influenza viruses have occurred. Therefore, surveillance and biological characterization of avian influenza viruses should be performed. Here, we present the genetic characterization of new avian-origin H1N1 influenza virus isolated from wild birds in South Korea.

Materials and Methods: H1N1 influenza virus was isolated from wild bird fecal samples obtained from Chungnam province of Korea in 2014. The virus was isolated in 9-11 day-old SPF (specific pathogen free) embryonated chicken eggs. Whole-genome sequencing was performed and the sequences were aligned in CLC sequence viewer 6.7. Phylogenetic trees were constructed with reference sequences from National Center for Biotechnology Information (NCBI). To evaluate the viral replication kinetics of H1N1 influenza, MDCK and A549 cells were inoculated with 0.01 MOI (multiplicity of infection) H1N1 viruses.

Results: The HA, NA and internal genes of H1N1 viruses belong to Eurasian lineage in phylogenetic analysis. The new avian-origin H1N1 virus could grow to high titers in mammalian cells and the viral growth kinetics of H1N1 influenza was similar to A/Puerto Rico/8/1934 (H1N1, PR8) virus.

Conclusions: The recombination of new avian-origin H1N1 virus and other influenza viruses could produce new influenza pandemic. Therefore, more research of this virus should be performed to evaluate the pathogenic threat for domestic poultry and human.

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Outbreak of avian botulismof wild birds in Seoul and pathogen detection from enviromental samples

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Introduction: Avian botulism isinflicting significant damage to wild birds. And the pathogen, *Clostiridumbotulinum* can be left in the vicinity environment. En masse died wild birdbodies were found in Gangseo-gu, Seoul Magog district reservoir in October,2014.

Materials and Methods: They were diagnosed withbotulism caused by *C.botulinum* type C/D by PCR test and bioassays. Find out the detectionlimit in the soil of *C.botulinum* type C/D from serial dilution method bySM buffer is called spiking. The toxin gene of the *C.botulinum* type C/D from collected environmental samples such as soil ofGangseo-gu, Seoul Magog district reservoir was detected by PCR. We also attempt anisolation of *C. botulinum* type C/D by subculture using Tryptone-Peptone-Glucose-Yeast(TPGY) broth and McClung toabe agar.

Results: Mouse bioassay is useddead filtered inoculum's supernatant from cecum ofsuspected animals. Hassurvived the mouse bioassay results antitoxin C and antitoxin D injected mice. Minimum detection ofspiking test concentration was 10^2/ml. *C. botulinum* type C/D was detected in collected environmental samplessuch as soil of Gangseo-gu, Seoul Magog district reservoir by PCR. The pathogen was isolatedfrom liver and its toxin type was C/D

Conclusions: En masse died wild birdsare correlated with external environment. However, the cause of *C.botulinum*type C/D could not be clearly seen. And *C. botulinum*type C/D may be remaining in the vicinity environment. So should be continued to monitorthis area carefully.

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Molecular characterization of Korean isolates of *Mycobacterium avium* subsp. *paratuberculosis* using MIRU-VNTR typing method

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Introduction: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is a causative agent of Johne's disease or paratuberculosis, a chronic debilitating disease in ruminants, characterized as incurable enteritis and persistent diarrhea. Genotyping is useful tool for differentiation of strains that is important to understand the epidemiology, pathogenesis, and transmission. The mycobacterial interspersed repetitive units-variable number tandem repeat (MIRU-VNTR) is a polymerase chain reaction (PCR) based genotyping method. MIRU-VNTR can differentiate subtypes by amplification of elements that have variable number of tandem repeats. It has been known to be fast and cheap method that showing high discriminatory power. The aim of this study was to identify the genetic diversity of MAP strains collected from Korean cattle using MIRU-VNTR method.

Materials and Methods: Total of 23 MAP strains were genotyped by MIRU-VNTR. 18 strains were isolated from Korean cattle farms located in different three regions. Rest of 5 strains were distributed from OIE reference laboratory for Paratuberculosis and for Avian tuberculosis (Veterinary Research Institute) in Czech Republic. That strains were originated from cows in different farms located in Czech Republic and one of them was isolated from Slovakia. The MIRU-VNTR typing was performed using eight polymorphic loci (292, X3, 25, 47,3, 7, 10 and 32). PCR results and type of the strains were identified according to INMV database (http://mac-inmv.tours.inra.fr/index.php).

Results: The 18 strains of Korean isolates were previously typed using IS1311 PCR-REA that can differentiate the strains to three types (cattle type, bison type, sheep type). Two MAP strains were identified as "cattle type" and rest of 16 were "bison type". MIRU-VNTR of 23 strains resulted in 4 INMV types (INMV1, INMV2, INMV5, INMV68). All of bison type strains isolated from Korea typed as INMV68, whereas

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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