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## Development of Diagnostic Methods for Bovine Herpesvirus 1 Infection

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It has long been recognized that the problems of disease control within the enormous population of animals needed to be reviewed in the broadest context before selecting and deciding a specific disease control program. The distribution of the viral diseases of livestock in Zambia, as well as the control measures and limited research that has been done, are described. Foot and mouth disease causes serious economic losses in the cattle industry. So far five serotypes (SAT1, SAT2, SAT3, O and A) of the virus have been isolated in Zambia. Other notifiable viral diseases are rabies, Rift Valley fever, lumpy skin disease, African horse sickness, bluetongue, African swine fever, Newcastle disease, Marek's disease, fowlpox and infectious bursal disease. Based on the reports of clinical and/or serological diagnoses, these are widespread in the country, although their precise incidence rates are not known. There is need for the development of simple, highly sensitive and specific methods of diagnosis applicable to the surveillance study of viral diseases.

It was recognized from the above review that bovine herpesvirus 1 (BHV-1) infections have a very high prevalence rate and cause one of the most serious problems to the cattle industry in Zambia. BHV-1 is perpetuated in populations by latent infections which can be reactivated by stress. With this view in mind the author established a highly sensitive and specific method of Immuno-PCR for the detection of antigens of and antibodies to BHV-1. The assay, based on the method of Sano et al. (Science 258: 120-122. 1992), combines the specificity of

monoclonal antibodies to the viral antigens in an ELISA and the sensitivity of the PCR. Biotinylated anti-species antibodies are used in the place of the streptavidin-protein A chimera (original method), followed by streptavidin and an antibody-borne biotinylated plasmid DNA is the indicator. For antigen detection in culture supernatant of BHV-1 infected cells the Immuno-PCR/Ag was  $10^{3.5}$ ,  $10^{7.0}$ , and  $10^{6.9}$  fold more sensitive than plaque forming assay using MDBK cells, ELISA, and PCR, respectively. The modified method (Immuno-PCR/Ab) was applicable to the detection of antibodies in rabbit anti-BHV-1 immune serum and showed enhanced sensitivity by a factor of  $10^{5.0}$  compared to that of ELISA. Nasal secretions from calves experimentally infected with BHV-1 were evaluated for the presence of viral antigen by Immuno-PCR/Ag. The Immuno-PCR maintained its high sensitivity during the testing of both antigens in the nasal secretion and antibodies in the serum samples. The nasal secretions at day 21 p.i. were negative by infectivity titration but positive by Immuno-PCR/Ag up to a dilution of  $1:10^4$ , confirming the sensitivity and specificity of the method. Hence, Immuno-PCR with a sensitivity greater than any existing antigen or antibody detection method is useful for the diagnosis of BHV-1 infections.

Diagnosis of latent infections presents an especially difficult problem. To obtain further information on the latent infection with BHV-1 in the natural host, the author examined the nasal secretions and various tissues of experimentally infected calves using virus isolation, PCR, and

Immuno-PCR/Ag assays. In the nasal secretions, viral DNA was detected in samples with virus titers of  $10^{4.3}$  TCID<sub>50</sub> or more by PCR. On the same samples, Immuno-PCR/Ag remained positive up to day 19 p.i., the last day of test. BHV-1 DNA was detected from the following tissues in all the calves at day 22 p.i. : trigeminal

ganglia, ovaries, lungs, nasal and tracheal mucosae, spleen, prescapular and precrucial lymph nodes, and PBL, but not skeletal muscles. Virus was not recovered from any of these tissues. The present findings suggest a possible role of leukocytes in BHV-1 latent infection.

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Mechanisms of immuno-suppression induced by  
Marek's disease virus and involvement of T lymphocytes  
in the protective effects of Marek's disease vaccine

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Several pathogenic agents, including bacteria, protozoa and viruses, induce some immunomodulations to escape from immune pressure of their hosts. Apoptosis of lymphoid cells is sometimes observed in viral infections, and highly correlated with the viral pathogenesis, especially immuno-suppression. Marek's disease virus (MDV) can suppress cellular immunity followed by development of T-cell lymphoma. Therefore, it has been believed that the immuno-suppression plays an important role in lymphoma formation. In this thesis, the author described that MDV induces apoptosis in peripheral CD4<sup>+</sup>T cells and CD4<sup>+</sup>CD8<sup>+</sup> immature thymocytes during the latent phase of the infection. Apoptosis in thymocytes was observed at the early stage of the latent phase when thymic atrophy was commonly observed. However, the spleen from MDV-infected chickens showed severe splenomegaly at the same time, and thymocytes did not show apoptotic changes when peripheral

CD4<sup>+</sup>T cells underwent apoptosis. These results suggest that immature thymocytes might be more susceptible to apoptosis than peripheral CD4<sup>+</sup>T cells, or that the induction mechanism of apoptosis might differ between cell populations.

Down-regulation in the expression of several cellular proteins is one of the immunomodulations induced by pathogenic agents for escaping from the immune pressure of hosts. In the case of MDV-infection, the expression of CD8 molecules on peripheral T cells and thymocytes were down-regulated. CD8 as well as CD4 molecules are necessary for class I or II major histocompatibility complex (MHC)-restricted recognition of foreign antigens, and for thymocyte development. Thus, CD8-down-regulation may be also important for immuno-suppression induced by MDV.

MDV-infected chickens have been reported to show functional deficiency in T cells, characterized by delayed rejections of transplantable