Main Contents

- Genetic analysis of **PRRSV (Porcine Reproductive & Respiratory Syndrome)** in Vietnam
- Genetic analysis of **PCV (Porcine Circovirus)** in the South of Vietnam
- Phylogenetic analysis of **Classical swine fever virus (CSFV)** in a Mekong Delta provinces
- Some methods for detecting and quantifying PRRSV, CSFV and PCV
  - PCR and RT-PCR
  - Realtime PCR
  - LAMP (loop-mediated isothermal amplification)
Why PRRSV, PCV and CFSV are the emerging diseases?

- They are a highly contagious viral disease and have immunosuppressive effects in pigs, it may increase their susceptibility to other agents causing diseases.
- Become the chronic disease → Hard to detecting by clinical findings. BUT, It is an implicit threat.
- Cause damage in some important organs → serious illness
- Swine industry is very important in Vietnam economy also in the world.

Genetic analysis of PRRSV in Vietnam

PRRSV in Vietnam???
Methode

RNA extraction
RT-PCR
Sequencing
Genetic analysis

PRRSV infected samples

RNA extraction

RT-PCR

Amplifying NSP2 gene

NSP 2 F: 5'-AAA GAC CAG ATG GAG GAG GA-3' 
NSP 2 R: 5'-GAG CTG AGT ATT TTG GGC GTG-3'

Vaccine

+ BSL-PS 100
+ Porcillis
+ AmerVac
+ IngelVac

Sequencing

Phylogenetic
Phylogenetic of PRRSV based on NSP2 gene

(Vo KH. And Nguyen NH., 2011)

Deletion of 87 nucleotide

Conclusion

- PRRSV isolates in Vietnam had deletion of 87 nucleotides and were the same to PRRSVs isolated from China in 2007.
- Homology between studied PRRSVs in Vietnam and Chinese type PRRSV isolated at China were high (93.9 – 96.1%) in the nucleotide sequence.
- PRRSV isolates from North Vietnam and PRRSV isolates from South Vietnam were high identity (91.4 – 99.1%).

➤ This study was undertaken to assess the regional variation of prevalent PRRSV and to establish a sequence database for PRRSV molecular epidemiological studies.
Genetic analysis of PCV in Vietnam

Post Weaning Multisystemic Wasting Syndrome

- wasting
- palpable lymphadenopathy

Intestina juice in breast cavity

Intestina juice in abdominal cavity

Stuffing spleen + hepatitis
Genetic analysis of PCV in Vietnam

PCV in Vietnam??

Phylogenetic of PCV based on ORF2
(Vo KH. and Nguyen NH., 2011)

The result show that all of the studied PCV isolates in Vietnam belong to PCV type 2b and 2d.
Conclusion

- All of the PCV isolates in Vietnam belong to PCV type 2b and 2d.
- The similarity between PCV2d isolates from studied regions and some regions of China is high (99.1-99.3%).
- Specially, PCV2 type b and PCV2 type d are simultaneously present at the same farm. The situation of PCV2 infection at some farm in Vietnam is complicated.
- Subgenotype PCV2 type d viruses are predominate genotype circulating in studied regions.
- More knowledge we get for prevention and treatment PMWS epidemic caused by PCV in Vietnam.

Phylogenetic of CSFV based on E gene

Classical swine fever (CSF) caused by a *pestivirus* (CSFV)

Distribution of CSFV in Asian
**Conclusion**

- All the studied CSFV isolates belonged to genogroup 2 and were clustered in subgroup 2.1 and 2.2. The isolates have high identity of nucleotide (97%-99%) compared to CSFV isolates from other regions in Vietnam (DN-VN, LD-VN, QN-VN). It is possible that the presence of these strains in the studied province may be the result of CSFV circulation among provinces in Vietnam territory.

- The attenuated live Thiveral vaccine and C vaccine strains were clustered in subgroup 1.1 and were very distantly related to the CSFV isolates found.
Methods for detecting and quantifying PRRSV

- RT-PCR and nested RT-PCR

Distinguishing EU and US PRRSV (Vo KH. And Nguyen NH., 2009)

Distinguishing vaccine virus and field virus of PRRSV by RLFP assay (Nguyen NH., 2010)
Methods for detecting and quantifying PRRSV

- **Realtime RT-PCR**

QUANTIFYING AND GENOTYPING OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS BY REAL-TIME RT-PCR USING EVAGREEN (Vo Khanh Hung et al, 2011)

![Image](image1)

**Conclusion**

- The sensibility of the assay was $10^1$ copies/ul.
- The specificity of the assay was confirmed by using PCV (porcine circovirus) and PRRSV-free blood of pig.
- Genotyping of EU PRRSV and US PRRSV was based on the difference of melt temperature (Tm) in melt curve analysis. The Tm of EU PRRSV amplicon was 83.4°C and that of US PRRSV amplicon was 84.4°C.

→ EvaGreen-based real time RT-PCR quick, sensitive and accurate results for quantifying and genotyping PRRSV, which was used to determined genotypes.
Methods for detecting and quantifying PRRSV

- **Realtime RT-PCR**

DEVELOPMENT OF ONE-STEP REAL-TIME QUANTITATIVE RT-PCR ASSAY BASED ON TAQMAN PROBE FOR DETECTION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS NA TYPE (Vo Kinh Hung et al., 2011)

![Graphs showing sensitivity test and standard curve](image1)

![Graph showing specific test](image2)

**Conclusion**

- The developed assay is specific for both NA type and Chinese type.
- Sensibility assay met $10^1$ copies/ml of sample from infected pigs.
- The developed method for detecting and quantifying PRRSV NA type is a realized method that is useful for preventing and controlling disease caused by PRRSV.
Methods for detecting and quantifying PRRSV
LAMP (loop-mediated isothermal amplification)
Notomi, 2000)

The mechanism of LAMP reaction
Methods for detecting and quantifying PRRSV

• LAMP (loop-mediated isothermal amplification)

Gel assay

Eye assay

specific test
Methods for detecting and quantifying PRRSV

- LAMP (loop-mediated isothermal amplification)

Gel assay
UV assay
Eye

sensitivity test

The targets of my group focused in

- Genetic analysis of PRRSV, PCV and CFSV all regions of Vietnam also in the world.
- Developing LAMP technique for detecting PRRSV, PCV and CFSV and producing LAMP kit for PRRSV, PCV, CFSV.
- Developing PCR assay for distinguishing vaccine virus and field virus of PRRSV, PCV also CFSV.
Reference

- Vo Khánh Hùng, Nguyễn Văn Chí, Lê Văn Thắng, Lê Nguyễn Ngọc Hạnh, Trần Thị Dân, quantifying and genotyping of porcine reproductive and respiratory syndrome virus by real-time rt-pcr using evagreen. Accepted by Journal of agricultural sciences and technology 2011.
Porcine Reproduction and Respiratory Syndrome (PRRS) caused by an Arterivirus is a highly contagious viral disease of pigs. The gene encoding glycoprotein 5 (ORF5) of porcine reproductive and respiratory syndrome virus (PRRSV) isolates from Ho Chi Minh city, Dong Nai and Ba Ria Vung Tau provinces of Viet Nam and a North American-type vaccine, BSL-PS100 (currently available in this country) were analysed by using RT-PCR and sequencing. Sequences were then compared with other PRRSV sequences available through GenBank. Results showed that, All the studied PRRSV isolates belonged to subgroup 2.2 of North American (NA) type and has high similarity to high virulent china isolates of PRRSV (98.5 - 99.7%). Meanwhile, the currently available vaccine in Viet Nam, BSL-PS100, were clustered in subgroup 2.1 and was distantly related to the PSSRV isolates from the studied provinces in Viet Nam (87.9 – 88.6%). These results may contribute to the knowledge of PRRSV epidemiology in HCMC, Dong Nai, Ba Ria Vung Tau of Viet Nam and may help to take into consideration for control and preventive measures by vaccinating to protect pigs in HCMC, Ba Ria Vung Tau and Dong Nai from PRRSV infection.
Nucleocapsid protein N (ORF7) protein bearing a RNA binding domain is suggested to play a role in viral replication, is highly antigenic and is the abundant protein in virions, and has been useful for the diagnosis of PRRS. To more fully understand the genetic diversity of PRRSV in south Vietnam, the ORF7 sequences of two PRRSVs isolated from HCMC, two PRRSVs isolated from Dong Nai province and four PRRSVs isolated from Ba Ria Vung Tau were analyzed, compared and used for building their phylogenetic tree. The results showed that almost studied Dong Nai, Ba Ria Vung Tau and HCMC isolates belong to subgroup 2.2 of the North American (NA) type and has high identity with PRRSVs isolated in mainland China from 2006 and 2007. Especially, there are two Ba Ria Vung Tau isolates (1 BRVT VN and 6 BRVT VN) separated from other studied isolates and other virus PRRSV on over the world and have the trend of setting up a new group in phylogenetic tree of PRRSVs. These isolates have low identity with other studied isolates (89.5 – 90.3 %) and BSL-PS vaccine (91.1 %). Moreover, the BSL-PS100 (Besta, Singapore) vaccine, were popularly used in Vietnam for PRRSV vaccination, were clustered in subgroup 2.1 and was distantly related to the PRRSV isolates from studied provinces. These results may contribute to the knowledge of PRRSV epidemiology in HCMC, Dong Nai, Ba Ria Vung Tau of Vietnam and may help to take into consideration for control and preventive measures by vaccine to protect pigs in Dong Nai, Ba Ria Vung Tau and HCMC from PRRSV infection.
Porcine Reproduction and Respiratory Syndrome caused by a *Arterivirus* (PRRSV) is a highly contagious viral disease of pigs. Most of detected Reproductive and Respiratory Syndrome virus in Vietnam belong to North American type and chinese type. To gain a better understanding of the genetic diversity and evolution of PRRSV in Vietnam, the nsp2 genes of 18 PRRSV strains collected in the North Vietnam (Ha Noi capital and Bac Giang province) and South of Vietnam (Ho Chi Minh city, Dong Nai province and Binh Duong province) in 2009 and 2010 were partially sequenced. These sequences were then analyzed along with some PRRSV strains that is isolated all over the world. The result indicated that Nsp2 gene of all 18 PRRSVs isolated in our studies had deletion of 87 nucleotides and were the same to PRRSVs isolated from China in 2007. Sequence analysis indicated that the homology between studied PRRSVs in Vietnam and chinese type PRRSV isolated at china in 2007 were high (93.9 – 96.1%) in the nucleotide sequence. On other hand, The identity of nsp2 genes between five isolates from North Vietnam (4 isolates from Bac Giang, 1 isolates from Ha Noi) and thirteen isolates from South Vietnam (8 isolates from Ho Chi Minh city, 4 isolates from Binh Duong and 1 isolates from Dong Nai) were high (91.4 – 99.1%). Beside that, the result show that almost isolates from each province is different in comparision with another province. Also, the result indicates that there were the genetic evolution of PRRSV along the time in 2009 and 2010. This study was undertaken to assess the regional variation of prevalent PRRSV and to establish a sequence database for PRRSV molecular epidemiological studies.

Postweaning multisystemic wasting syndrome (PMWS) is an emerging disease in swine. Increasing evidence indicates that a variant strain of porcine circovirus (PCV), designated type 2 PCV (PCV-2), is responsible for PMWS. So, detecting, typing and analysing phylogenetic tree of PCV2 is important for diagnosis, typing and molecular epidemiological study on PCV2. A part of ORF2 that encoded capsid protein of PCV2 was sequenced and used for genetic analysis. In this study, 13 isolates from Dong Nai province and Ho Chi Minh city (HCMC) was sequenced and used to build phylogenetic tree with some reference isolates in over the world from genebank. The result show that all of the isolates belong to PCV type 2b and 2d. Specifically, 1 isolate from HCMC and 4 isolates from Dong Nai province belong to PCV2 type b (with the similar is 98.8 – 99.5 % in nucleotid sequence); 1 PCV isolate from HCMC and 7 isolates from Dong Nai province belong to PCV2 type d which is new subgenotype prevalent in china. The similarity between PCV2d isolates from studied regions and some regions of China is high (99.1-99.3%). Specially, PCV2 type b and PCV2 type d are simultaneously present at the same farm. This result show that the situation of PCV2 infection at some farm in Dong Nai province and HCMC is complicated. Of 13 isolates in this study, the majority belonged to PCV2 type d (61.54%), indicating that subgenotype PCV2 typd d viruses are predominante genotype circulating in studied regions. The more studies in molecular epidemiologic of PCV2 in other regions in Vietnam the more knowledge we get for prevention and treatment PMWS epidemic caused by PCV in Vietnam.
Porcine reproductive and respiratory syndrome (PRRS) caused by PRRSV is detrimental to economy. There are two types of PRRSV, being North American (NA) type and Europe (EU) type. EvaGreen-based real time RT-PCR protocol was established for quantifying and genotyping PRRSV virus, in which F7 and R7 primers were designed on ORF7 of the virus. A standard curve used in the assay was created by a plasmid DNA template. The whole ORF7 of PRRSV genome was inserted into pGEM T Easy plasmid, then, cloned into E.coli DH5α. The sensibility of the assay was $10^1$ copies/ul. The specificity of the assay was confirmed by using PCV (porcine circovirus) and PRRSV-free blood of pigs. Genotyping of EU PRRSV and US PRRSV was based on the difference of melt temperature (Tm) in melt curve analysis. The Tm of EU PRRSV amplicon was 83.4°C and that of US PRRSV amplicon was 84.4°C. The results showed that EvaGreen-based real time RT-PCR assay could offer quick, sensitive and accurate results for quantifying and genotyping PRRSV, which was used to determined genotypes and viral load of blood samples and swab from post weaning pigs.