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P-102

Application of thromboelastorgraphy for the prediction of mortality for intensive care patients

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Introduction: Oneof the leading causes of death for intensive care patients is hemostatic dysfunctionincluding disseminated intravascular coagulopathy and bleeding. Clinicopathologicalexams to assess the function of coagulative system are PT, aPTT, and D-dimer. However, abnormal status of the coagulative system cannot be properly determined byeither PT or aPTT because these tests are negative until fall belowapproximately 30% of normal value. Thromboelastography provides more globaltest that help facilitate accurate evaluation of hemorrhagic or thrombotic risks.Therefore, the present study was performed to evaluate the thromboelastography forthe prediction of mortality in intensive care patients.

Materials and Methods: Thestudy population was divided into two groups. Group I involved stable caninepatients with potential hypercoagulable diseases including cancer,pancreatitis, pyometra, and hyperadrenocorticism. Group II enrolled thepatients with multiple organ dysfunction syndrome or the patients who diedwithin 72 hours of admission. Whole blood was collected from jugular vein. Bloodsample in EDTA tube was subjected to complete blood count by Advia 2120i (SiemensHealthcare, Erlangen, Germany). To obtain a dilution of whole blood to sodiumcitrate of 9:1, 3.2% sodium citrate tube was used. PT, and aPTT were measuredusing Coag Dx Analyzer (IDEXX Laboratories Inc, ME, USA). Thromboelastrographywas performed by TEG 5000 Thromboelastograph Hemostasis Analyzer System(Haemonetics Corporation, MA. USA). In some patients, D-dimer was also measuredafter centrifugation.

Results: Thirty-six dogs presented to SNU-VMTH wereenrolled in the present study. Group I included 30 patients, and Group II had 6patients. PT had no significant difference between Group I and II, however, aPTTlevel was significantly increased in Group II (mean: 100.7 ± 6.2 seconds;,range: 81-126 seconds) compared to Group I (mean: 87.2 ± 2.0 seconds;,range: 73-116 seconds) ($P < .05$). MPV value was also significantly increasedin Group II patients ((mean: 12.02 ± 0.92 minutes; range: 9.0-15.1 minutes) comparedto Group I (mean: 10.81 ± 0.63 minutes; range: 7.0-21.9 minutes). Amongthromboelastography results, the R value demonstrated a significant differencebetween Group I and II ($P < .05$). Group II dogs had a significantlyincreased R value (mean: 8.63 ± 1.36 minutes; range: 4.6-14.2 minutes)than that in Group I (mean: 5.71 ± 2.48 minutes; range: 0.8 ± 10.3minutes) ($P < .05$).

Conclusions: This study demonstrated that R value, representing the initial formationof the clot, was the only TEG marker predictive of mortality. Increase in Rvalue was associated with an increased risk of death. Therefore, R value couldbe used as an indicator of prognosis of patients associated with highmortality. However due to small population, further studies may be warranted todetermine the significance of thromboelastography abnormalities.

P-103

Successful Generation of a Complete Recombinant H9N2 Avian Influenza Virus by modifying Hoffmann's Reverse Genetics Vector System

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Introduction: Reverse genetics system of influenza A virus (IAV)has advanced to the bidirectional 8-plasmid system which has human promoters ofpolymerase l and ll, resulting in synthesis of viral RNA(vRNA) and mRNA from one template(Hoffmann *etal.*, 2001). This system is very efficient for recombinant virus generationdue to transfection of only 8 plasmids encoding each genome segment of IAV intocell. Because all IAVs have conserved terminal sequences in both ends of vRNA,universal primer set containing conserved sequence and segment specificsequence can be used to cloning genome of diverse subtype viruses. Through thismethod, various subtypes of avian IAVs (AIVs) have been generated easily asreassortants with six genomes of high yielding A/PR/8/34 (PR8) virus. However,there was a limit that recombinant viruses having whole genome of some AIVs arevery difficult to rescue. Recently we found some polymerase genes were notadapted to mammals and could not support efficient virus replication inmammalian cells (Kim *et al*, 2014; L*eet al*, 2017). Furthermore, we foundthe constellation of the fourth nucleotide at the 3'end of viral genome (C4,weak promoter and U4, strong promoter) were important for

efficient virusreplication and mammalian pathogenicity (Lee et al, 2017). The polymerasegenomes (PB2, PB1 and PA) of Hoffmann's vector system possess the weakpromoter, C4. Therefore, in this study we demonstrated the hypothesis that promoterchange from C4 to U4 in polymerase genomes can generate a complete recombinantH9N2 AIV.

Materials and Methods: Each genome of A/chicken/Korea/01310/2001 (H9N2) (01310) virus CE2and CE20 was cloned into the bi-directional pHW2000 vector. 4thnucleotide in Non-coding region (NCR) of polymerase genes were mutated from Cto U by site-directed mutagenesis kit. Recombinant 01310 viruses (r01310) weregenerated by transfecting 8 plamids to 293T cell. The culture medium wasinjected into 10-day-old SPF embryonated chicken eggs via allantoic fluidcavity and harvested allantoic fluid is tested by HA assay using 1% chickenRBC.

Results: In contrast topolymerase genes with C4 complete recombinant virus, r01310 was generated successfullyby using polymerase genes with U4. But, when PA plasmid was solely mutated intoU4, r01310 was not rescued.

Conclusions: Changing weak(C4) to strong (U4) promoters of polymerase genomes of Hoffmann's vector systemwas enough to generate a complete recombinant AIV, r01310, and our method maybe useful to generate wild and mutant AIVs for studies on genotype-phenotypecorrelation.

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P-104

Minimum Infectious Dose Determination of the K40/09Infectious bronchitis Virus vaccine candidate strain by spray administration

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Introduction: Infectious bronchitis virus (IBV) causes an acute and highly contagiousviral disease of chickens that is characterized by respiratory signs,nephritis, and reduced egg reproduction. Current IBV vaccine strains do notcross-protect against new viral strains because of the extensive geneticdiversity and high mutation rate of IBV. Therefore, the best protective effectthat can be achieved would be only against strains of the same serotype orgenotype. In our previous study, the natural recombinant nephropathogenic IBV strainK40/09 was heat-adapted as a live attenuated vaccine and showed significantlyhigh levels of immunogenicity and protective ability against many different typesof IBV.
In the field, IBVvaccine is delivered by mass applications but mostly delivered via hatcheryspray cabinet and eye drop administration. So we sought to determine the minimum infectious dose of K40/09 IBV vaccine candidate strain by spray-administrationin one-day-old chick.

Materials and Methods: One-day-oldspecific pathogen free(SPF) chicks were divided into 9 Groups. 4 Group vaccinatedwith attenuated K40/09 vaccine candidate strain(CE60) either a $10^{1.5}$,$10^{2.5}$, $10^{3.5}$, $10^{4.5}$ EID50 dose and 3 controlGroups were vaccinated with commercial H120 IBV vaccine as 0.01x, 0.1x, 1x ofmanufacturer's recommend dose using a fine-spray cabinet (Three Shine, dropletsize = 50 um), whereas the other 2 groups were kept as nonimmunized controls.Three weeks after vaccination, serum samples were collected and level ofIBV-specific antibodies were determined by enzyme-linked immunosorbent assay(ELISA). After that all of the birds were challenged intraocularly with $10^{4.5}$EID50 of each homologous IBV strain K40/09 and M41, respectively. Fivedays after challenge, the challenge virus was re-isolated from the trachea andkidney of the birds by inoculating 9 to 11d old embryonated SPF chicken eggs toevaluate the protective efficacy of vaccines.

Results: Group of chickensimmunized with K40/09 showed protection of the respiratory tract and kidneywhen vaccinated over $10^{2.5}$ EID50 dose. And ELISA titer also showedpositive over $10^{2.5}$ EID50 dose
Group of chickensimmunized with H120 vaccine showed protection of the respiratory tract andkidney when vaccinated over 0.1x of manufacturer's recommend dose but ELISAtiter was low at all dose. Compared with the non-vaccinated control group, andthere were no Clinical sign between vaccination groups and control group showed100% re-isolation of challenge strain in the respiratory tract and kidney.

Conclusions: In summary, we havedemonstrated the minimum infectious dose for the attenuated K40/09 infectiousbronchitis virus vaccine candidate strain by spray administration is $10^{2.5}$EID50. This study will be helpful of developing future vaccinationprotocols and implementing them properly.