to humans through food chain ultimately causing a significant public health hazard.

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A *Vibrio vulnificus* VvpM induces necrotic cell death coupled with IL-1β production via spatial targeting distinct ANXA2

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Introduction: An inflammatory response is a hallmark of necrosis evoked by gram-negative bacterium *Vibrio (V.) vulnificus* (WT), but the virulence factor required for IL-1β production by this pathogen has not been identified. In the present study, we identified that VvpM, an extracellular metalloprotease, is essential for *V. vulnificus* pathogenicity and investigated the cellular mechanism of VvpM with regard to IL-1β production of *RAW 264.7 murine macrophage cells*.

Materials and Methods: *In vivo* role of VvpM in inflammation and intestinal colonization was assessed by the mutation and complementation ofthe *vvpM* gene from *V. vulnificus* in mouse infection models. Gain- and loss-of-function approaches were used to identify the mechanism of VvpM in the promoting of necrotic cell death coupled with IL-1β production .

Results: Mutation of the *vvpM* gene among various mutants from WTappeared to play major role in prevention of IL-1β production, whereas the recombinant protein (r) VvpM caused cytotoxicity mainly via necrosis coupled with IL-1β production. The necrotic cell death induced by rVvpM is highly susceptible to the knockdown of ANXA2 located in both membrane lipid and non-lipid raft parts. rVvpM acting on non-lipid raft part induced NLRP3 inflammasome via Atg5-mediated autophagy. rVvpM acting on lipid raft induced the recruitment of NOX enzymes coupled with ANXA2 to facilitate the ROSproduction that are responsible for activation of redox-sensitive PKCα, JNK,and NF-κBp65. In an *in vivo* model, VvpM increased autophagy activation and canonical inflammation pathway for IL-1β production.

Conclusions: These findings delineate novel rVvpM-dependent pathogenic signaling pathway that facilitates necrotic cell death coupled with IL-1β production via spatial targeting distinct ANXA2 within the cell.

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Establishment of differential diagnosis between LOM vaccine and B^{Erns} marker vaccine of Classical Swine Fever Virus used

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Introduction: Classical swine fever (CSF) is a highly contagious disease of swine that has caused significant economic losses in industrialized pig producing countries around the world [1]. Recently, new marker vaccine was developed against CSFV, it requires the establishment of a differential diagnosis with traditional LOM-vaccine. Neutralising peroxidase-linked assay (NPLA) is a gold standard method for serological differential diagnosis, but it needs a lot of the processing time [2, 3]. We developed competition ELSIA using two different vial antigens to promote more rapid specific diagnostic against CSFV.

Materials and Methods: Two cELISA (for differential diagnosis toward LOM, field strain and marker vaccine strain) were designed which having two different recombinant E^{rns} antigen proteins (CSFV and BVDV). Sows and piglets serum from 8 farms was performed different E^{rns}-ELISA, E2-cELISA (CSFV) and SN (serum neutralization) assay. Time-dependent test was performed, focusing on the determination of diagnostic sensitivity, specificity and differential diagnosis between two different types of vaccines.

Results: We confirmed two different (CSFV and BVDV)-cELISA test gave 90% sensitivity and 99.3% specificity compared with the NPLA in sows and piglets serum. Serum from sows vaccinated with marker vaccine showed positive result in E2-cELISA(CSFV) and SN (serum neutralization) assay. However, CSFV cELISA showed negative result and BVDV showed positive results. Piglets serum showed similar results with sow sera when tested with same manner. Therefore, the two different antigens (CSFV and BVDV)-cELISA proved to be a sensitive and specific novel discriminatory test in conjunction with vaccination using the marker vaccine.

Conclusions: Two different E^{rns}-cELISA proved to be a sensitive and specific novel discriminatory test with vaccination using the marker vaccine. The E2-ELISA can be used in combination with two different type (CSFV and BVDV)-cELISA to enhance the overall specificity of the test system. Thus, the ELISAs represent promising differential diagnostic tools, as well as an alternative to traditional LOM vaccine differentiation by serum neutralization test.

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Comparison among three antibody diagnostic methods against Classical Swine Fever Virus

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Introduction: Classical Swine Fever (CSF) is severe systemic swine disease with high mortality, fever, erythema, diarrhea etc. Many countries give a lot of efforts to eradicate CSF. Vaccination policy is used to control the CSF by inducing high level of antibody against field virus. Veterinary service laboratories use Indirect-ELISA (I-ELISA) to detect antibody against CSFV to process large amount of field samples. In this study, we compared the specificity of I-ELISA from other diagnostic methods to improve currently used method which is the I-ELISA.

Materials and Methods: Three antibody diagnostic methods were compared; I-ELISA, Comparative ELISA (C-ELISA), and Serum Neutralization test (SNT). SNT is considered as gold standard method. I-ELISA (MEDIAN Diagnostic, CSFV AB ELISA) and C-ELISA (MEDIAN Diagnostic, CSFV AB C-ELISA) were conducted following manufactor's instruction. SNT was perfomed following the protocol from OIE manual of classical swine fever. The 532 serum samples (128 sows, 404 piglets)were collected from nationwide and tested in this study.

Results: From the sow serum, SNT, C-ELISA and I-ELISA showed 82% (105/128), 91.4% (117/128), 95.3% (122/128) of positive percentage, respectively. The positive rate from SNT, C-ELISA, and I-ELISA showed 90% (364/404), 92.1% (374/404), 95.9% (387/404) in piglets respectively. The positive pattern was similarity between sow and piglet.

Conclusions: I-ELISA showed higher positive ratio than C-ELISA and SNT. Especially SNT, the standard method, showed significant differences from I-ELISA, 13% in sow, 5.7% in piglet. From this findings, we concluded that I-ELISA has high sensitivity and low specificity compared to other methods. False positive value was detected in I-ELISA when sample have positive/negative cut off (0.14). This result maybe due to the technical problem including antigen clean-up and antigen coating. Therefore, additional quality control process for I-ELISA test is need to improve specificity of the diagnostic method.

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Immune responses in pigs by vaccination of transgenic plant-expressed classical swine fever virus E2 protein

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Introduction: Classical swine fever virus (CSFV) is a member of the *Pestivirus* genus of the family of *Flaviviridae* and its infection results in highly contagious and severe disease in pigs. The CSFV possesses a positive-stranded RNA genome encoding a polyprotein that is subsequently processed into for structural protein (nucleocapsid protein C, envelop glycoprotein E^{rns}, E1, and E2) and eight nonstructural proteins. The glycoprotein E2 is the most immunogenic envelope protein. In previous studies, we have produced the CSFV glycoprotein E2 developed using recombinant protein expression system in transgenic Arabidopsis (*A. thaliana*) and found its potential as an E2 subunit vaccine. The aim of this study is to evaluate immune response of E2 vaccine after challenge and to provide its optimal vaccine dose to obtain effective protection against CSFV infection

Materials and Methods: The vaccine contained transgenic-plant E2 proteins prepared in a 1:1 water-in-oil emulsion with the adjuvant IMS1313. In first experiment to find optimal vaccine dose, ten-week-old piglets were randomly divided to five vaccine groups as follows: a negative control and different vaccine administration groups with 5, 10, 25, 50, or 100 μg of E2 subunit vaccine per one piglet (n=3 per each group). Vaccine groups were immunized with one dose of vaccine twice at 2-week intervals. All pigs were challenged with virulent YC11 strain at 2-weeks post of vaccination. In second experiment to test efficiency of one vaccination, E2-vaccined piglets were challenged with different periods as 7, 11, and 14 days of post vaccination (n=4 per each group).

Results: All E2-immunized piglets (5, 10, 25, 50, or 100 μg of vaccine dose) mounted an anamnestic response after booster vaccination with neutralizing antibody titers range from 1:512 to 1:2048 after twice vaccination in 2-weeks interval. After