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Analysis of the glycoprotein 25 coding region
of the bovine viral diarrhea virus gene

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Cattle persistently infected with bovine viral diarrhea virus (BVDV) excrete the virus continuously without any clinical signs, and are serious sources of infection of BVDV in a herd. To diagnose the BVDV infection accurately and rapidly, polymerase chain reaction (PCR) has been utilized. By the use of specific primers for detecting p14 and gp25 coding regions of the BVDV gene, it was possible to discriminate the viral serotypes based on the PCR-amplification patterns. However, PCR-amplification patterns of several field isolates from Hokkaido did not correspond to any Japanese BVDV strains. In this study, to clarify the relation between PCR-amplification patterns and viral serotypes, a new primer coding the gp25 region of the BVDV gene was designed and PCR was performed on Japanese BVDV strains, field isolates, and bovine leukocytes. Then nucleotide sequences and de-

duced amino acid sequences of the PCR products were compared. Nucleotide sequences and deduced amino acid sequences of the PCR products were highly homologous. Therefore gp25 seemed not to be involved in the different PCR-amplification patterns. Analysis of the amino acid sequences indicated that the hydrophobicity of gp25 was highly conserved among PCR products examined in this study. This region might be a transmembrane domain of gp25, and function as an anchor for gp53 and gp48, which induce neutralizing antibodies.

Additionally, a gene highly homologous with gp25 of BVDV was detected in the bovine genome in leukocytes. This gene was recognized in not only BVDV-infected cattle but also in uninfected cattle. This gene might play an important role in BVDV attachment to or invasion of host cells.

Isolation and identification of the rumen bacteria responsible for
the onset of onion-induced hemolytic anemia in ruminants

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Onions (*Allium cepa*) are known to cause hemolytic anemia in many domestic animals, especially in cattle. The mechanism of onion-poisoning, however, has not been fully under-

stood. In the present study, some rumen bacteria capable of producing certain toxic compounds from onions were isolated and identified by the following procedure.

First, various colonies from ruminal fluid were formed on Müller-Hinton agar plates or Soybean-casein-digest agar plates containing 5% sheep blood after incubation at 37°C for 1~2 days under aerobic or anaerobic conditions. Each colony formed on the plates was inoculated to a sterile medium consisting of 2 ml of ruminal fluid from sheep and 500 µl of onion juice, and incubated at 38.5°C for 48 hours under anaerobic conditions. After incubation, 2 ml of the supernatant of the medium was partitioned with diethylether. After the ether was removed, the remaining dry materials were added to a sheep erythrocyte suspension. The suspension was incubated at 38.5°C for 2 hours, and the methemoglobin concentration of sheep erythrocytes was measured. The colonies that formed a high methemoglobin concentration (more than 60%) were selected as "active bacteria" strains.

Consequently, 5 strains from 2 sheep, 11 strains from 2 cows, and 1 strain from 5 goats were isolated as active bacteria. One strain of active bacteria, derived from sheep, was identified as *Lactococcus lactis lactis*, and the others were identified as *Streptococcus bovis*.

Antimicrobial sensitivity tests were performed for the *Streptococcus bovis* isolated from sheep. These tests showed that *Streptococcus bovis* was susceptible to penicillin G, ampicillin, dicloxacillin, cefalexin, tetracycline, erythromycin, lincomycin and chloramphenicol.

From the results, it was demonstrated that *Streptococcus bovis* was mainly responsible for the onset of onion-induced hemolytic anemia in ruminants. In addition, the antibiotics described above may be useful for the treatment of the disease.

Identification of a genetic defect and the establishment of gene diagnosis of cutaneous asthenia in a Holstein cow

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A defect of the core protein of dermatan sulphate proteoglycan was suspected to account for the pathogenicity of a Holstein cow affected with cutaneous asthenia (P1). By genetic analysis of the core protein in P1, a point mutation was detected. The mutation was a G to A transition at nucleotide position 254, which resulted in a Ser to Asn substitution of the bovine proteoglycan core protein. This substitution occurred in one of the Ser-Gly dipeptide repeats, which was considered the binding portion of dermatan sulphate. The dermatan sulphate chain can bind to Ser residues by an O-glycosyl bond, but can not

bind to an Asn residue because of the different pattern of the glycosyl bond. Cutaneous asthenia in P1 is, therefore, a disorder with mutation of the gene for the core protein of dermatan sulphate proteoglycan.

An attempt at genetic diagnosis of this type of cutaneous asthenia was then made using restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR). The results of RFLP and PCR indicated that P1 was a heterozygote of an abnormal gene of core protein. Thus, it was strongly suggested that the cutaneous asthenia in P1 was an autosomal