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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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Introduction: Foot and mouth disease (FMD) is the most contagious disease ofcloven-hoofed animals, and has a great potential for causing severe economicloss. Detection of antibodies to non-structural protein (NSP) has been used toidentify past or ongoing infections with any of the existing serotypes of FMDV.Surveillance activity consists of passive epidemiological surveillance systemof investigating reported disease activity and active epidemiologicalsurveillance system of examining statistically selected and targeted sampleswithin host populations. In this study, we have summarized serologicalsurveillance results for the detection of NSP antibody across the country in2014.

Materials and Methods: A totalof 37,336 sera from 6,965 farms were tested for statistical surveillance. Atotal of 93,893 sera from 4,613 farms were tested for targeted surveillance. Thosesera were tested by NSP ELISA (Median Diagnostics or Bionote) accordingto the manufacturer's instructions.

Results: Results: A total of 33 samplesfrom 20 farms were NSP antibody positive with 28 cattle, 1 pig and 4 goats instatistical serological surveillance. Targeted serological surveillance foundout 127 samples NSP antibody from 18 farms. When we traced back those NSPantibody positive farms, any evidence of virus circulation was not found. Inaddition, all the samples from 617 wild animals such as wild boars and so onwere negative by the NSP ELISA.

Conclusions: Theanimals with NSP antibody positives did not show any clinical sign and all negativeby PCR for antigenic samples.

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Characterization of MonoclonalAntibodies Against VP2 and VP4 of Foot-and-Mouth Disease Virus

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Introduction: Foot-and-mouth disease (FMD) is one of the most highly contagiousdiseases of cloven-hoofed animals including cattle, swine, sheep and goats. Thecausative agent is a FMD virus (FMDV), which is classified into sevenserotypes; O, A, Asia1, C, South African Territory1 (SAT1), SAT2 and SAT3. FMDVis surrounded by an icosahedral capsid that comprises 60 copies of fourstructural proteins: VP1, VP2, VP3 and VP4. As a serological method, virusneutralization test (VNT) is a standard test. It, however, takes several daysto complete and requires a containment facility. These aspects make the VNTinappropriate for mass serological surveillance. The other prescribed test atOIE manual is the liquid-phase blocking (LPB) ELISA which requires training andexperience to yield reproducible results and use an inactivated FMDV as adiagnostic antigen. To address these limitations, recombinant proteins derivedfrom FMDV P1 precursor and 3C protease genes of FMDV type O was developed inour previous study. In this study, monoclonal antibody was developed againstVP2 and VP4 of FMDV. We suppose that these mAbs can be applied tocharacterization of FMD recombinant antigens.

Materials and Methods: VP2,VP4 genes were cloned into Rhamnose SUMO vector separately and expressedrecombinant proteins using Rhamnose SUMO Cloning Expression System in BL21 DE3 *E.coli*strain. Soluble VP2 and VP4 proteins were purified using Ni-NTA affinitychromatography and the purified proteins were confirmed by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). MAbs against VP2and VP4 were produced by immunizing mouse (Balb/c) with each recombinant VP2and VP4 proteins.

Results: VP2 and VP4proteins were purified using Ni-NTA affinity chromatography and the purifiedproteins were confirmed by sodium dodecyl sulfate polyacrylamide gelelectrophoresis (SDS-PAGE). To obtain mAbs, the purified proteins VP2 and VP4were immunized to mouse followed by cell fusion, and selection according to thegeneral mAb production protocol. As a result, we screened 6 of VP2 and 1 of VP4monoclonal antibodies using ELISA and fluorescence analysis (FA) and alsocharacterized using isotyping kit respectively.

Conclusions: Therecombinant VP2 and VP4 proteins were expressed using Rhamnose SUMO CloningExpression System and monoclonal antibodies against VP2 and VP4 were alsoproduced with the recombinant proteins. We suppose that these mAbs can beapplied to characterization of FMD recombinant antigens.

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