeds of cattle and buffaloes tested in this study coded for Gly at the position in exon VI, which corresponds to the same amino acid of the murine Nramp1-resistant phenotype at position 169. Based on these substitutions in the sequence, a phylogenetic tree was constructed as shown in Fig. 1. The phylogenetic relationship among the different breeds showed a cluster comprised mainly of cattle and another one mainly of buffaloes.

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Table 1. Nucleotide substitutions in the region between the positions 1071 and 1852 (781 bp) of the NRAMP1 gene from the different bovine and buffalo breeds. The amino acid substitution from Thr to Ile at position 1202 is indicated.

| Position | Intron 4 | Exon 5 | Intron 5 | | | | | |
|----------|---------|-------|---------|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Breed    | 1130    | 1140  | 1202    | 1260    | 1290  | 1316  | 1319  | 1341  | 1348  | 1405  | 1418  | 1426  | 1462  | 1634  | 1664  |
| Jap. Blk | g       | g     | c<sup>Th</sup> | t     | c     | t     | t     | c     | t     | g     | a     | g     | g     | a     | a     |
| Korean   | -       | -     | t<sup>Fr</sup> | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| Holstein | -       | -     | t<sup>Fr</sup> | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| Angus    | t       | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| N’Dama   | t       | t<sup>Fr</sup> | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| Phil.Swamp | c    | c     | -     | -     | -     | c     | -     | c     | a     | -     | -     | -     | -     | -     | -     |
| Indo.Swamp | c | t<sup>Fr</sup> | c     | -     | -     | c     | -     | c     | a     | -     | -     | -     | -     | -     | -     |
| Bang.Swamp | t    | -     | -     | a     | c     | -     | a     | c     | a     | c     | -     | -     | -     | -     | -     |
| Bang. River | c | t<sup>Fr</sup> | c | a | - | g | c | - | a | - | - | - | - | - | - |

Abbreviations are as follows: Japanese black (Jap. Blk.), African N’dama (N’Dama), Philippine (Phil.), Indonesia (Indo.), Bangladesh (Bang.), Swamp-type buffalo (Swamp), and River-type buffalo (River).

Fig. 1. A phylogenetic tree of bovine NRAMP1 was constructed using the neighbor-joining method [11].