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Serosurvey for antibody to egg drop syndrome virus in wild waterfowl species in Korea

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Introduction: Egg drop syndrome virus (EDSV), known as duck adenovirus serotype 1, is the sole member of the genus *Atadenovirus* in the family *Adenoviridae*. Single serotype has been recognized. The EDSV causes egg drop syndrome (EDS) with increased abnormal eggs in laying hens. Outbreaks of egg drop syndrome caused by EDSV resulted in substantial economic loss in laying hens during late 1980s. The disease disappeared later in poultry farms due to intensive vaccination and then re-emerged recently in some of laying hen farms in Korea since 2010. It is believed that the natural hosts for the virus are waterfowl birds such as ducks and geese. Nevertheless, the ecology of EDSV infection in wild waterfowl birds still remains unclear. In the study, serological study for EDS was performed, as a part of exploring the ecology of EDSV and investigating EDSV infection in wild waterfowl birds.

Materials and Methods: Serum samples from wild ducks and geese were collected at stopover wintering sites in Korea during the migration seasons in 2012 and 2013 and examined for antibodies to EDSV. Wild waterfowl birds captured were eight species including Baikal teal (*Anas Formosa*, n=12), Common teal (*Anas crecca*, n=53), European wigeon (*Anas Penelope*, n=30), Mallard (*Anas platyrhyncho*, n=285), Mandarin duck(*Aix galericulata*, n=138), Pintail (*Anas acuta*, n=83), Spot-billed duck (*Anas poecilorhyncha*, n=113) and White-fronted goose (*Anser Albifrons*, n=21).The history of each bird including geographical location, capture date and bird species was recorded and the blood samples were taken from the jugular vein of the birds. Sera were maintained at −70 C until the use for serologic assay. EDSV antibodies were determined by hemagglutination inhibition (HI) test employing four hemagglutination units (HAU) of EDSV antigen (EDS virus 127 strain) in V-bottom microtiter plates as described previously. The HI antibody titer was determined as the reciprocal of the last dilution of serum that completely inhibited hemagglutination, and HI titers ≥ 8 were considered positive. All tests were repeated twice.

Results: A total of735 serum samples from wild waterfowls collected in the study were tested for antibodies to EDSV by HI test. EDSV antibodies were detected in seven of eight species tested in the study. The overall prevalence of antibodies against EDSV was 14.1% (95% CI 11.6～16.7%) for the 735 serum samples consisting of eight wild bird species captured in the study. Most of positive birds (96/104) were mandarin(38.0%, 95% CI 29.6～45.8%), mallards (11.2%, 95% CI 7.6～14.9%) and spot-billed ducks (11.0%, 95% 4.9～14.9%). The low prevalence of antibodies to EDSV was observed in European wigeon (6.7%, 95% CI 0～15.7%), White-fronted goose (4.8%, 95% CI 0.0～14.1%), common teal 3.8%, 95% CI 0～7.9%) and Pintail (3.6%, 95% CI 0～7.7%) in the order. EDSV antibodies were detected in none of Baikal teal birds captured in the study. When analyzed the antibody titers, sero-positive birds of Mandarin, Mallards and Spot-billed ducks had mean HI titers (log_2) of 4.6±1.3, 3.6±1.0 and 4.3±1.6, respectively.

Conclusions: EDSV antibodies were detected in seven of eight speciesin the Order of *Anseriformes* examined in the study. Of them, mandarin, mallards and Spot-billed ducks showed the serological prevalence of 10% or greater duringwintering period.This suggests that these three waterfowl speciesincluding Mandarin, Mallards and Spot-billed ducks in the Order Anseriformes mightbe likely to be major natural reservoir of EDSV in Korea.

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Local Mucosal Immune Responses in Turkeys Following Respiratory versus In ovo Exposure to Avian Metapneumovirus Subtype C (aMPV/C)

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Introduction: Avian metapneumovirus subtype C (aMPV/C) causes severe upper respiratory tract (URT) infection in turkeys (1, 2). Clinical signs include depression, coughing, sinusitis, airsacculitis, and mortality (2). aMPV/C outbreaks in the United States were first reported in 1996 in Colorado and has spread to Midwestern states the following year. The aMPV strain isolated in Minnesota was identified as subtype C (aMPV/C), which has low sequence identity with subtypes A and B prevalent in Europe and other countries (3). aMPV/C replicates in the ciliated epithelial cells of nasal turbinates and infraobital sinuses (4). The lesions characterized by lymphoid cell infiltration are restricted to the URT (1). In this study, we examined and compared the local mucosal immune responses against aMPV/C exposed by respiratory or in ovo route.

Materials and Methods: Two-week-old turkeys, free of antibodies against aMPV/C, were inoculated oculonasally (O/N) with live aMPV/C. At 5 and 7 days post inoculation (DPI), lymphoid cells infiltrating the mucosal lining of turbinate of virus-exposed and control turkeys were isolated

by enzymatic treatment. Cytokine gene expressions were detected by real time quantitative RT-PCR (qRT-PCR). aMPV/C-specific IgG or IgA ELISA were also performed in serum and bile. For in ovo administration, aMPV/C antibody free commercial turkey eggs were inoculated at embyronation day 24 via the amniotic route with live aMPV/C. At the day of hatch (embyronation day (ED) 28)and 5 days post hatch (DPH), virus replication was detected by RT-PCR in various tissue. In addition, CD4+/CD8+ cells in URT, cytokine gene expressions and aMPV/C-specific IgG and IgA were detected as described above.

Results: Control turkeys had resident CD4+ and CD8+ cells in the URT mucosa. Respiratory exposure to aMPV/C increased the proportion of CD8+ cells but not of CD4+ cells in the turbinate. At 5 and 7 DPI, upregulated gene expressions of CD8, IFN-γ and IL-10 were detected in the turbinate. The level of aMPV/C-specific Ig G was also increased in the serum and aMPV/C-specific IgA was detected in bile and serum after two O/N exposures. After in ovo exposure of aMPV/C, hatchability of the inoculated eggs was not affected. At ED28 and 5DPH, virus replication was detected in the turbinate and trachea, but not in the thymus or the spleen. Although the relative proportion of T cell phenotypes (CD4/CD8) was not affected. Gene expression of IL-10, not IFN- γ, was upregulated in the turbinate tissue. Poults hatching from virus-exposed eggs had aMPV/C specific-IgG in the serum and tears.

Conclusions: Our data demonstrate that different administration route of aMPV/C may modulate the local mucosal immunity in the URT of turkeys. This results may contribute to the selection of optimal vaccination route to control of aMPV/C in turkeys.

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Ovine ine interferon tau: atype I interferon effect against *foot and mouth disease*

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Introduction: Foot and mouth disease is an economically importantdisease in most parts of the world. In acute FMD outbreak situation, there arein need of new therapeutic agents which can protect the animals before the hostimmune response to the vaccination. Several interferons have been tried fortheir antiviral activity against FMDV. Hence in our study we evaluated theantiviral activity of recombinant ovine interferon τ4 (ovIFN-τ4) in LFBK cellsand survivalrate in ICR suckling mice.

Materials and Methods: Ovine interferon tau 4 (Geneaccession No.X56341.1) wassynthesized into pBHA vector by Bioneer Corp (Daejeon, Republic of Korea). Aproduct of 567 bp of IFN-τ was amplified in order to clone into Expresso®Rhamnose SUMO Cloning and Expression System according to manufacture'sinstruction (Lucigen Corp, WI). The antiviral dose of ovIFN-τ4 in LFBK cells,tenfold serially diluted recombinant protein from 1500ng/ml till 0.015ng/mlwere added in duplicates and challenged with FMDV O1manisa 24 hours posttreatment to prevent Cytopathic effect caused by the virus as well as the virusmultiplication rate was determined. Suckling mice were received 500ng ofovIFN-τ4 in 50 ul PBS and 250ng of ovIFN-τ4 in 50 ul PBS and 50 ul PBS ascontrol. The route of inoculation was intraperitonium for all the differentgroups. All the suckling mice were challenged with 50ul of 100$LD_{50}$of FMDV O Andong, 24 hours post therapy with interferon. The survival rate ofsucking mice against FMDV infection was monitored for 10 days post challenge.

Results: The highest dilution of ovIFN-τ4 to protect the LFBKcells from CPE due to FMDV was 0.15ng/ml giving 100% protection from CPE. Viralcopy number were reduced to 2-log titer between 0.15ng/ml and virus control,even though there was protection against CPE but 0.15ng/ml could not able todestroy all the virus RNA. The dose between 1500ng/ml till 1.5ng/ml there was4-log titre reduction in the viral copy number compared with the virus control.In suckling mouse the dose of 500ng was protecting the animals until 5days postchallenge and giving 90% of protection till 10^{th} day of postchallenge.

Conclusions: In conclusion, we propose that this ovIFN-τ4 may beused as an alternative or in combination with other interferon or antiviralagents at the time of outbreak or prior to vaccine immune response.

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