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The molecular epidemiology investigation of the hemocytozoon distributed in South-East Asia and Southern Africa

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Benign Theileria group, represented by *T. sergenti/buffeli/orientaris* and related species, are classified into 4 types, Chitose (C), Ikeda (I), Werwick/Essex (*T. buffeli*: B), and Thailand (Thai) types, based on their sequences of the major piroplasm surface protein (MPSP) gene. In this study, by using allele-specific polymerase chain reaction which specifically amplifies the MPSP genes of those 4 types, molecular epidemiological analyses of the *Theileria* protozoa isolated in South-East and West Asia were carried out. Moreover, identification of protozoa species distributed in Southern Africa was carried out since various wild animals inhabit African Continent and various hemocytozoons which are parasitic to them also exist. Phylogenetic analysis was performed based on their nucleotide sequences of variable (V 4) regions of the 18 S ribosome RNA genes, which were conserved among species.

In Cambodia, Theileria protozoa were detected in 43 out of 56 samples obtained from Kompong speu, and in 26 out of 81 samples from Ratanakiri distinct. Mixed infections either by 3 types, C, B, and Thai types, or by 2 types, C and B types, were frequently detected. In Vietnam, *Theileria* protozoa were detected in 11 out of 40 samples, but all of them were classified as the Thai type. The I type was not detected in both Cambodia and Vietnam. No benign *Theileria* was detected in samples obtained from Pakistan. From these results, it is clear that the genetically different benign *Theileria* protozoa were distributed to those countries at the various proportion though they are geographically close to each other.

In Zambia, the 18 S ribosomal RNA genes of *Theileria* species were detected in 9 out of 117 samples derived from cattle, and they were classified into 4 types determined by nucleotide sequences. Comparison of them with known sequences in the database showed that these 4 types were similar to *T. mutans, T. buffeli, T. velifera*, and *T. sp.* which is similar to *T. mutans*, respectively. Among the wild animals in South Africa, a new parasitic which is closely related to *Babesia gibsoni* was found in hyena determined by phylogenetic analysis of the nucleotide sequences of the V 4 region. The 18 S ribosomal RNA genes were also detected in DNA samples derived from sable antelopes, elands, impalas, giraffes, and buffaloes. Based on the nucleotide sequences, the protozoa derived from buffalo were identified as *T. parva, T. mutans, T. velifera* and *T. sp.*, similar to *T. sp.* from Zambia. In the other animal species, mixed infection of *Theileria* protozoa in the wild ruminant as well as cattle were confirmed. This study showed that the various hemocytozoons which are parasitic to wild animals are distributed in African Continent, and it was also considered that the protozoa would be present in other animal species

which are not yet examined. To understand epidemiology of theileriosis, studies on vector tick distribution should be carried out.

Cloning of genes encoding animal cell growth factors and the expression of these genes in transgenic plants

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Epidermal growth factor (EGF) is known to naturally enhance the intestinal mucosal immunity, while transforming growth factor-β (TGF-β) plays a role in immunoglobulin (Ig) class switch to IgA, and may reduce intestinal inflammatory response. Therefore, oral administration of those factors, which could enhance the intestinal mucosal defenses, would benefit the prevention and treatment of gastrointestinal infections or the autoimmune diseases.

Expression of proteins with potential medical applications in transgenic plants has advantages over other expression systems because of low costs for production and of the possible application of the recombinant products for edible drugs. However, the major draw back at the present time is the inability to achieve the high amount of protein expression in transgenic plants. In this study, molecular cloning of dog and cat EGF was performed since EGF has been known to promote gastrointestinal maturation, and thus could be useful for the prevention of gastrointestinal infections. In addition, aiming at higher level expression of the recombinant products in transformed plants, bovine TGF-β1 gene fused with the legumin signal gene which can heighten the transgene expression in plants was introduced into tobacco.

For the cloning of dog and cat EGF genes, polymerase chain reaction (PCR) was carried out using specific primers designed from a region of the EGF gene conserved among other species. The resultant PCR products were sequenced, and identified as dog and cat EGF genes based on their similarities to known EGF sequences. The open reading frames of dog and cat EGF genes encode proteins with 1,216 and 1,210 amino acids, respectively, and showed very high homology to human, mouse and rat EGF genes.

Bovine TGF-β gene was introduced into tobacco using the *Agrobacterium tumefaciens*-mediated transformation system. The insertion of the TGF-β gene into tobacco genomic DNA was confirmed in all of the selected transformants by PCR. Only 2 % of the transformants were confirmed to express detectable levels of the recombinant protein, determined by ELISA. No significant difference was observed in the frequency of transformants positive for protein expression between legumin-positive and-negative transformants. However, the levels of protein expression were increased by the addition of the legumin signal.

By improving the expression levels of pro-