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Introduction: Anaplasmaphagocytophilum is an obligatoryintracellular and Gram-negative bacterium that encodes the 44-kDa major outer membraneproteins (p44). The main hosts are ruminants, dogs, horses, and rodents. Thisbacterium was cause tick-borne Anaplasmosis in ruminants, characterized byfever, lethargy, anorexia, arthritis, and thrombocytopenia. Recently, thechance of Anaplasmosis infection has been increased because of tick populationgrowth as a result of global warming. The aim of the study is the production ofrecombinant protein p44 for rapid diagnostic methodusing Immunochromatography assay.

Materials and Methods: Inthis study, *A. phagocytophilum* Minnessotastrain was cultivated in the HL 60 Human promyelocytic cell line in 175cm^{2}in RPMI1640 supplemented with 20%inactivated fetal bovine serum in a humidified 37℃ incubator with 5% CO_2.Its genomic DNA was extracted and amplified major surface protein (MSP) codinggene by Polymerase chine reaction. Amplified MSP product was cloned into the expressionvector pET-32a by *BamH*I and *Sal*I site. The recombinant pET-32avector was transformed into *Escherichiacoli* BL21. Recombinant MSP was over-expressed in *E.coli* system and purified by His-tag purification kit. Thispurified recombinant MSP will be used for development of rapid diagnostic toolfor *A. phagocytophilum.*

Results: MSP(p44) was purified His-tagging system based on high affinity chromatographybetween immobilized Ni-ion and histidine amino acid. Expressed MSP (p44) was confirmedon 44kDa and antigenicity SDS-PAGE gel and Western blotting.

Conclusions: In this study, we aimed to develop prduct specificantigen, p44 for rapid detection method using immunochromatography assay. MSP (p44) was prepared by purification andrecombinant protein system. This purified recombinant MSP (p44) will beused for the generation of monoclonal antibody to develop a diagnosis tool for *A.phagocytophilum* infection in bovine.

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Expressionand Purification of the 120-kDa Immunodominant Protein of *Ehrlichiachaffeensis*

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Introduction: *Ehrlichiachaffeensis* is obligatoryintracellular and gram-negative bacteria and infect various animal hostsincluding deer, horses, sheep, cattle, wild rodents, and human. The 120-kDa outermembrane protein (p120) is one of the immunodominant proteins of *E. chaffeensis* thatstimulates production of specific antibodies in infected animal. Recently, the chance of Ehrlichiosisinfection has been increased because of tick population growth as a result ofglobal warming. Thus, this study is aim the production of recombinant proteinp120 for diagnostic method using immunochromatography assay.

Materials and Methods: Inthis study, *E.chaffeensis* Arkansas strain was cultivated in DH82 dogmacrophage cell line in 175cm$_2$ in Minimal essential medium supplementedwith 10% inactivated fetal bovine serum, MEM NEAA and HEPES in a humidified37℃ incubator with 5% CO_2. Its genomic DNA was extracted and amplified outer membraneprotein (p120) coding gene byPolymerase chine reaction. Amplified outer membrane protein (p120) product was cloned into expression vector. Recombinantoutermembrane protein (p120) was over-expressed in *E.coli*system and purified by his-tag purification kit. This purified recombinant MSPwill be used for development of rapid diagnostic tool for *E. chaffeensis.*

Results: Outermembrane protein (p120) was purified His-tagging system based on high affinity chromatographybetween immobilized Ni-ion and histidine amino acid. Expressed outer membrane protein(p120) wasconfirmed on 45kDa and antigenicity SDS-PAGE gel and Western blotting.

Conclusions: In this study, we aimed to develop prduct specificantigen, p120 for rapid detection method using immunochromatography assay. Outer membrane protein (p120) was prepared bypurification and recombinant protein system. This purified recombinant outer membrane protein(p120) willbe used for the generation of monoclonal antibody to develop a diagnosis toolfor *E.chaffeensis* infection in bovine.

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Molecular Characterization of *Avibacterium paragallinarum*, the Causative Agent of Infectious Coryzain South Korea

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Introduction: *Avibacterum paragallinarum* (*Av. paragallinarum*, previously called *Haemophilus paragallinarum*) is the causative agent of infectious coryza, an acute respiratory disease of chickens. The greatest economic losses associated with infectious coryza result from retarded growth in growing birds and marked reduction (10 to 40%) in egg production of laying and breeding hens. The objective of this study is to characterize the Korean field isolates of *Av. paragallinarum* at the molecular level based on the genotyping by enterobacterial repetitive intergenic consensus-based PCR (ERIC-PCR) and phylogenetic analysis of bacterial 16S ribosomal RNA (rRNA) and hemagglutinin antigen (*hag*A) genes.

Materials and Methods: Bacteria were isolated from chickens in Korea during the period from 2011 to 2014 and confirmed by HPG-2 PCR known to be specific for *Av. paragallinarum*. Genomic DNA was extracted from the 24-48hr grown bacterial culture by boiling or using a genomic DNA extraction kit. The genomic typing of the isolates was performed by the ERIC-PCR and the amplification and phylogenetic analysis of bacterial 16S ribosomal RNA (rRNA) and hemagglutinin antigen (*hag*A) genes were performed using primers and conditions as previously described.

Results: We have characterized the thirteen Korean field isolates of *Av. paragallinarum* with ERIC-PCR and phylogenetic analysis of 16S rRNA and *hag*A genes. Isolates were identified by specific HPG-2 PCR. A total of three different ERIC-PCR patterns were recognized in Korean field isolates and eleven nicotinamide adenine dinucleotide (NAD) dependent Korean isolates had the same ERIC-PCR pattern which was distinguished from eleven reference strains for the Page and Kume serovars. Phylogenetic analysis showed that thirteen Korean isolates were divided into two distinct groups based on the NAD requirement. The similarity of Korean isolates was at least 97% and 96% in the 16S rRNA and hagA genes, respectively. The isolates did not cluster according to Page and Kume serotyping schemes.

Conclusions: The present study revealed that there are two lineages of *Av. paragallinarum* isolates in Korea that are distinct in their phenotypic and genotypic characteristics. ERIC-PCR fingerprints analysis and phylogenic analysis of 16s rRNA and hagA genes would be useful molecular typing tools for outbreak detection and epidemiological surveillance of infectious coryza in Korea.

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Serotyping and Antimicrobial Sensitivity of *Avibacterium paragallinarum* Isolates from South Korea

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Introduction: *Avibacterium paragallinarum (Av. paragallinarum)* is the causative agent of infectious coryza, an acute respiratory disease of birds, causing poor growth performance in chickens and reduction (10-40%) in egg production. The Page scheme is the most widely applied serotyping based on a plate agglutination test for *Av. paragallinarum*. Using this scheme, a total of three different serovars, A, B, and C, are recognized. Serotyping and an appropriate selection and application of antibiotics can provide the necessary information for control of infectious coryza. In this study, we investigated the hemagglutinin serotypes and antimicrobial resistance of *Av. paragallinarum* isolates from South Korea.

Materials and Methods: Thirteen field isolates from South Korea during 2011-2014 and three reference strains 0083, 0222, and Modesto were used in this study. The identity of field isolates was confirmed by specific PCR and multiplex PCR for serotyping was performed by as previously described. The isolates were serotyped according to the Page scheme by a hemagglutination- inhibition (HI) test with specific antisera as previously described. The disk diffusion test using 16 antimicrobial drugs was performed as previously reported, with slight modifications.

Results: All thirteen isolates were confirmed as *Av. paragallinarum* by PCR**.** The results of the multiplex PCR and serotyping showed that all isolates were recognized to be serovar A except two NAD-independent isolates, non-typeable. More than 70% of the isolates were susceptible to amoxicillin, amoxicillin-clavulanic acid, ampicilin, ceftiofur, cloxacillin, doxycycline, enrofloxacin, erythromycin, gentamicin, lincomycin, neomycin, oxytetracycline, penicillin, sulfamethoxazole-trimethoprim, spectinomycin and tylosin. Especially, all isolates were susceptible to amoxicillin-clavulanic acid, ceftiofur, gentamicin and spectinomycin. However, all isolates were resistant to lincomycin.

Conclusions: The present study identified only serovar A in the Korean isolates of *Av. paragallinarum* except two untypable NAD independent isolates and the isolates were highly susceptible to most antimicrobials used. To our best knowledge, this is the first report showing the distribution of serovars of *Av. paragallinarum* in Korea, although the number of isolates tested was limited.

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Serological Surveillance for the Detection of Foot-and-MouthDisease Virus Nonstructural Protein Antibodies in 2014

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