two cattle type strains were identified as INMV2. 4 strains isolated from Czech Republic revealed as INMV1, whereas Slovakia strain revealed as INMV5. Discrimination index (DI) of MIRU-VNTR method was calculated as 0.498.

Conclusions: In the present study, genetic diversity of MAP field strains isolated from Korean cattle farms were analyzed by comparing to other strains isolated from different countries. Korean strains were discriminated as only two types but they were different from Czech and Slovakian strains. Although the number of strains were low, it is supposed to be that bison type (INMV68) strains are predominant in Korea because all of six farms had this strains. This have epidemiological importance because the bison type strains have been rarely reported in worldwide. However, subtypes of bison type strains couldn't be identified with MIRU-VNTR. Therefore, new genotyping methods which are highly discriminatory should be applied in further study. This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ00897001)" Rural Development Administration, the BK21 PLUS Program for Creative Veterinary Science Research and the Research Institute for Veterinary Science, Seoul National University, Republic of Korea.

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Development of an immunochromatography assay for antibody detection of Anaplasma phagocytophilum in Bovine

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Introduction: Anaplasma phagocytophilum is an obligatory intracellular and Gram-negative bacterium that encodes the 44-kDa major outer membrane proteins (p44). The main hosts are ruminants, dogs, horses, and rodents. This bacterium was causing tick-borne Anaplasmosis in ruminants, characterized by fever, lethargy, anorexia, arthritis, and thrombocytopenia. Recently, the chance of Anaplasmosis infection has been increased because of tick population growth as a result of global warming. The aim of the study is the development of an immunochromatographic strip for antibody detection of Anaplasma phagocytophilum using the 44-kDa major outer membrane proteins.

Materials and Methods: A. phagocytophilum DNA was extracted and amplified p44 coding gene by Polymerase chine reaction. Amplified p44 was cloned into the expression vector pET-32a by BamHI and SalI site. The recombinant pET-32a vector was transformed into Escherichia coli BL21. Recombinant p44 was over-expressed in E.coli system and purified by His-tag purification kit. this purified recombinant p44 was confirmed to antigencity by western-blotting. we performed that immunochromatographic assay using purified recombinatn p44 and antibody-colloial gold particle for the rapid detection of the A. phagocytophilum. in the detection test, purified recombinatn p44 and anti-rabbit IgG were blotted on the nitrocellulose membreane for the test and control lines, respecively. Serum samples diluted 10-fold with PBS and applied to the sample pad, and the solution migrates toward the absorbent pad. Protien A labled colloidal gold and p44 antigen were responded after 10 minutes.

Results: Expressed major surface protein was observed aroudn 44kDa and purified using His-tagging system based on high affinity chromatography between immobilized Ni-ion and histidine amino acid. Purified p44 was confirmed to antigencity by western-bloting and indirect ELISA. purified recombinatn p44 was blotted on the nitrocellulose membreane for the test lines. and than protein A labeled with colloidal gold was used the detector. The immunochromatographic test strip provides clear positive or negative results with the field samples. With a positive sample the antibody binding to the antigen conjugated forming a gold-antigen-antibody complex, which binds to p44 and giving a red colored band at the test line.

Conclusions: Dipstick assay based on the strip is rapid and easy to perform with no requirement of professional skills and equipment. So we was carried out to develope the immunochromatography assay for the ripid detection of the A. phagocytophilum. The immunochromatographic test strip provides clear positive or negative results with the field samples. These results show that the possibility of developing a rapid immunochromatography kit of A. phagocytophilum. However, further study will perform sensitivity and specificity test.

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Productions of inflammatory cytokines by *Brucella abortus* mutants with different growth rates

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