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Title	Analysis of diversity in the genes of Theileria sergenti major piroplasm surface protein by denaturing gradient gel electrophoresis
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ies against the HA of A/duck/Hong Kong/301/78 (H 7 N 1) were prepared to develop a rapid diagnostic method for H 7 influenza virus infection by detecting H 7 HA antigen in clinical samples. Monoclonal antibody 521 reacted with all H 7 viruses including horse, seal, and avian origin and not with any of H1, H3, H4, H5, or H 9 virus strains tested. The results indicate that monoclonal antibody 521 recognizes an epitope specific for the H 7 HA, being useful for diagnosis specific for H 7 influenza virus infection.

Equine herpes virus (EHV) 1 is an important pathogen to cause abortion in the pregnant mares. EHV 4 serologically cross-react with EHV 1 but scarcely causes abortion. Since both viruses distribute among horse populations, a simple and rapid method to differentiate them have been demanded. In the present study, monoclonal antibodies were raised against TH20 strain of EHV 4 and examined their biological activities for the purpose of the development of a rapid method of diagnosis of EHV 1 or EHV 4 infections. The present six monoclonal antibodies recognized gC glycoprotein of EHV4. They reacted with all of 10 EHV 4 strains isolated from horse during 1957 to 1997 in Japan as well as an EHV 1 strain isolated in Japan in 1967. Monoclonal antibody 247-4 to the EHV 1 GC molecule, provided by Dr. M. Shimizu at the National Institute of Animal Health, Japan, did not react with any of the EHV 4 strains tested. The results indicate that the present monoclonal antibodies could be used in combination with 247-4 for rapid diagnosis of EHV 1 and EHV 4 infections.

Analysis of diversity in the genes of *Theileria sergenti* major piroplasm surface protein by denaturing gradient gel electrophoresis

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Bovine theileriosis is a tick-borne disease of cattle caused by the protozoan parasite *Theileria sergenti* that causes anemia as intraerythrocytic piroplasma. On the surface of piroplasms, an immunodominant major piroplasm surface protein (MPSP) is expressed, and this molecule is thought to be a possible target for vaccine development. It was shown that MPSP gene shows genetic diversity that lead to antigenic diversity. This study was aimed at elucidating biological significance of MPSP gene diversity through the analysis based on denaturing gradient gel electrophoresis (DGGE) that is proved to be enough sensitive to detect even a single point mutation in a DNA fragment.

After the optimal conditions of DGGE for the analysis of *T. sergenti* MPSP gene were determined, DNA samples of field isolates were analysed. As most stocks of *T. sergenti* in Japan have been proven to be mixtures of two genotypes of MPSP, named by Chitose (C) and Ikeda (I) types, DGGE condition was optimized for each subtypes. The DGGE analysis revealed that C or I type was usually consisted of a single population, but sequence

variations of C-type MPSP gene were observed among the isolates. In some isolates, C types consisted of one major and several minor populations.

To analyse parasite population dynamics during serial passages between calves, and during persistent infection in a single host, parasite DNAs obtained from six calves infected with the same stock of C type *T. sergenti* were systemically analysed. Comparison among parasites that appeared at the first parasitemia peak in each animal by DGGE revealed that the parasite population was different in each animal. This result indicated that a certain population of *T. sergenti* selectively grew in a particular host. Between the first and second parasitemia peaks in individual calves, changes in populations were not detected.

In the experimental infection of cattle with a C-type parasite clone, mutations on MPSP gene were detected. However, this appearance of MPSP variants seemed to be due to random mutations in MPSP gene and these variants did not seem to undergo positive selection in infected calves during the persistant infection.

The method of mutation or population analysis based on DGGE developed in this study may be a good tool to analyse molecular epidemiology and to develop effective control methods against *T. sergenti* infection.

Expression of tumor necrosis factor-α receptors mRNA in sheep infected with bovine leukemia virus

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Bovine leukemia virus (BLV) is associated with enzootic bovine leukosis (EBL), which is the most common neoplastic disease of cattle. The BLV infection can remain clinically silent with cattle in an aleukemic state, or it can emerge as a persistent lymphocytosis (PL), characterized by an increased number of B lymphocytes, and more rarely, as B-cell lymphomas in various lymph nodes after a long latent period.

Many studies have reported factors involved in the disease progression of BLV infection, including major histocompatibility complex (MHC) haplotypes, the mutation in the *p53* gene and the changes in cytokine profiles. The importance of proinflammatory cytokines, such as tumor necrosis factor (TNF) -α has been documented for the antiviral and antiparasitic immune responses. In addition, the expression of TNFα receptors (TNFα R 1 and 2) on B cells in patients of chronic B- lymphatic leukemia were analyzed providing evidence that the importance of R 2 for B cell activation. It has also been reported that TNFα R 1 leads to programmed cell death through their respective death domains while TNFα R 2 mediates the inhibition of apoptosis. The aim of this study was to investigate the relationship between the expressions of TNFα and TNFα receptor mRNA from peripheral blood mononuclear cells (PBMCs) and disease progression of BLV