Journal of Agricultural Science and Technology A 7 (2017) 282-287
doi: 10.17265/2161-6256/2017.04.006

Investigation on Infectious Agents of Aborted Pig Fetuses and Its Correlation with PRRSV MLV Vaccine

Woo-Taek Oh[1], Van-Giap Nguyen[2], Hyung-Joon Moon[3], Jee-Hoon Lee[1], Hye-Kwon Kim[4], Seong-Jun Park[5], Hee-Chun Chung[1] and Bong-Kyun Park[1]

1. Department of Veterinary Medicine Virology Lab, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Daehak-ro 1, Gwanak-gu, Seoul 08826, Korea

2. Department of Veterinary Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi 50877, Vietnam

3. Viral Infectious Disease Research Unit, Green Cross Veterinary Products, Yongin 17066, Republic of Korea

4. Viral Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Korea

5. Forensic Medicine Division, Daegu Institute, National Forensic Service, Chilgok 34054, Korea

Abstract: Infectious agents causing aborted fetus problems in domestic pigs were investigated in this study. More than 10 different infectious agents were known to cause abortion in swine and the major eight viruses among them were inspected. One hundred twelve samples of aborted fetuses from nine provinces in South Korea were collected during April to November, 2013 in this study for the diagnosis of infectious agents causing abortions in pigs. Eight major infection viruses were examined in this study mainly using various diagnostic kits and reverse transcriptase polymerase chain reaction (RT-PCR). Positive rate of the detection differed from each viruses. In this study, the main focus was the porcine reproductive and respiratory syndrome virus (PRRSV), which took the second large portion in the positive rate of detection, and then its *ORF5* gene was compared with modified live virus (MLV) vaccine strain to figure out the influence of vaccine on disease. Between four positive samples' sequence, two of them were 99.9%-100% similar to MLV vaccine strain and two other samples were 88.6%-92.7% similar. Similarity rate of the sequences between the vaccine and virus from aborted fetuses are very crucial, because it implies that abortion in swine can be made due to the usage of vaccine not only by the infection of field virus, and if MLV vaccine actually do have an impact on the infection, usage of the vaccine should be reconsidered.

Key words: Abortion, infectious agents, domestic pig, PRRSV, MLV vaccine.

1. Introduction

There are many agents known to cause abortions in sows, including viruses. Some RNA or DNA viruses can cause massive loss to swine producing industries all over the world, because it matters directly with the reproduction of pigs [1, 2], such as porcine circovirus type 2 (PCV2), Aujeszky's disease virus (ADV), porcine parvovirus (PPV), classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV) and encephalomyocarditis virus (EMCV) [3-5]. Also, family of Flaviviridae (Japanese encephalitis virus, JEV) has been reported to cause severe abortion and many potential zoonotic problems [6].

Among those viruses, one of the most important virus to study is PRRSV, which was already well known to cause abortion in pigs as it was named for. PRRSV is a small, enveloped RNA virus, which is included in genus *Arterivirus* and family Arteriviridae [7]. The genome of this virus is consisted of at least nine open reading frames (*ORF*) and its size is approximately 15 kb [8]. Based on the sequence of PRRSV, two genetic lineages were recognized: European genotype and North American genotype [9].

Corresponding author: Bong-Kyun Park, professor, research fields: veterinary medicine and virology.

Those two genotypes show homology of 60%-70% and distinct differences between two genotypes stand for the difference of virulence and pathogenicity of virus in two groups [10]. Since PRRSV caused the majority of serious abortion problems in swine industries globally, many phylogenetic, pathologic and immunologic studies were done briskly in many countries [11-13]. Also, the usage of PRRSV vaccine is globally commercialized due to the economic losses made by abortions, and it becomes prerequisite for farmers who domesticate pigs to use both attenuated and inactivated vaccines of PRRSV for their farms [14, 15].

Modified live virus (MLV) vaccines are vitally used for the prevention of major diseases, such as PRRSV, PCV2 and SIV in swine industries, and it is critical for these vaccines to neither spreading nor reverting virulence of the virus. But, sometimes safety issues concerning the usage of MLV virus vaccines are made, because the virulence of vaccine itself can affect the swine's health [16, 17]. Correlation between the vaccine and disease is essential for every swine farm in most countries, because it is directly related with economic losses in swine industries [18]. So this study focused on commercialized vaccines of PRRSV in South Korea and the phylogenetic studies were performed using the sequences of the vaccine and PRRSV gained in the investigation of diagnosis of infectious agents causing abortions in pigs. Among nine *ORFs* in gene of PRRSV, *ORF5* was known to play an important role immunologically and be related directly with neutralizing antibody formation [19]. This study aimed to investigate abortion problems of sows in South Korea on the basis of regional and individual farm surveillance and focused particularly on PRRSV and its *ORF5* gene sequence to compare them with sequence of PRRSV vaccine strains commercialized in South Korea.

2. Materials and Methods

During April to November 2013, fetuses (n = 112) from swine farms caused abortion in all nine provinces were randomly collected. Fetuses were pooled mainly from organs, including kidney, liver, lymph node, heart and spleen. DNA and RNA extraction were individually done from each tissue, and all samples were conducted for the extraction of DNAs as follows. Proteinase K solution 8 uL, DNA lysis buffer 500 uL and a stool sample 200 uL were mixed. Next, the mixture was thoroughly vortexed and incubated for 1 h. Then, 200 uL of phenol-chloroform-isoamyl alcohol (25:24:1) was added into the preparation, thoroughly vortexed and centrifuged 10 min at 13,000 rpm. DNA in aqueous phase was precipitated with an equal volume of isopropanol and centrifuged again. The resulting DNA pellet was washed with 1 mL of 70% ethanol, centrifuged, dried and resuspended in 30 uL TE buffer. Total RNA was extracted by using viral RNA mini kit (QIAGEN Ltd., Manchester, UK) following the manufacturer's instructions. The RNA was then converted into cDNA with the use of random hexamers and commercial M-MLV reverse transcriptase kit (Invitrogen, USA) following the manufacturer's protocol.

For the exact abortion problem investigation, median diagnostics usual commercial kits were used following its own protocols: PCV2, ADV, PPV, PRRSV, CSFV, SIV, EMCV and JEV detailed kits information (PCV2: VDx® PCV2 ORF2 PCR Cat. No. 3023; ADV and PPV: VDx® Abortion MP PCR Cat. No. 3031; PRRSV: VDx® PRRSV ORF RT-PCR Cat. No. 3024, VDx® PRRSV ORF RT-PCR Cat. No. 3024, Europe type, VDx® PRRSV NA/EU typing Nested PCR, VDx® PRRSV NA/EU typing nested PCR; CSFV: VDx® CSFV 5'NCR RT-PCR Cat. No. 3011; SIV: VDx® SIV RT-PCR; JEV and EMCV: VDx® MP RT-PCR II (JEV/EMCV), Cat. No. 3033). Also, PRRSV *ORF5* complete gene were detected using nested PCR (specific primers one round: FR9, RR9, second round: RF5, RR5), which followed former study protocols [2]. The band amplicon 716 bp was purified by using the gel-extraction method and

further processed for TA cloning and transformation. The phylogenetic analysis of *ORF5* complete sequences for PRRSV were performed with reference strains of lineage 1 to lineage 9 and lineage KOR (from Genbank).

3. Results and Discussion

As shown in Table 1, among investigated viruses, the virus which showed the highest positive rate (16.96%) was PCV2. Both SIV (H1N1) and PRRSV showed positive rate of 3.57%, and other viruses (ADV, PPV, CSFV, EMCV, JEV) were all negative. The complete *ORF5* gene of PRRSV from four samples which were positive in PRRSV detection was sequenced. The information about the positive samples was shown in Table 2. Those samples were from all different provinces in Korea from May to July and every farm has common features that they were vaccinated for PRRSV and PPV. Also all four sequences were classified in type II PRRSV and turned out to be negative on PPV HI test, except one farm from Chungnam. The result showed that all of four sequences were clustered to lineage 5, which are already predominant in Korea (Fig. 1) [14]. *ORF5* gene of PRRSV was very variant compared to other *ORFs*, and it is also important for the manufacture of vaccine, because this part is related with neutralizing antibody formation. Total 10 lineages were classified globally and four positive samples belonged to the same group with MLV vaccine strain. Also both CP296-3 and PF2516-2 (gene accession No. KP317085 and KP317086) showed 99.9%-100% similarity with

Table 1 Fetus samples positive for investigation of abortion by RT-PCR or PCR, South Korea, 2013.

Pathogen	Positive rate (%)	Typing	No. of positive (positive rate %) with total n = 112							
			April (n = 11)	May (n = 22)	June (n = 22)	July (n = 8)	August (n = 8)	September (n = 14)	October (n = 19)	November (n = 8)
PCV2	19 (16.96)	Type II	5 (45.45)	5 (22.73)	6 (27.27)	1 (12.50)	0 (0.00)	0 (0.00)	0 (0.00)	2 (25.00)
ADV	0 (0.00)		0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
PPV	0 (0.00)		0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
PRRSV	4 (3.57)	Type II	0 (0.00)	1 (4.55)	1 (4.55)	2 (25.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
CSFV	0 (0.00)		0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
SIV	4 (3.57)	H1N1	0 (0.00)	1 (4.55)	2 (9.09)	1 (12.50)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
EMCV	0 (0.00)		0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
JEV	0 (0.00)		0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

PCV2: porcine circovirus type 2; ADV: Aujeszky's disease/pseudorabies virus; PPV: porcine parvovirus; PRRSV: porcine reproductive and respiratory syndrome virus; CSFV: classical swine fever virus; SIV: swine influenza virus; EMCV: encephalomyocarditis virus; JEV: Japanese encephalitis virus.

Table 2 PRRSV positive samples' information.

Fetus No.	Sample collected		Farm information			Antigen test (PCR, RT-PCR)	Antibody test (ELISA, HI)
	Month	Province	Farm name	Sow scale	Vaccinated		
CP266-2	May	Gyeongbuk	SS	450	PRRSV, PPV	PRRSV (positive)	PPV HI test < 20 (negative)
CP293-3	June	Gyeonggi	SS	1,000	PRRSV, PPV	PRRSV (positive)	PPV HI test < 20 (negative)
PF2516-2	July	Chungnam	YM	600	PPV, PRRSV, JEV	PRRSV (positive)	1. PRRSV ELISA IDEXX XR3 kit: 2.95 (positive); 2. PPV HI: 160 (positive)
PF2576-2	July	Jeonbuk	DNDN	200	PPV, PRRSV	PRRSV (positive)	PPV HI test <20 (negative)

PPV HI test is for measuring antibody titers in the serum samples and range below 20 meant negative in antibody test.

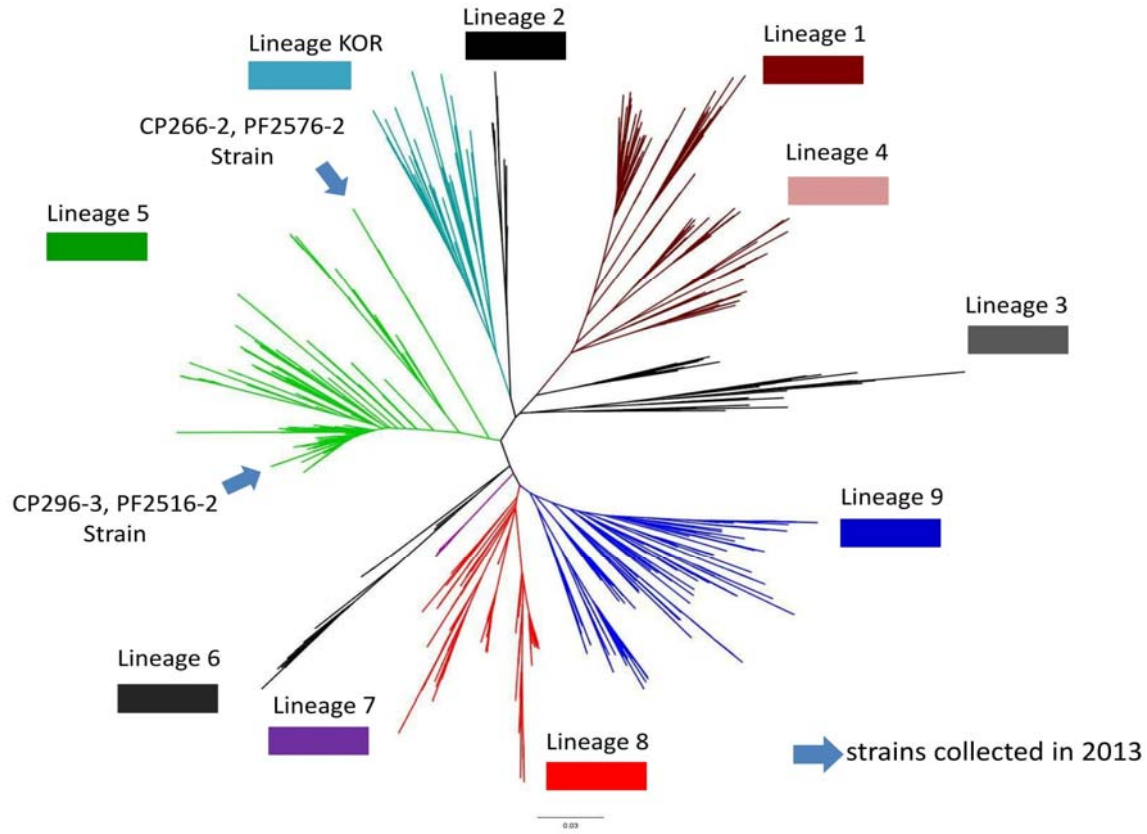

Fig. 1 Maximum likelihood phylogenetic tree using Fast Tree program, with the general time reversible nucleotide substitution model.

Phylogenetic analysis with reference PRRSV strains of lineage 1 to lineage 9, and lineage KOR (gene accession No. KP317084, KP317085, KP317086 and KP317087 for fetus No. CP266-2, CP296-3, PF2516-2 and PF2576-2, respectively).

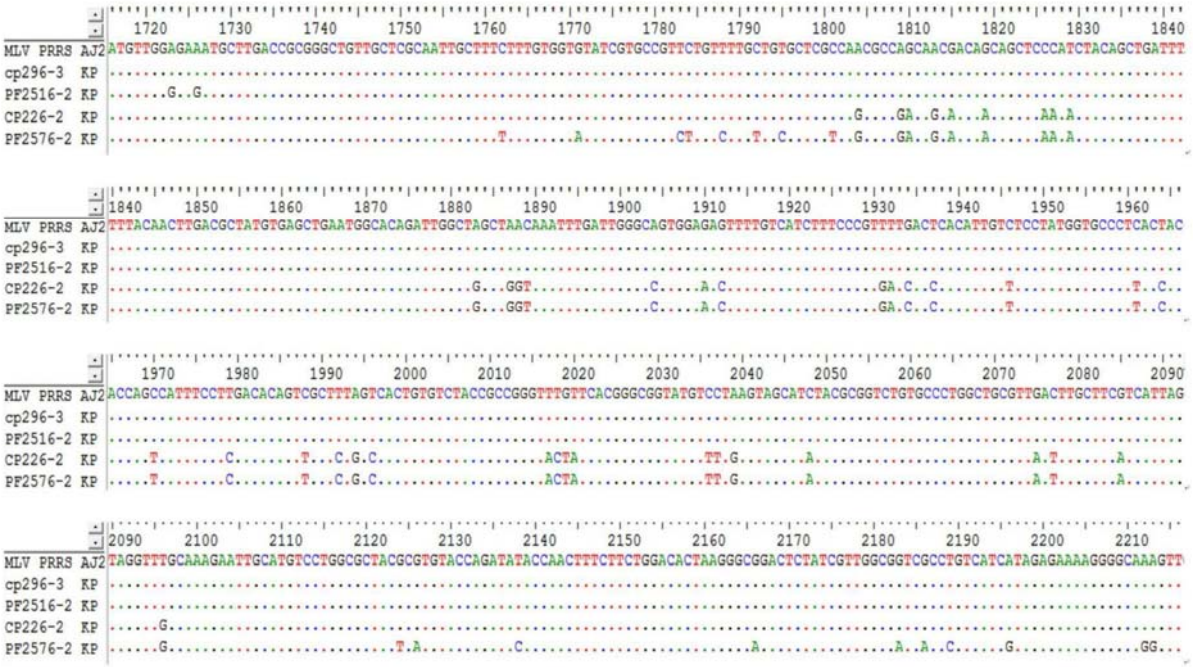

Fig. 2 Comparison between MLV vaccine strain (gene accession No. AJ223082) and four positive sample sequences (KP317084, KP317085, KP317086 and KP317087, respectively).

MLV vaccine strain of type II PRRSV (gene accession No. AJ223082). Only two nucleotides were changed in PF2516-2 and CP296-3 was completely identical compared to MLV vaccine strain. But the other two strains CP266-2 and PF2576-2 (gene accession No. KP317084 and KP317087) showed 88.6%-92.7% similarity with MLV vaccine strain, which were totally different to vaccine strain. It means the former two strains may have been influenced by MLV vaccine and they were completely different to the rest two strains which are field viruses.

The similarity among four positive sequences was compared in Fig. 2. In general, MLV vaccines influence only immune system of the individual weakly and make primary antibodies to protect body from the real infection by making the secondary antibodies, but if the vaccines influence the immune system heavily by any reason and play as virulence factor, the consequence for the young fetuses can be fatal. Although vaccines may be tested for hundreds of times for the estimation of influence on individual animal by clinical trials and followed the same process of manufacture as other MLV vaccines, there may be other environmental factors or co-infection of other infectious agents affecting the virulence of the vaccine. In this study, four positive samples positive for the antigen detection of PRRSV showed negative results in PPV antibody test, PCV2 antigen detection and detection of other viruses. Also, the four farms where each sample came from were not infected by the PRRSV before and all four farms used both inactivated and live attenuated vaccines for the prevention of infection of PRRSV. Owners of four farms also insisted that they followed the vaccination program accurately and their farms were supervised cleanly by veterinarians employed individually. From these circumstances, it can be inferred that MLV vaccine may have influenced some of the cases of abortions occurring in domestic pig industry, but their influences are not really related with the sanitary situations of the farm or co-infection of other viruses. It can not be concluded the causes of infections were 100% by the virulence of the vaccines, but other information about the co-infection status and sanitary conditions of the farms indicate that there may be some possibilities for vaccines to be related directly with the occurrences of disease. Thus, it is important to figure out whether this vaccine actually has an influence on some of the other abortion cases, and further studies are needed for the full level of explanation as the vaccine to act as a pathogenic virus. This study just implies some possibilities of relations between the vaccine and emergence of disease.

4. Conclusions

In conclusion, PRRSV was known to be one of the most important causes for inducing the reproductive failure in swine industries, and South Korea is also the country where PRRSV was prevalent from the past to nowadays and many economic losses were still made due to the infection of PRRSV. Although the usage of vaccines is essential factor for every swine farm, eradication of PRRSV was not easily made and still many problems were generated by this virus globally. Many causes will exist in this issue, but there may be some relationships between a few abortion cases and vaccine itself excluding the influence of field viruses. Thus, it may be better for swine industries to reconsider the usage of vaccine, whose strains were completely identical to the PRRSV isolated from the aborted fetuses itself.

Acknowledgments

This study was supported by the BioGreen 21 Program, Rural Development Administration (grant No. PJ011184). This study was supported through BK21 PLUS Program for Creative Veterinary Science Research. The authors would like to thank Hye Jung Yang and Jung Ah Kim for excellent technical assistance.

References

[1] Niewold, T. A., Van Essen, G. J., Nabuurs, M. J. A., Stockhofe-Zurwieden, N., and Van Der Meulen, J. 2000. "A Review of Porcine Pathophysiology: A Different Approach to Disease." *Veterinary Quarterly* 22 (4): 209-12.

[2] Zhou, L., and Yang, H. 2010. "Porcine Reproductive and Respiratory Syndrome in China." *Virus Res.* 154: 31-7.

[3] Kassimi, L. B., Boutrouille, A., Gonzague, M., Mbanda, A. L., and Cruciere, C. 2002. "Nucleotide Sequence and Construction of an Infectious cDNA Clone of an EMCV Strain Isolated from Aborted Swine Fetus." *Virus Res.* 83: 71-87.

[4] Kimman, T. G., De Wind, N., Oei-Lie, N., Pol, J. M. A., Berns, A. J. M., and Gielkens, A. L. J. 1992. "Contribution of Single Genes within the Unique Short Region of Aujeszky's Disease Virus (Suid Herpesvirus Type 1) to Virulence, Pathogenesis and Immunogenicity." *Journal of General Virology* 73: 243-51.

[5] Opriessnig, T., Patterson, A. R., Meng, X. J., and Halbur, P. G. 2009. "Porcine Circovirus Type 2 in Muscle and Bone Marrow Is Infectious and Transmissible to Naive Pigs by Oral Consumption." *Vet. Microbiol.* 133: 54-64.

[6] Neyts, J., Leyssen, P., and De Clercq, E. 1999. "Infections with Flaviviridae." *Verhandelingen —Koninklijke Academie Voor Geneeskunde Van Belgie* 61 (6): 661-97.

[7] Tian, K., Yu, X. L., Zhao, T. Z., Feng, Y. J., Cao, Z., Wang, C. B., Hu, Y., Chen, X. Z., Hu, D. M., Tian, X. S., Liu, D., Zhang, S., Deng, X. Y., Ding, Y. Q., Yang, L., Zhang, Y. X., Xiao, H. X., Qiao, M. M., Wang, B., Hou, L. L., Wang, X. Y., Yang, X. Y., Kang, L. P., Sun, M., Jin, P., Wang, S. J., Kitamura, Y., Yan, J. H., and Gao, G. F. 2007. "Emergence of Fatal PRRSV Variants: Unparalleled Outbreaks of Atypical PRRS in China and Molecular Dissection of the Unique Hallmark." *PloS ONE* 2 (6): e526.

[8] Meulenberg, J. J. 2000. "PRRSV: The Virus." *Veterinary Res.* 31 (1): 11-21.

[9] Geldhof, M. F., Vanhee, M., Van Breedam, W., Van Doorsselaere, J., Karniychuk, U. U., and Nauwynck, H. J. 2012. "Comparison of the Efficacy of Autogenous Inactivated Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Vaccines with That of Commercial Vaccines against Homologous and Heterologous Challenges." *BMC Veterinary Res.* 8: 182.

[10] Forsberg, R., Storgaard, T., Nielsen, H. S., Oleksiewicz, M. B., Cordioli, P., Sala, G., Hein, J., and Bøtner, A. 2002. "The Genetic Diversity of European Type PRRSV Is Similar to That of the North American Type But Is Geographically Skewed within Europe." *Virology* 299 (1): 38-47.

[11] Martelli, F., Toma, S., Di Bartolo, I., Caprioli, A., Ruggeri, F. M., Lelli, D., Bonci, M., and Ostanello, F. 2010. "Detection of Hepatitis E Virus (HEV) in Italian Pigs Displaying Different Pathological Lesions." *Res. Vet. Sci.* 88 (3): 492-6.

[12] Sookhoo, J. R., Brown-Jordan, A., Blake, L., Holder, R. B., Brookes, S. M., Essen, S., Carrington, C. V., Brown, I. H., and Oura, C. A. 2017. "Seroprevalence of Economically Important Viral Pathogens in Swine Populations of Trinidad and Tobago, West Indies." *Tropical Animal Health and Production.* doi:10.1007/s11250-017-1299-3.

[13] Zhang, L. J., Feng, Y., Martin, D. P., Chen, J., Ma, S. X., Xia, P. G., and Zhang, G. P. 2017. "Genetic Diversity and Phylogenetic Analysis of the *ORF5* Gene of PRRSV from Central China." *Res. Vet. Sci.* 115: 226-34.

[14] Kim, H. K., Park, S. J., Rho, S. M., Han, J. Y., Nguyen, V. G., and Park, B. K. 2011. "One Year's Study of Dynamic and Evolution of Types I and II PRRSV in a Swine Farm." *Vet. Microbiol.* 150: 230-8.

[15] Cano, G., Cavalcanti, M. O., Orveillon, F. X., Kroll, J., Gomez-Duran, O., Morillo, A., and Kraft, C. 2016. "Production Results from Piglets Vaccinated in a Field Study in Spain with a Type 1 Porcine Respiratory and Reproductive Virus Modified Live Vaccine." *Porcine Health Management* 2 (1): 22.

[16] Berton, P., Normand, V., Martineau, G. P., Bouchet, F., Lebret, A., and Waret-Szkuta, A. 2017. "Evaluation of Porcine Reproductive and Respiratory Syndrome Stabilization Protocols in 23 French Farrow-to-Finish Farms Located in a High-Density Swine Area." *Porcine Health Management* 3 (1): 11.

[17] Sirisereewan, C., Nedumpun, T., Kesdangsakonwut, S., Woonwong, Y., Kedkovid, R., Arunorat, J., Thanawongnuwech, R., and Suradhat, S. 2017. "Positive Immunomodulatory Effects of Heterologous DNA Vaccine-Modified Live Vaccine, Prime-Boost Immunization, against the Highly-Pathogenic PRRSV Infection." *Vet. Immunol. Immunopathol.* 183: 7-15.

[18] Renson, P., Fablet, C., Le Dimna, M., Mahé, S., Touzain, F., Blanchard, Y., Paboeuf, F., Rose, N. and Bourry, O. 2017. "Preparation for Emergence of an Eastern European Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Strain in Western Europe: Immunization with Modified Live Virus Vaccines or a Field Strain Confers Partial Protection." *Vet. Microbiol.* 204: 133-40.

[19] Zimmerman, J. J., Yoon, K. J., Wills, R. W., and Swenson, S. L. 1997. "General Overview of PRRSV: A Perspective from the United States." *Vet. Microbiol.* 55: 187-96.