

proteins were expressed in Sf9 insect cells and simplified purification, immunized intramuscularly into 8- to 9-week-old SPF chickens with the adjuvant ISA 70. The 3 weeks post injection, chickens were boosted for increasing antibody against NA. The NI titer of antiserum was measured by the conventional TBA NI assay.

Results: The nine recombinant NA proteins were showed NA activity. The antisera were identified 28 AIV strains with low cross-reactivity against each NA subtype. The NI titer in antiserum was similar or slightly higher than conventional vaccination.

Conclusions: The production method of NA reference antisera using baculovirus expression system is simple, and safer than those of whole virus, without antibodies to other influenza viral proteins.

References

- [1] CW Lee, DA Senne, DL Suarez. (2006) Development and application of reference antisera against 15 hemagglutinin subtypes of influenza virus by DNA vaccination of chickens. *Clin Vaccine Immunol.* 2006. Mar;13(3):395-402.
- [2] IC Brett, BE Johansson. (2005) Immunization against influenza A virus: Comparison of conventional inactivated, live-attenuated and recombinant baculovirus produced purified hemagglutinin and neuraminidase vaccines in a murine model system. *Virology.* 2005. Sep 1;339(2):273-80.

P-57

Genetic Comparison of H5N1 Highly Pathogenic Avian Influenza Virus between Korea and Vietnam

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Introduction: Highly pathogenic avian influenza virus (HPAIV) has circulated in poultry populations of many countries in Eurasia, Africa and spreads to new areas caused by migratory bird and movement of poultry. Among these countries, Vietnam is the most of country occurring H5N1 viruses and various H5N1 viruses were isolated since 2003 including Clade 1.1, 2.3.2, 2.3.4, 3, 5, 7 viruses. In this study, H5N1 viruses isolated in Vietnam were analyzed to compare genetic characteristic with H5N1 of Korea isolates.

Materials and Methods: Five H5N1 viruses were introduced from NCVD (National Center for Veterinary Diagnosis) in Vietnam. Each sample was inoculated into SPF eggs and virus isolation was determined by HA assay. Viral full genes were sequenced and analyzed according to Hoffmann et al. (2001). Assembly of the sequencing contigs were performed using Chromas pro program (Technelysium Pty Ltd). A phylogenetic tree was constructed using the neighbor-joining method with 1000 bootstrapping replicates.

Results: Phylogenetic analysis with nucleotide sequences revealed that Vietnam isolates belong to clade 1.1, 2.3.2.1, 2.3.4.3, 7.1 viruses whereas Korea isolates belong to clade 2.2, 2.5, 2.3.2.1 viruses. Most of internal genes of Vietnam and Korea isolates

clustered with viruses isolated in Eurasia. H5N1 of Vietnam and Korea isolates closely related to the HPAIV isolate at China, Mongolia.

Conclusions: H5N1 viruses circulating in Vietnam have shown that multiple clades of the virus have introduced into Vietnam over past several years and it is possible that these viruses spreads to new areas. Therefore, to predict genetic characteristics of HPAIV which available introduce is important to prevent re-introduce of H5N1 viruses into Korea.

References

- [1] I. Hoffmann, E. J. Stech, Y. Guan, R. G. Webster, and D. R. Perez. 2001. Universal primer set for the full-length amplification of all influenza A viruses. *Archives Virology* 146: 2275-2289.

P-58

Plasmid-Mediated Quinolone Resistance in *Escherichia coli* Isolates from Wild Birds and Chickens in Korea

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Introduction: Plasmid-mediated quinolone resistance (PMQR) determinants including *qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *aac(6)-Ib-cr*, and *qepA* have been found in clinical isolates of *Enterobacteriaceae*. These PMQR determinants are usually carried on mobile elements and can be transferred among different bacterial strains. In addition to the clinical use in human medicine, quinolones are widely used in livestock production in many countries. The aims of this study were to investigate the prevalence and molecular characteristics of PMQR genes in *Escherichia coli* strains isolated from various specimens of wild birds and chickens in South Korea.

Materials and Methods: A total of 1,630 nonduplicated isolates of *E. coli* were obtained from both wild birds and commercial chickens in South Korea. Strains were tested for antimicrobial susceptibility by disc diffusion assay and MICs. The PMQR genes (*qnrA*, *qnrB*, *qnrS*, *aac(6)-Ib-cr*, and *qepA*) were amplified by PCR and confirmed by both direct or full length sequencing of the purified PCR products. Clonality of the isolates was determined by pulse-field gel electrophoresis (PFGE). Plasmids carrying *qnr* determinants were transferred by conjugation and characterized by PCR-based replicon typing.

Results: Fifty-four (3.3%) of the total *E. coli* isolates were identified as *qnr*-positive by PCR screening. The *qnr*-positive of the 790 *E. coli* isolates from wild birds was twenty-six (3.3%) and twenty-eight (3.3%) of the 840 *E. coli* isolates from chickens were *qnr*-positive. The most common subtype was *qnrS1* (42/54, 77.8%), which account for 76.9% (20/54) in the isolates from wild birds and 78.5% (22/54) in the isolates from chickens, respectively. The prevalence of *qnrB* was 18.5% (10/54), which account for 9.3% in the isolates from wild birds and chickens, respectively. Among these, the five new *qnrB* variants (*qnrB43*, *qnrB44*, *qnrB45*, *qnrB46*, and *qnrB47*) were characterized by full-length sequence analysis. The resistance rates to nalidixic-acid and ciprofloxacin against *qnrS*-positive isolates were higher in

chickens isolates rather than wild birds. The plasmids carrying *qnrS* in 14 (25.9%) of the total *qnr*-positive isolates including one (1.85%) *qnrB* were conjugally transferred to the recipient strain *E. coli* RG488. The replicon type IncK was the most prevalent, which account for 12 (46.2%) and 7 (25.0%) in the *qnr*-positive isolates from wild birds and chickens, respectively. Southern hybridization showed that all plasmids from the transconjugants carried the *qnrS* gene and all transconjugants belonged to the IncK replicon type. All isolates carrying *qnr*-determinant were genetically classified into forty-one subgroups by PFGE analysis. The PFGE groups XVIII and XXXV from wild birds belonged to same bird species, whereas the XXXII group was highly homologous between two bird species. Some of the *qnrS1*-positive *E. coli* strains isolated from slaughter house and chicken farms were classified into four groups (XIV, XXI, XXXVII and XXXVIII). Of these, group XXXVII (ECQ39 and ECQ57) was regionally different clones.

Conclusions: The most prevalent *qnr* subtype was *qnrS1* among *qnr*-positive *E. coli* strains and the new allele *qnrB* subtypes were discovered in isolates of the both wild birds and chickens in South Korea. Some highly homologous clones of *qnr*-positive strains among each bird family were distributed in same bird species and chicken farms in their inhabiting environment.

References

- [1] 1. Huang SY, Dai L, Xia LN, Du XD, Qi YH, Liu HB, Wu CM, Shen JZ. 2009. Increased prevalence of plasmid-mediated quinolone resistance determinants in chicken *Escherichia coli* isolates from 2001 to 2007. *Foodborne Pathog Dis.* 6:1203-1209. *Escherichia coli*

P-59

Occurrence of *Campylobacter* spp. in Free-living Wild Birds from Korea

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Introduction: *Campylobacter*s are emerging as a significant cause of human infection, and the role that wild birds have in the spread of this disease is beginning to be elucidated.

Materials and Methods: We evaluated the occurrence of *Campylobacter* species in 2,173 fecal samples of wild birds (representing 72 species and 28 families) captured throughout the Korean peninsula.

Results: Overall, the prevalence was 15.2% (332/2173). The highest isolation rate was found in 9 of 30 members of the Charadriidae family (30.0%), followed by 9 of 34 Ardeidae (26.4%), 9 of 41 Turdidae (21.9%) and 252 of 1642 Anatidae (15.3%). Also, the prevalence of *Campylobacter* spp. differed significantly when migratory habits were looked at. Stopover birds were the most commonly infected (19.6%), followed by the winter migratory (16.7%), and the summer migratory birds (12.3%), whereas indigenous birds had very a low prevalence (1.6%). Broth microdilution was used to test antimicrobial susceptibility of 213 isolates to nine antimicrobials. *Campylobacter*

jejuni isolates (n=169) were resistant to nalidixic acid (5.3%), ciprofloxacin (3.0%) and tetracycline (1.8%). The *C. lari* isolate displayed resistance to nalidixic acid and ciprofloxacin. However, all *C. coli* isolates (n=20) were susceptible to the tested antimicrobials. This is the first account on the isolation of *Campylobacter* species from wild birds that seasonally or indigenously inhabit the Korean peninsula that has been reported in the literature.

Conclusions: Our results indicate that there is an overall moderate prevalence of *Campylobacter* in wild birds, and birds may also serve as a significant reservoir for campylobacteriosis.

References

- [1] Keller et al. 2011. Prevalence of *Campylobacter* in wild birds of the Mid-Atlantic Region, USA, *J. Wildlife Diseases* 47: 750-754.
- [2] Van Dyke et al. 2010. The occurrence of *Campylobacter* in river water and waterfowl within a watershed in southern Ontario, Canada. *J. Applied Micro.* 109: 1053-1066.

P-60

Prevalence of *Salmonella enterica* Isolated from Poultry between 2009 and 2010 in Korea

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Introduction: *Salmonella* is the most common causative agent of foodborne disease in human. *Salmonella* infections of poultry have been shown to be of critical importance in many countries. Commercial chickens and ducks are one of the main sources of potentially dangerous *Salmonella* bacterial infections. The purpose of this study was to investigate the frequency of isolation of *Sal. enterica* contaminated from chickens and ducks in Korea. **Materials and Methods:** *Salmonella* strains were isolated using fecal samples collected from both 172 chicken farms (7 provinces) and 152 duck farms (5 provinces) during 2009-2010 in Korea. Fecal samples were pre-enriched in 225 ml of buffered peptone water broth at 37°C for 18 hours and then 0.1 ml of enrichment culture transferred to 9.9 ml of tetrathionate broth and incubated at 42°C for 48h. The cultures were streaked onto Rambach agar or xylose-lysine desoxycholate agar as selective mediums. Serotyping for *Sal. enterica* identification was conducted using *Salmonella* O-antisera and H-antisera (Difco).

Results: A total of 171 nonduplicated isolates of *Salmonella*, including more than 2 different serotypes identified from several farms were isolated from all surveyed farms. Sixty-seven strains of *Salmonella* were isolated from 60 (34.9%) of the 172 chicken farms and one hundred and four strains were isolated from 77 (50.7%) of the 152 duck farms. Fifteen and Eighteen different *Salmonella* serovars were found in the fecal samples collected on the chicken and duck farms, respectively. Among serovars, thirty-one (46.2%) strains were identified as *Sal. Enteritidis*, which were predominant in chickens, whereas thirty-seven (35.6%) strains from duck farms were identified as *Sal.*