and virulence of Streptococcussuis from diseased pigs in Korea

Materials and Methods: A total of 12 S. suisfield strains isolated from pigs with presenting clinical signs of infectionwere used in this study. Seven S. suis housekeeping genes, a 5-enolpyruvylshikimate3-phosphate synthase (aroA), a 60 kDa chaperonine (cpn60), aperoxide resistance protein (dpr), a glucose kinase (gki), a DNAmismatch repair protein (mutS), a homologous recombination factor (recA)and an aspartokinase (thrA), were amplified for the 12 S. suis genomic DNA preparations usingthe primer sequences.Each PCR product was purified using PCR purification kits or agarose gelextraction kits (Bioneer, Korea). The sequences of both strands of theindividual fragments were determined by automated DNA sequencer. MLST alleles of the individual genes, sequence types (STs)and clonal complexes (CC) were identified using the S. suis MLSTdatabase and eBURST Web application (http://ssuis.mlst.net/). The presence of arcA, bay046, epf, hyl, mrpand sly was determined by conventional and multiplex PCR. Amplicons were electrophoresed on 1.2% agarose gel andvisualized using an UV transilluminator. Consensussequences were aligned by CLUSTERW. Phylogenetic tree was constructed withMEGA5 program (USA) by neighbor-joining methodwith branch lengths estimated by the Maximum Composite Likelihood method.Branch quality was assessed by bootstrap test using 1,000 replicates.

Results: Twelve isolates could beassigned into serotypes 2 (2 isolates), 13 (6 isolates), 20 (1 isolate), 23 (1isolate) and 31 (2 isolates). The 12 isolates could be assigned into 4 knownSTs [ST25 (1 isolate), ST27 (4 isolates), ST28 (1 isolate), and ST117 (1isolate)] and 5 novel STs [ST625 (1 isolate), ST626 (1 isolate), ST627 (1 isolate),ST628 (1 isolate), and ST630 (1 isolate). ST25 belonged to the ST25 complex, andST27, ST28 and ST117 belonged to the ST28 clonal complex. All the 12 strainswere subjected to PCR-based screening of six virulence markers, namely, mrp,sly, epf, hyl, acrA, and bay046. acrA and bay046were detected in all the 12 strains. sly and hyl were found in the novel ST strains (ST626 and ST630) but not inthe other strains. mrp was found inonly ST27.

Conclusions: In summary, 12 S.suis strains were isolated from diseased pigs in Korea. Major serotypeswere 13, 2, 31, 20 and 23 and could be assigned into into 4 known STs [ST25 (1isolate), ST27 (4 isolates), ST28 (1 isolate), and ST117 (1 isolate)] and 5novel STs [ST625 (1 isolate), ST626 (1 isolate), ST627 (1 isolate), ST628 (1isolate), and ST630 (1 isolate). Virulence genes were detected in all the 12 strains.sly and hyl were found in thenovel ST strains (ST626 and ST630) but not in the other strains. mrp was found in only ST27. Theseresults illustrated that the zoonotic strains of S. suis in Korea arecontinually evolving; therefore, increased surveillance of S. suis infarm-raised pigs should be conducted.

Ackwledgements: This research was supported by TechnologyDevelopment Program (Project No. 315046-2) for Bio-industry, Ministry forAgriculture, Food and Rural Affairs, Republic of Korea.

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P-137

Distribution of *Brucella canis* in body of dogs

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Introduction: Caninebrucellosis caused by B. canis, whichis one of important disease in kennels. B.canis has been reported from the America, European and Asian countries. Inclinical sign, most abortions occur late and fever is rare. Many infected dogsremain asymptomatic. This bacterium is mainly transmitted by contact with the placenta and vaginal dischargesafter an abortions, stillbirths, and mating dogs with epididymitis. Withregard to this disease, there has been currently introduced on diagnosticmethods; culture method, serological and genetic assay. Serological assay is liketo rapid slide agglutination (RSAT), rapid slide agglutination with2-mercaptoethanol (2-ME RSAT) and immunochromatography test (ICT) is mostcommonly used in South Korea. But, these sensitivity and specificity werecomparatively known to low (about 80-90%). For confirmation of disease,cultural methods are required to essential. Therefore we surveyed distributionof B. canis in body of dogs.

Materials and Methods: The blood samples were collected and tested by RSAT,2-ME RSAT and ICT. Of them, 24 dogs (female 15 and male 9) wereselected by serological results with clinical manifestation and sacrificed toanalyze distribution of B. canis inbody. The whole blood and tissues were inoculated on tryptic soy broth (TSB) supplementedwith

5% bovine serum at 37°C for20 days and they were cultured on blood agar and/or modified Brucella selective (MBS) medium by 7-10 day intervals. Brucella suspect colonies wereidentified by classical biotyping methods and molecular assays including thedifferential multiplex-PCR.

Results: Of specimens,B. canisstrains was isolated in all inguinal lymph-node(LN) and whole blood, each 20/24(83.3%) in retropharyngeal LN and spleen, 17/24 (70.8%) in liver, 15/24 (62.5%)in lung, 10/24 (41.7%) in kidney and 7/24 (29.2%) in urine, respectively. Of 17female, B. canis were isolated from10 uteri (66.7%) and in male, prostate gland 5/7(71.4%), epididymis 4/7 (57.1%)and testis 3/7 (42.9%), respectively. Also, the isolation proportion of female and malewas shown no difference.

Conclusions: Specimens to isolate B. canisfrom dogs were recommended to whole blood, inguinal LN, retropharyngeal LN,spleen, uterus and prostate gland.

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P-138

A virological investigation of infectious agents in aborted pig fetuses, South Korea, 2013

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Introduction: There are many agents were known to caused abortion of sows. Especially the is sue of RNA, DNA viruses which causes massive loss for swine producing sector throughout the world (1). Several viruses already known to associate with porcine abortion, such as porcine circovirus type 2 (PCV2), Aujeszky's disease virus(ADV), Porcine parvovirus (PPV), Porcine reproductive and respiratory syndromevirus (PRRSV), Swine influenza virus (SIV), Encephalomyocarditis virus (EMCV). Also, family of Flaviviridae (Japanese Encephalitis Virus, JEV) and Bunyaviridae (Gouleakovirus, GOLV; Herbert virus, HEBV) which cause potential zoonotic problems. This study aimed to investigate abortion problems of sows in South Korea, on the basic of regional and individual farm surveillance.

Materials and Methods: During April to November 2013, we randomly collected fetus (n=112) from swine farms in 9 provinces for detection viruses associated with abortion problems. The fetuses' organs (kidney, liver, tonsil, heart and spleen) were pooled, homogenized, and extracted of nucleic materials(DNA and RNA). We used both pathogen specific primers and commercial diagnostic kits (Median kits) for this investigation. Among the positive viruses, PRRSV used MLV usual live vaccinated at sow, we conduct detail survey about farm vaccinated situation and information. All each of 4 PRRSV positive farmshad vaccinated with PRRSV, PPV, PCV2, PPV. Also, we had isolated PRRS ORF 5 complete gene for important pathogen gene using nested PCR of specific primers (one round; FR9, RR9, second round; RF5, RR5) followed protocol's (2). We perform phylogenetic analysis of ORF 5 complete sequences for PRRSV with reference strains of lineage 1 to lineage 9, and lineage KOR (extracted genbank).

Results: Among investigated viruses, 19/112 samples (16.96%) was PCV2 positive. It was followed by GOLV which showed positive rate of 8.93% (10/112 samples). Both SIV (H1N1) and PRRSV showed positive rate 4/112 (3.57%). TTV was also found in 3/112 aborted fetuses(2.68%). The lowest positive rate was HEBV which was detected in 2/112 samples. Others viruses were not detected (ADV, PPV,CSFV, EMCV, JEV, PEV-A, PEV-B, PAV, and PTV). We had sequenced the ORF5 complete gene of PRRSV from 4 samples (cp266-2, cp296-3,PF2516-2, and PF2576-2). All of our 4 sequences are type 2 PRRSV. The result showed that all of 4 sequences cluster to lineage 5, which are predominant in Korea. Both cp296-3 and PF2516-2 are 99.98~100% similarity with MLV vaccine strain of type II PRRSV. The cp266-2 and PF2576-2 are 88.6~92.7% similarity with MLV vaccine strain, and was field isolates.

Conclusions: In 2013, a virological investigation of abortion problem detected the presence of six viruses. The positive rate was in order from highest to lowest: porcine circovirus type 2, gouleako virus, porcine reproductive and respiratory syndrome virus, swine influenza virus, torque teno virus, and herbert virus.

Acknowledgments: This study was supported by a grant (No. PJ011184) from BioGreen 21 Program, Rural Development Administration.

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