2013 International PRRS Symposium and PCVAD(PCV2)

Final Program

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May 20-22, 2013

Organized By:

Chinese Association of Animal Science and Veterinary Medicine
Kansas State University
China Agricultural University
National Swine Industry Technology System

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David A. Benfield IPRRSS Travel Fellowship

David A. Benfield received his BS and MS degrees from Purdue University and a PhD from the University of Missouri-Columbia. He has a distinguished 23 years in research related to virus diseases of food animals. In 1990, he was the co-discoverer of the cause of "mystery swine disease" or porcine reproductive and respiratory syndrome virus (PRRSV). He has remained a role model and mentor to many of those who are currently in the PRRS field. He is a member of the American Society for Virology, American Association of Veterinary Laboratory Diagnosticians, and Honorary Diplomat of the College of Veterinary Microbiologists, American Association of Swine Veterinarians and the American Association for the Advancement of Science. Currently, he is the Associate Director of the Ohio Agricultural Research and Development Center, The Ohio State University and a Professor in the Food Animal Health Research Program in the College of Veterinary Medicine. It is his generous donation that initiated this fellowship program. It is his hope that these fellowships provide students with the experience of attending the International PRRS Symposium to present their work on PRRS.

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2013 International PRRS Symposium and PCVAD(PCV2) Program

Monday, May 20	
	Workshop: Modern technologies for the field with focus on industrial applications: Information for, industry, veterinarians and producers Session Chairs: Hanchun Yang and Jane Christopher-Hennings
0800	Hanchun Yang, China Agricultural University - *PRRS situation in China-the past, present and future*
0830	Guangzhi Tong, Shanghai Veterinary Research Institute - *Development and application of a dual-marker vaccine for highly pathogenic porcine reproductive and respiratory syndrome*
0900	Robert Morrison, University of Minnesota - *Control of PRRS on farm and regional level*
0930	Jane Christopher-Hennings, South Dakota State University - *Porcine reproductive and respiratory syndrome (PRRSV) diagnostics in the United States: Past, present and future*
1000	*Tea Break*
1030	David McLaren, PIC - *Breeding for PRRS resistance*
1055	Enric Mateu, Universitat Autònoma de Barcelona - *PRRS immunology*
1120	Pablo Lopez, IDEXX China - *Diagnostic testing in China*
1145	*Lunch* - Round table discussion on PRRS Vaccines in China *Moderator:* Jishu Shi, Professor, College of Veterinary Medicine at Kansas State University *Panel Members:* • Guangzhi Tong, Director-General, Shanghai Institute of Veterinary Research, Chinese Academy of Agricultural Sciences (CAAS) • Hua Wu, Professor, Institute of Special Animal and Plant Research, CAAS • Kegong Tian, Director, National Center for Veterinary Drug Technologies • Ping Jiang, Professor, Nanjing Agricultural University • Xuehui Cai, Professor, Harbin Institute of Veterinary Research, CAAS • Wenhai Feng, Professor, College of Life Sciences, China Agricultural University • Shishan Yuan, Director, Asian Veterinarian R&D Center, Boehringer Ingelheim Animal Health • Shucheng Zhang, Associate Director, Research & Development, Zoetis
	Session Chairs: Tahar Ait Ali and Ying Fang
1330	Jay Calvert, Zoetis - *Fostera™ PRRS: Modulation of interferon-alpha and induction of protective immunity two weeks following vaccination*
1400	Tanja Opriessnig, Iowa State University - *Luminex application for PRRS and PCV2*
1430	Ganfeng Yi, Beijing Dabeinong Technology Group - *Scale-up and transformation of Chinese swine production in the next decade and analysis of high-efficacy hog production strategies*
1500	*Tea Break*

1520 IPRRS Opening Ceremony
Bob Rowland and Hanchun Yang

- Dr. Bingsheng Ke, President China Agricultural University - Opening remarks
- Dr. Cynthia Duerr, Agriculture Attaché U.S. Embassy - Beijing - Opening remarks
- Dr. Kangzhen Yu, National Chief Veterinarian of China Ministry of Agriculture - Opening remarks
- Dr. Bob Rowland, Executive Chair Kansas State University - Special Honor to David Benfield-Co-discoverer of PRRSV
- Dr. Hanchun Yang, Chair China Agricultural University - Welcome speech

1600 IPRRS Opening Keynote Address

- Keynote Speaker: Luis Enjuanes, Centro Nacional de Biotecnología-CSIC - *Vaccine Vectors Based on Coronavirus Genomes to Protect Against PRRSV and SARS-CoV*

1700 *Break (if desired)*
or
Alex Morrow and Hans Nauwynck, DEFRA (Department for Environment, Food & Rural Affairs), UK - *Presentations and Discussion: International Collaborative Network for PRRS*

1900 Reception-Mixer - Finger food and drinks

Tuesday, May 21

0800 Keynote Presentation

- Ying Fang, South Dakota State University - *The PRRSV Replicase: Structure, Function and Implications*

0900 Section-1: Virus replication-protein structure-function
Session Chairs: Dongwan Yoo and Luis Enjuanes

- Invited Speaker: Dongwan Yoo, University of Illinois at Urbana-Champaign - *Structure and function of PRRSV proteins*

0935 Abstract Talk: H.C. Liu, North Carolina State University - *Discovery and characterization of the involvement of a cellular membrane fusion protein in PRRSV replication*

0955 Abstract Talk: Mingyuan Han, University of Illinois at Urbana-Champaign - *Non-structural protein 1-mediated interferon modulation is a common strategy for immune evasion in arteriviruses*

1015 *Tea Break*

1030 *Section-2:* Vaccines and immunity
Session Chairs: Shishan Yuan and Enric Mateu

- Invited Speaker: Shishan Yuan - *Towards a better PRRS vaccine: what are the missing links?*

1100 Abstract Talk: Marga Garcia Duran, Ingenasa - *The immunization of pigs with a Gp3 subunit vaccine elicits specific antibody response but does not protect against PRRSV infection after challenge*

1120 Abstract Talk: Renukaradhya Gourapura, Ohio State University - *Development and validation of porcine reproductive and respiratory syndrome virus specific neutralizing antibody detection assay in pig oral fluid samples*

1140	Abstract Talk: Simon Graham, AHVLA - *Characterisation of the T cell response induced by infection with subtype 1 or 3 European porcine reproductive and respiratory syndrome viruses*
1200	*Lunch*
	Poster Session 1 - Vaccines, Immunity, Evolution
1430	Section-3: Genetic heterogeneity and evolution Session Chairs: Tomasz Stadejek and Ping Jiang • Invited Speaker: Tomasz Stadejek, Warsaw University of Life Sciences - *Molecular evolution of PRRSV in Europe*
1500	Abstract Talk: Chao Fang, Nanjing Agricultural University - *A Novel isolate with deletion in GP3 gene of Porcine Reproductive and Respiratory Syndrome virus from the mid-east of China*
1520	Abstract Talk: Dongsheng He, South China Agricultural University - *swIFN-beta promotes genetic mutation of porcine reproductive and respiratory syndrome virus in Marc-145*
1540	Abstract Talk: Charlotte Hjulsager, Technical University of Denmark - *Experimental infection of pigs with two East European variants of Type 1 PRRSV*
1600	*Tea Break*
1620	Abstract Talk: Gerard Eduard Martin Valls, Universitat Autonoma de Barcelona - *Recombination events are common in genotype 1 and 2 PRRS virus worldwide and may generate mosaic viruses*
1640	Abstract Talk: Andrew Suddith, Kansas State University - *A novel strain of PCV2 isolated from vaccinated pigs suffering PCVAD causes 40% mortality in CDCD pigs in the presence of PPV*
1800	Banquet
Wednesday, May 22	
0800	Keynote Presentation Session Chairs: Joan Lunney and Enmin Zhou • Keynote Speaker: Michael Murtaugh, University of Minnesota - *Immunological Interactions of PRRSV and Pigs*
0900	Section-4: Genetics • Invited Speaker: Joan Lunney, USDA ARS BARC - *Genetics of host resistance to PRRSV infection: Progress of the PRRS Host Genetics Consortium*
0930	Abstract Talk: Li Gao, China Agricultural University - *MicroRNA-181 suppresses PRRSV infection by targeting its receptor CD163*
0950	Abstract Talk: Li Zhang, Nanjing Agricultural University - *Toll-like receptor ligands enhance the protective effects of vaccination against porcine reproductive and respiratory syndrome virus in swine*
1010	Abstract Talk: Bob Rowland, Kansas State University - *The role of host genetics in vaccine development*
1030	*Tea Break*

1050	Section-5: Virus-host interaction (pathogenesis) Session Chairs: Hans Nauwynck and Guangzhi Tong • Invited Speaker*:* Hans Nauwynck*,*University of Ghent - *The pathogenesis of PRRS is fully determined by the intriguing interplay of PRRSV with monocytic cells*
1120	Abstract Talk: Qing He, China Agricultural University - *Nonstructural proteins 1beta and 11 play an important role in differential TNF-alpha production induced by different PRRSV strains*
1140	Abstract Talk: Yongming Sang, Kansas State University - *Genome-wide analysis of marker genes related to antiviral regulation in PRRSV infected porcine macrophages at different activation statuses*
1200	*Lunch*
	Poster Session 2 - Genetics, Pathogenesis, Epidemiology
1430	Section-6: Epidemiology and disease control Session Chairs: Marlon Linatoc and Falko Steinbach • Invited Speaker: Marlon Linatoc, Zoetis - *Principles of controlling PCV2-The Importance of Reducing Viremia*
1500	Abstract Talk: Frederick Leung, The University of Hong Kong - *Recombination leads to the re-emergence of highly pathogenic PRRSV in China*
1520	Abstract Talk: Emanuela Pileri, Universitat Autonoma de Barcelona - *Quantification of PRRSV transmission: effect of pig vaccination*
1540	Abstract Talk: Rolf Rauh, Tetracore - *Development of best practices to diagnose Porcine Reproductive and Respiratory Syndrome (PRRS) Virus subtypes with high sensitivity in swine*
1600	Conclusion: Hanchun Yang, CAU, Bob Rowland, KSU - Announce BIVI PRRS Research Award Winners
1800	Organizing committee and invited speaker dinner

2013 International PRRS Symposium and PCVAD(PCV2) Abstracts

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Epidemiology and Disease Control

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Vaccines and Immunity

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70	Characterisation of the T cell response induced by infection with subtype 1 or 3 European porcine reproductive and respiratory syndrome viruses	H. Mokhtar[1], M. Pedrera[1,2], J.P. Frossard[1], S.E. Essler[3] and S.P. Graham[1*], [1]Virology Department, Animal Health and Veterinary Laboratories Agency, Addlestone, UK, [2]Department of Comparative Pathology, Veterinary Faculty, University of Córdoba-Agrifood Excellence International Campus, Córdoba, Spain, 3Institute of Immunology, Department of Pathobiology, University of Veterinary Medicine, Vienna, Austria	p. 99
71	Development of a multiplex fluorescent microsphere immunoassay for diagnosis of porcine respiratory disease complex	S.R. Lawson*, R.J. Langenhorst, R. Ransburgh, L. Zhu, Y. Fang, Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD 57007	p. 100
72	Evaluation of the efficacy of an attenuated live vaccine against highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) in young pigs	X. Leng[1], Z.G. Li[1], M.Q. Xia[2], Y.L. He[2], H. Wu[2*], [1]Institute of Special Wild Economic Animal and Plant Science, CAAS, [2]Sinovet (Beijing) Biotechnology Co., Ltd	p. 101
73	Virulence evaluation of a modified live highly pathogenic porcine reproductive and respiratory syndrome virus vaccine by serial passages in pigs	X. Leng[1], Z.G. Li[1], M.Q. Xia[2], F.X. Wang[1], Y.J. Wen[1], W. Wang[2], Y.L. He[2], H. Wu[2*], [1]State Key Laboratory for Molecular Biology of Special Economic Animals, Institute of Special Wild Economic Animal and Plant Science, CAAS, [2]Sinovet (Beijing) Biotechnology Co., Ltd.	p. 102

74	Changes of different lymphocyte contents in peripheral blood from pigs co-infected with porcine circovirus type 2 and porcine parvovirus	H.Y. Li, Y.Y. Liu, Q.Y. Song*, L.G. Chen, Y.Q. Li, W.Z. Yuan, College of Veterinary Medicine, Agricultural University of Hebei, Baoding, Hebei 071001, China	p. 103
75	Peptide nanofiber hydrogel: A novel adjuvant for Porcine Reproductive and Respiratory Syndrome virus modified live vaccine	X. Li[1*], A.J. Galliher-Beckley[1], J.C. Nietfeld[2], H. Huang[3], X. Sun[3], K.S. Faaberg[4], and J. Shi[1], [1]Department of Anatomy and Physiology, [2]Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, [3]Department of Grain Science and Industry, College of Agriculture, Kansas State University, Manhattan, KS, [4]Virus and Prion Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA	p. 104
76	Attenuation of interferon antagonizing function of PRRSV by targeted mutations in a highly conserved motif of nsp1beta protein	Y.H. Li[1*], L.C. Zhu[1], S.R. Lawson[1], Y. Fang[1,2], [1]Department of Veterinary and Biomedical Sciences, [2]Department of Biology-Microbiology, South Dakota State University, Brookings, SD 57007, USA	p. 105
77	Comparative respiratory pathogenicity and dynamic distribution of highly pathogenic porcine reproductive and respiratory syndrome virus and its attenuated vaccine strain in piglets	C. Liu[1,2*], C. Ying[1,3], W.L. Zhang[1], H.G. Wang[1], Y.B. Ning[1], [1]China Institute of Veterinary Drug Control, Beijing, China, [2]China Agricultural University, Beijing, China, [3]Huazhong Agricultural University, Wuhan, China	p. 106
78	Construction and biological characteristics of a recombinant porcine circovirus type 2 expressing the VP1 neutralizing epitope of foot-and-mouth disease virus	F.Y. Zhang*, Q.H. Tang, L.P. Huang, Y.W. Wei, H.L. Wu, L.J. Guo, Y.J. Fu, C.M. Liu*, Division of Swine Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, The Chinese Academy of Agricultural Sciences, Harbin 150001, China	p. 107
79	Enhanced protective immune response to PCV2 subunit vaccine by co-administration of recombinant porcine IFN-gamma in mice	Y.P. Wang*, D. Liu, L.J. Guo, Q.H. Tang, Y.W. Wei, H.L. Wu, J.B. Liu, S.B. Li, L.P. Huang, C.M. Liu*, Division of Swine Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, China	p. 108
80	Immunity induced by an experimental vaccine based on the recombinant PRRSV GP5 protein without ADE epitope expressed in adenovirus in pigs	M. Yang*, S. Sylla, X. Li, Z. Ding, College of Veterinary Medicine and Key laboratory of Zoonosis Research, Ministry of Education, Institute of Zoonosis, Jilin University, Changchun China	p. 109
81	Modulation of swine interleukin-4 to induce virus-specific immune responses elicited by modified live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine	J. Peng[1,3*], J.B. Wang[1,2,3], J. Yu[2,3], J.Q. Wu[2,3], Y.J. Du[2,3], J. Li[2,3], Z.K. Guo[1,3], S.J. Xu[2,3], Y.Y. Zhang[2,3], W.B. Sun[2,3], X.Y. Cong[2,3], J.L. Shi[2,3], [1]College of Veterinary Medicine, Shandong Agricultural University, Taian, China, [2]Institute of Animal Science and Veterinary Medicine, Shandong Academy of Agricultural Sciences, Jinan, China, [3]Shandong Key Laboratory of Animal Disease Control and Breeding, Jinan, China	p. 110

82	Construction and immunogenicity of DNA vaccine coexpressing GP3 and GP5 proteins of European-type Porcine Reproductive and Respiratory Syndrome virus	J.Q. Ren*, H.J. Lu, S.B. Wen, W.C. Sun, H. Liu, S. Yuan, J. Jing, F.L.Yan, F. Lu, K.S. Jin*, N.Y. Jin, Military Veterinary Institute, Academy of Military Medical Science, Changchun 130122, China	p. 111
83	Inhibition of porcine circovirus type 2 replication by cap protein gene DNA vaccine in mice	S.Sylla*, M. Yang, H. Liu, Z. Ding, Collage of Veterinary Medicine, and Key Laboratory of Zoonosis Research, Ministry of Education, Institute of Zoonosis, Jilin University, Changchun China	p. 112
84	Preliminary study of recombinant Bordetella Bronchiseptica expressing cap protein of Porcine Circovirus type 2 as a candidate vaccine against porcine Circovirus disease	S. Jin, X. Xin, F. Cuihua, W. Bin*, State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, People's Republic of China	p. 113
85	Isolation of the European genotype porcine reproductive and respiratory syndrome virus (PRRSV) in Kazakhstan	K.K. Tabynov[1*], M. Mambetaliyev[1], A.Zh. Azhibayev[1], M.B. Orynbayev[1], V.L. Zaitsev[1], A.A. Kopochenya[2], A.A. Azanbekova[1], A.R. Sansyzbay[1], [1]Research Institute for Biological Safety Problems (RIBSP), SC ME&S RK, Gvardeiskiy, Zhambylskaya oblast, Republic of Kazakhstan, [2]Boehringer Ingelheim RCV GmbH & Co KG, Vienna, Austria	p. 114
86	Effect of vaccination with Ingelvac Circoflex® on fattening performance in a farm in north China	A. Wang[1], J. Zhao[2], L. Zhu[1], T. Tan[1], [1]Boehringer Ingelheim Int'l Trading (Shanghai) Co. Ltd, Beijing100004, China, [2]Henan Agricultural University, Zhengzhou450008, China	p. 115
87	Construction of recombinant PRRSV expressing granulocyte-macrophage colony-stimulating factor	L.X. Yu*, Y.J. Zhou, Y.F. Jiang, W. Tong, G.Z. Tong*, Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai 200241, China	p. 116
88	Evidence for a broadly neutralizing antibody response to PRRSV	B.R. Trible[1*], A. Hess[2], Y. Wang[1], N. Boddicker[2], M.A. Kerrigan[1], J.C.M. Dekkers[2], and R.R.R. Rowland[1], [1]Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA, [2]Department of Animal Science, Iowa State University, Ames, Iowa, USA	p. 117
89	Host recognition of the structural form of the PCV2 capsid protein results in protective immunity or immunopathogenesis	B.R. Trible* and R.R.R. Rowland Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA	p. 118
90	The comparison of immune efficacy between combined PCV2, MH vaccine inactivated and the corresponding single vaccines	G.H. Wang*, Y.J. Che, B. Tang, G.H. Yan, Y. Zhao, Animal Medicine Research Center of Da Bei Nong Group, Beijing,China	p. 119

91	Identification of a B-cell epitope in the glycosylated protein 3 of highly pathogenic porcine reproductive and respiratory syndrome virus HuN4 strain	Y. Sun[1], C.L. Leng[1], J.M. Peng[1], T.Q. An[1], J.Z. Chen[1], H.Y. Zhao[1], D. Chang[1], G.Z. Tong[2*], Z.J. Tian[1*], [1]Division of Swine Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, China, [2]Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai, China	p. 120
92	Anti-idiotypic antibody vaccine against highly pathogenic PRRSV infection	C. Zhang[1*], Y. Yu[1], S. Xiao[1], G. Wang[2], N. Kong[1], X. Wang[1], H. Ni[1], C. Ma[1], H. Liu[1], N. Li[1], J. Gao[1], Q. Zhao[1], En. Zhou[1], [1]College of Veterinary Medicine, Northwest A&F University, Yangling, China, [2]Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, China	p. 121
93	The nonstructural protein 1 papain-like cysteine protease was not necessary for porcine reproductive and respiratory syndrome virus nonstructural protein 1 to inhibit interferon-beta function	X.B. Shi[1,2], Q.M. Li[1], Y.Z. Chang[1,2], J. Chen[1,2], C. Wang[3], P. Zhao [4], J.F. Yang[1], S.Z.Yang[1], G.P. Zhang[1,2,*], [1]Henan Provincial Key Laboratory of Animal Immunology, Henan Academy of Agricultural Sciences, Zhengzhou 450002, China, [2]College of Veterinary Medicine and Animal Science, Henan Agricultural University, Zhengzhou 450002, China, [3]Department of Preventive Veterinary Medicine, College of Veterinary Medicine, Northwest A&F University, No. 22 Xinong Road, Yangling, Shanxi 712100, China, [4]Department of Animal Science and Technology, Henan Institute of Science and Technology, Xinxiang 453003, China	p. 122
94	Toll-like receptor ligands enhance the protective effects of vaccination against porcine reproductive and respiratory syndrome virus in swine	L.L. Zhang, J. Bai, J. Liu, X. Wang, Y.F. Li, P. Jiang*, Key Laboratory of Animal Disease Diagnostic and Immunology, Ministry of Agriculture, Nanjing Agricultural University, Nanjing, Jiangsu, China	p. 123
95	The changes of the lymphocyte subpopulations in peripheral blood of the pigs inoculated with highly pathogenic PRRSV GD wild and vaccine type	W.L. Zhang[1*], H.G. Wang[1], F.T. Sun[2], C. Liu[1], Y.B. Ning[1], [1]China Institute of Veterinary Drug Control, Beijing, China, [2]Huazhong Agricultural University, Wuhan, China	p. 124

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97	A novel isolate with deletion in GP3 gene of Porcine Reproductive and Respiratory Syndrome virus from the mid-east of China	B.C. Fan, H. Wang, J. Bai, L.L. Zhang, P. Jiang*, Key Laboratory of Animal Disease Diagnostic and Immunology, Ministry of Agriculture, Nanjing Agricultural University, Nanjing, Jiangsu, China	p. 126

98	swIFN-beta promotes genetic mutation of porcine reproductive and respiratory syndrome virus in Marc-145	D.S. He[1,2*], X.Y. Niu[1], Y.L. Wang[1], Z.X. Xiong[1], C. Rui-ai[1,2], [1]Guangdong Dahuanong Animal Health Product Co.,Ltd., Guangdong Xinxing,527400, China, [2]College of Veterinary Medicine, South China Agricultural University, Guangdong Guangzhou, 510642, China	p. 127
99	Experimental infection of pigs with two east European variants of Type 1 PRRSV	C.K. Hjulsager[1*], L.E. Larsen[1], P.M.H. Heegaard[1], T. Stadejek[2], A. Bøtner[1], J. Nielsen[1], [1]National Veterinary Institute, Technical University of Denmark, Denmark, [2] Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Poland	p. 128
100	Fast and robust methods for full genome sequencing of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Type 1 and Type 2	L.K. Kvisgaard[1*], C.K. Hjulsager[1], U.Fahnøe[2], S.Ø. Breum[1], T. Ait-Ali[3], and L.E. Larsen[1], [1]National Veterinary Institute, Technical University of Denmark, DK-1870 Frederiksberg C, Denmark, [2]DTU National Veterinary Institute, Technical University of Denmark, Lindholm, Denmark, [3]The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, UK	p. 129
101	The diversity of Porcine Reproductive and Respiratory Syndrome virus type 1 and 2 in Denmark	L.K. Kvisgaard[1*], C.K. Hjulsager[1], C.S. Kristensen[2], M.S. Brar[3], F.C.C. Leung[3,4], and L.E. Larsen[1], [1]National Veterinary Institute, Technical University of Denmark, DK-1870 Frederiksberg C, Denmark, [2]Pig Research Centre, Danish Agriculture & Food Council, DK-8620 Kjellerup, Denmark, [3]School of Biological Sciences, University of Hong Kong, Hong Kong, China, [4]Bioinformatics Center, Nanjing Agriculture University, Nanjing, China	p. 130
102	The evidence of porcine respiratory and reproductive syndrome virus evolved under pressure from deaminases	C.L. Leng[1, 2], Y.D. Tang[1], J.G. Dong[1], Y.X. Hou1, D. Chang[1], X.H. Cai[1], J.H. Zhou[1], G.H. Zhang[2], Z.J. Tian[1]*, G.Z. Tong[2,3*], [1]Division of Swine Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, 150001, China, [2]College of Veterinary Medicine, South China Agricultural University, Guangzhou, 510642, China, [3]Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai, 200241, China	p. 131
103	Genomic sequencing and genetic variation analysis of highly pathogenic porcine reproductive and respiratory syndrome virus	C. Liu[1,2*], C. Ying[1,3], W.Z. Gong[1], Y.B. Ning[1], [1]China Institute of Veterinary Drug Control, Beijing, China, [2]China Agricultural University, Beijing, China, [3]Huazhong Agricultural University, Wuhan, China	p. 132
104	Recombination events are common in genotype 1 and 2 PRRS virus worldwide and may generate mosaic viruses	G.E. Martin-Valls[1*], A.J. Burgara-Estrella[2], L. Darwich[1,3], M. Tello3, J. Hernández[2], E. Mateu[1,3], [1]Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain [2]Centro de Investigación en Alimentación y Desarrollo (CIAD AC), Hermosillo (Sonora), Mexico [3]Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA, campus UAB, 08193 Bellaterra, Spain	p. 133

105	Evolution dynamics of highly pathogenic type 2 PRRSV: analysis of envelope protein coding genes	V.G. Nguyen[1,2], H.K. Kim[3], H.J. Moon[4], S.J. Park[5], H.C. Chung[1], M.K. Choi[1], B.K. Park[1*], [1]Department of Veterinary Medicine Virology Laboratory, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul, 151-742, Korea, [2]Department of Veterinary Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Hanoi University of Agriculture, Hanoi, Vietnam, [3]Research Evaluation Team, Institute for Basic Science, Daejeon, Korea, [4]Research Unit, Green Cross Veterinary Products, Yongin, Korea, [5]Viral Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea	p. 134
106	Phylogentic analysis and molecular characteristics of five variant Chinese field isolates of PRRSV	Z.F. Pei, R.A. Chen, D.S. He, X.H. Zhang, H.P. Liu, Guangdong Dahuanong Animal Health Products Co., Ltd, Xinxing, China	p. 135
107	Phylogenetic analysis for PRRS evolution in Romania	V. Petrovan*, M. Zaulet, L. Buburuzan, University of Bucharest, Department of Biochemistry and Molecular Biology, 91-95 Splaiul Independentei, 5th district Bucharest, Romania	p. 136
108	A cell culture-derived deletion in the nsp2 region of a North American PRRSV overlaps with a unique nsp2 deletion reported in a Chinese PRRSV isolate	R. Ransburgh*, Z. Sun, Y. Li, Y. Fang, Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD	p. 137
109	A novel strain of PCV2 isolated from vaccinated pigs suffering PCVAD causes 40% mortality in CDCD pigs in the presence of PPV	A.W. Suddith[*1, 2] and Richard Hesse[1, 2], [1]Kansas State Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, KS, [2]Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS	p. 138
110	A monoclonal antibody against the N of a HP-PRRS vaccine strain(HuN4-F112) could not recognize its virulent strain(HuN4-F5) not due to their amino acid sequence difference	Q. Wang[1,2], T.Q. An[1], J.M. Peng[1], J.Z. Chen[1], C.L. Leng[1], Y. Sun[1], Z.J. Tian[1*], H.C. Yang[2], [1]Division of Swine Infectious Diseases, National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, China, [2]Key Laboratory of Zoonosis of Ministry of Agriculture, College of Veterinary Medicine and State Key Laboratory of Agrobiotechnology, China Agricultural University, Beijing, China	p. 139
111	Molecular basis associated with the pathogenicity of a genotype 1 PRRSV isolate	X.C. Wang*, R. Zhou, X.R. Yang, S.C. Yao, Y. Liu, L. Zhou, X.N. Ge, X. Guo, H.C. Yang, Key Laboratory of Animal Epidemiology and Zoonosis of Ministry of Agriculture, College of Veterinary Medicine, China Agricultural University, Beijing, P.R. China	p. 140

119	Antigenic epitope mapping using the monoclonal antibody against the recombinant Rep' protein of porcine circovirus type 2	D. Liu[1,2], J.B. Liu[1], H.L. Wu[1], Y.P. Wang[1], Y.W. Wei[1], Q.H.Tang[1], S.B. Li[1], P. Wei[2*] and C.M. Liu[1*], [1]Division of Swine Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, [2]College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, China	p. 148
120	Characterization of two chimeric viruses by exchanging ORF2 gene of porcine circovirus type 1 (PCV1) and type 2 (PCV2)	C.M. Liu*, Y.H. Lu, D. Liu, Division of Swine Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, China	p. 149
121	Natural recombinant mutants, resulting from replacement of an open reading frame 2 (ORF2)-containing genomic fragment of porcine circovirus (PCV) genotype 2b with that of genotype 2a, showed significantly enhanced viral replication and altered antigenicity in vitro	L.J. Guo, Y.H. Lu, Y.W. Wei, L.P. Huang, H.L. Wu, C.M. Liu*, Division of Swine Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences, Harbin 150001, China	p. 150
122	Discovery and characterization of the involvement of a cellular membrane fusion protein in PRRSV replication	J.A. Hicks[1], D. Yoo[2], H.C. Liu[1*], [1]Department of Animal Science, North Carolina State University, Raleigh, NC. [2]Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL	p. 151
123	Amino acid mutations in the capsid protein produce novel porcine circovirus type 2 neutralizing epitopes	J.B. Liu*, L.P. Huang, Y.W. Wei, Q.H. Tang, D. Liu, Y.P. Wang, S.B. Li, L.J. Guo, H.L. Wu, C.M. Liu*, Division of Swine Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, China	p. 152
124	RNA interference targeting nucleocapsid protein (N) inhibits porcine reproductive and respiratory syndrome virus replication in Marc-145 cells	M. Yang*, X.D. Zhang, X. Li, S. Sylla, Z. Ding, College of Veterinary Medicine, and Key Laboratory of Zoonosis Research, Ministry of Education, Institute of Zoonosis, Jilin University, Changchun China	p. 153
125	Functional analysis of ORF4 protein encoded by porcine circovirus type 2	N. Zhou*, J.L. He, H.J. Wang, C. Lin, Y.L Jin, J.Y. Zhou, Key Laboratory of Animal Virology of Ministry of Agriculture, College of Animal Sciences, Zhejiang University	p. 154
126	ORF4 protein: a newly discovered virus protein in PCV2 replication	J.L.He, N. Zhou*, J.J. Cao, Y.L. Jin, J.S. Wu, J.Y.Zhou**, Key Laboratory of Animal Virology of Ministry of Agriculture, College of Animal Sciences, Zhejiang University	p. 155
127	Co-infection study between porcine reproductive and respiratory syndrome virus and a bacteria led to the discovery of a possible new bacteria antiviral.	C. Provost*, Y. Hernandez Reyes, C. Lévesque, J. Labrie, M. Jacques, C.A. Gagnon, Groupe de recherche sur les maladies infectieuses du porc (GREMIP), Centre de recherche en infectiologie porcine (CRIP), Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada	p. 156

128	Impact of co-infection by porcine circovirus (PCV) and porcine reproductive and respiratory syndrome virus (PRRSV) in porcine pulmonary epithelial cell line	C. Provost*, F. Alvarez, N. Music, M. Jacques, C.A. Gagnon, Groupe de recherche sur les maladies infectieuses du porc (GREMIP), Centre de recherche en infectiologie porcine (CRIP), Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada	p. 157
129	The correlation of cyclin A expression level with porcine circovirus type 2 propagation efficiency	Q.H. Tang*, S.B. Li, H. Zhang, Y.W. Wei, L.P. Huang, H.L. Wu, J.B. Liu, Y.P. Wang, D. Liu, Z.H. Zhang, C.M. Liu, Division of Swine Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, The Chinese Academy of Agricultural Sciences, Harbin 150001, China	p. 158
130	Dissection for the structure and function of a 5'-proximal prominent stem-loop in Porcine Reproductive and Respiratory Syndrome virus	F. Gao*, J.Q. Lu, Z.Z. Wei, S.S. Yuan, Y.J. Zhou, Y.F. Jiang, G.Z. Tong*, Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences	p. 159
131	Signal peptide cleavage from GP5 of PRRSV: Only a minor subfraction of molecules retains the decoy epitope, a presumed molecular cause for viral persistence	B. Thaa[1], B.C. Sinhadri[1], C. Tielesch[1], E. Krause2, M. Veit[1*], [1]Institute of Virology, Faculty of Veterinary Medicine, Free University Berlin, Germany, [2]Mass Spectrometry Unit, Leibniz Institute of Molecular Pharmacology (FMP), Berlin	p. 160
132	Non-structural protein 2 of the porcine reproductive and respiratory syndrome (PRRS) virus: a crucial protein in viral pathogenesis, immunity and diagnosis	F.X. Wang[1], N. Song[1], L.Z. Chen[1], S.P. Cheng[1], H. Wu[2*], Y.J. Wen[1**], [1]State Key Laboratory for Molecular Biology of Special Economic Animals, Institute of Special Economic Animal and Plant Sciences, Chinese Academy of Agricultural Sciences, No. 4899 Juye Street, Changchun Jilin 130112, P. R. China, [2]Sinovet (Beijing) Biotechnology Co., Ltd., Beijing, P. R. China	p. 161
133	Role of non-structural protein 2 in the regulation of the replication of the porcine reproductive and respiratory syndrome virus in MARC-145 cells: effect of gene silencing and over expression	F.X. Wang, Y.J. Wen, B.C. Yang, Z. Liu, X.C. Shi, X. Leng, N. Song, H. Wu*, L.Z. Chen, S.P. Cheng, State Key Laboratory for Molecular Biology of Special Economic Animals, Institute of Special Economic Animal and Plant Sciences, Chinese Academy of Agricultural Sciences, No 4899 Juye Street, Changchun Jilin 130112, China	p. 162
134	The effect of the position of N-linked glycosylation sites within hypervariable region of GP5 on virus neutralization.	Z.Z. Wei*, F. Gao, Y.F. Jiang, Y.J. Zhou, S.S. Yuan, G.Z. Tong*, Department of Swine Infectious Diseases, Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai 200241, China	p. 163
135	The role of antibody in PRRSV infection PAM	Q.Y. Yang, Y.J. Qin, Y.N. Zhang, N. Li, D.M. Wang, D.Y. Du, P.A. Xia*, M.F. Yang, B.A. Cui, The College of Animal Husbandry and Veterinary, Henan Agricultural University, Zhengzhou 450002, China	p. 164
136	Regulation of host cell gene expression and cell cycle progression by nsp11 of PRRS virus	Y. Sun[1], D. Li[1], S. Giri[2], S. G. Prasanth[2], D. Yoo[1*], [1]Department of Pathobiology,[2]Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA	p. 165

137	Concurrent highly pathogenic porcine reproductive and respiratory syndrome virus infection accelerates Haemophilus parasuis infection in conventional pigs	J. Yu[1,2], J.Q. Wu[2,3], Y.Y. Zhang[2,3], L.H. Guo[2,3], X.Y. Cong[2,3], Y.J. Du[2,3], J. Li[2,3], W.B. Sun[2,3], J.L. Shi[2,3], J. Peng[2,3], F.F. Yin[2,3], D.P. Wang[2,3], P.W. Zhao[2,3], J.B. Wanga[2,3*], [1]Shandong Normal University, Jinan, People's Republic of China, 250014, [2]Shandong Academy of Agricultural Sciences, Jinan, People's Republic of China, 250100, [3]Shandong Key Lab of Animal Disease Control and Breeding, Jinan, People's Republic of China, 250100	p. 166
138	Mutagenesis analysis of porcine reproductive and respiratory syndrome virus nonstructural protein 7	M.Z. Zhang†, Z. Huang†, J.X. Xie†, W.J. Zhu, L. Sun, S. Su and G.H. Zhang*, MOA Key Laboratory of Animal Vaccine Development, Ministry of China, College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, China	p. 167

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140	The application of deep sequencing for the analysis of mutations within hypervariable regions of PRRSV	N. Chen[1*], S.L. Carpenter[2], R.R.R. Rowland[1], [1]Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, [2]Department of Animal Science, College of Agriculture and Life Sciences, Iowa State University, Ames, IA, United States	p. 169
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1
EUROPRRS: Still paving the way?

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Back in 2007, debates within the European PRRS community prompted the need for the development of an effective entity to understand and control PRRS at European level. Since 1992, PRRS research in Europe has been fragmented and operated via national research programs. Such programs lacked wider vision and were inefficient due to the international nature of the disease. Furthermore, a gap existed between national teams developing strategies to combat PRRS disease solely based on vaccine development and those that exploited host genetics. With the creation of the EuroPRRS network, 2009 has to be seen as a landmark year for the European PRRS community. With a core group of 5 countries in its initial stage, EuroPRRS was joined by 22 European countries. The network has enabled the development of collaborative links between national PRRS research groups with their different areas of expertise and improved cross-discipline communication. An example of such interaction is the creation of the FP7 European PoRRScon project in 2010. Including regular workshops in control and diagnostics and the organisation of an annual conference, EuroPRRS has actively promoted training schools in PRRSV diagnostics and control and genomics. Most of these activities have been greatly enhanced from the constant support of first class PRRS specialists from China and the USA, as well as from the industry. Finally, multiple national PRRS research projects have significantly profited from an active short term scientific missions exchange programme for young scientists.

Four years on the EuroPRRS network has clearly contributed to strengthen Europe's PRRS capacity, thus paving the way towards a greater understanding of the PRRS disease.

2
Fostera™ PRRS: Modulation of interferon-alpha and induction of protective immunity two weeks following vaccination

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a highly infectious RNA virus which causes abortions and stillborn/mummified fetuses in sows, and respiratory distress with poor growth in young pigs. PRRSV remains one of the most economically important diseases in the global swine industry. Fostera™ PRRS received a USDA product license in February 2012, and is the only PRRSV vaccine to earn the label claim "… as an aid in preventing disease associated with PRRSV, Respiratory Form". A study was designed to evaluate the protective effect of vaccinating three week old pigs with Fostera PRRS, when challenging with virulent PRRSV strain NADC20 two weeks post-vaccination. The primary variable in determining prevention of disease was mean percent lung with lesions at 10 days post-challenge. Clinical observations and body weight were analyzed as supportive data. Cytokine data were also collected as an indicator of the immune response. The Fostera PRRS vaccinated group had a significantly lower ($P = 0.0177$) mean percent of lung involvement than the mock-vaccinated control group. In addition, significant differences ($P = 0.0001$) were seen in body weight at the day of necropsy (day 24) when comparing the Fostera PRRS vaccinated group to controls. Abnormal clinical signs were also reduced in the vaccinated group compared to the mock-vaccinated control group, though statistical comparisons were not made. In addition, interferon-alpha levels were significantly elevated ($P = 0.0001$) in the Fostera PRRS vaccinated group compared to mock-vaccinated controls just before challenge (day 13) and the vaccinated group avoided a high spike (>1000 pg/mL) in interferon-alpha levels that correlated with clinical disease following challenge with the virulent NADC20 strain ($P < 0.0001$). The data demonstrate that Fostera PRRS vaccination of three week old pigs helps provide a protective effect against challenge 14 days later. This protective effect is likely due to a combination of acquired and innate immunity. All experiments involving animals were carried out in compliance with national legislation and subject to review by the local Institutional Animal Care and Use Committee (IACUC).

3
Porcine Reproductive and Respiratory Syndrome (PRRSV) diagnostics in the United States: Past, present and future

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A historical, present day and futuristic viewpoint in the development and use of reagents and diagnostic tests for PRRSV will be presented. Currently, PRRSV elimination and control programs are being implemented in the US. Even though PRRSV is not eliminated in the US, it should be noted that the causative agent of PRRSV was first identified only about 20 years ago. By comparison, in the US, pseudorabies (PRV) was discovered in swine in 1909 with eradication programs starting in 1989, with much of the eradication completed by 2005; classical swine fever (CSF/Hog cholera) was first reported in 1810 with much of the eradication completed between 1962 and 1976, and *Brucellosis* in swine was identified in 1914 and is nearly eradicated at this time. In these cases, between 100-200 years passed before elimination or near elimination was completed after identification of these swine pathogens.

For PRRSV control and elimination programs, convenient, economically viable and welfare conscious surveillance methods are now available to obtain and test samples. These include pooling of samples and obtaining oral fluids or blood swabs from swine. Virus isolation (VI) from serum was originally used for identification of the virus, but a disadvantage of this method included delayed diagnosis compared to detection with the polymerase chain reaction (PCR) which can now be completed within a few hours after specimen submission. However, PRRSV specific monoclonal antibodies have been available and shorten the virus isolation procedure and offer specific confirmatory results. Automated nucleic acid extractions and commercial tests for real-time PCR are also available which allow for rapid, sensitive and specific diagnosis whereby results are reported to the veterinarian the same day the specimen is submitted. These improvements simplify the procedures and allow for better quality control, repeatability and high throughput. In addition, DNA sequencing of open reading frame (ORF) 5 is routinely performed in the diagnostic laboratory for epidemiologic purposes. For detecting antibodies to PRRSV, high throughput enzyme linked immunosorbent assays (ELISA) are available and have replaced older methods of indirect immunofluorescent assays (IFA) to detect PRRSV exposure. Antibody ELISAs are higher throughput, more specific and less subjective than IFAs which are also subject to false negative results due to antigenic variation. Currently, Luminex multianalyte profiling (xMAP®) assays are being developed to detect multiple antigens (PRRSV, swine influenza (SIV), porcine circovirus (PCV2), etc.) or antibodies simultaneously within a single sample. This methodology should be helpful for rapid, routine surveillance and for evaluating immune responses to vaccine constructs.

4
Vaccine vectors based on coronavirus genomes to protect against PRRSV and SARS-CoV

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Eliciting long-term protection against virus-induced diseases requires the development of attenuated viruses and efficient vectors. In some cases, this is possible because the live modified virus does not have dominant components that interfere with the induction of a protective immune response, as we will describe for the severe and acute syndrome (SARS) virus (SARS-CoV). In contrast, in other cases, such as for the porcine respiratory and reproductive syndrome virus (PRRSV), this is a less clear possibility as a consequence of the presence of many viral proteins that inhibit the onset of a protective immune response. In these cases, protection could be induced by the selective expression of the viral antigens using a vector that, ideally, should be specific for the animal species that we try to protect. Progress on coronavirus replication, transcription, virus host-interaction, and on coronavirus reverse genetics to design vaccine vectors has been made to control viruses relevant in human and animal health. Our laboratory has made significant progress on: (i) the development of reverse genetics systems to engineer coronavirus genomes as vaccine vectors; (ii) the control of coronavirus replication and transcription to increase protective antigen expression levels; and (iii) the identification of genes involved in the virulence of transmissible gastroenteritis virus (TGEV) and SARS-CoV to generate modified live attenuated viruses. Coronavirus transcription is guided mainly by the free energy released in the base pairing between an RNA sequence motif located in the viral genome, and a complementary sequence synthesized during transcription. In addition, our group has identified a transcription enhancer that is very useful to increase expression levels of protective antigens.

Studies on TGEV and SARS-CoV virulence led to the identification of genes responsible for virus attenuation. The mechanism involved in these processes showed that defined viral proteins regulated inflammation induction by TGEV and SARS-CoV. Using information from these basic studies we have engineered the first recombinant vaccine that protects against SARS-CoV. Also, significant progress has been made in the development of a vaccine for PRRSV, although in this case additional work is still required to overcome the partial stability of the heterologous genes in the vaccine vector. To generate attenuated SARS-CoVs lacking E gene (SARS-CoV-ΔE) adapted to grow in mice were constructed. SARS-CoV E protein down-regulated the stress and unfolded protein responses. Infection of mice with a mouse adapted SARS-CoV reproduced many aspects of the human disease. The expression of proinflammatory cytokines was clearly reduced in the lungs of mice infected with SARS-CoV-ΔE compared to SARS-CoV-infected mice. Furthermore, a reduction of lung inflammation was observed in mice infected with mouse-adapted SARS-CoV-ΔE, probably contributing to the lower degree of inflammation. This deletion mutant provided protection against the challenge with homologous and heterologous pathogenic SARS-CoV strains in both young and old Balb/c mice, indicating that SARS-CoV-ΔE is a very promising vaccine candidate.

5
The PRRSV replicase: structure, function and implications

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Our knowledge about the structure and function of the nonstructural proteins (nsps) encoded by the arterivirus replicase gene has advanced significantly in recent years. The continued characterization of arterivirus nsps has not only corroborated several important functional predictions, but also revealed various novel features of arteriviral replication. For porcine reproductive and respiratory syndrome virus (PRRSV), based on bioinformatics predictions and experimental studies, a processing map for the pp1a and pp1ab replicase polyproteins has been developed. In addition, a novel ORF (TF) overlapping the central region of ORF1a was discovered, which expresses a previously undiscovered protein, nsp2TF, through an unprecedented -2 programmed ribosomal frameshift mechanism. Crystal structures have been resolved for three of the PRRSV nonstructural proteins that possess proteinase activity (nsp1α, nsp1β and nsp4), and the structure of the highly conserved fourth protease (PLP2 in nsp2) was recently solved for equine arteritis virus. The functional characterization of key enzymes for arterivirus RNA synthesis, the nsp9 RNA polymerase and nsp10 helicase, has been initiated. In addition, progress has been made on nsp functions relating to the regulation of subgenomic mRNAs synthesis (nsp1), the formation of replication-associated membrane rearrangements (nsp2 and nsp3), and an intriguing replicative endoribonuclease (nsp11) for which the natural substrate remains to be identified. In addition to their natural role as "the engine of viral RNA synthesis", a steadily increasing number of nsp-host interactions are being uncovered, with clear implications for studies into the host immune response and viral pathogenesis. Specifically, nsp1α/β, nsp2, nsp4 and nsp7 have been investigated for their role in the modulating immune responses to PRRSV infection. Using PRRSV reverse genetic systems and our advanced knowledge on the structure and function of specific nsps, the rational design of genetically engineered PRRS vaccines is now being explored. Furthermore, several PRRSV nsps have been tested as potential new antigens for the development of diagnostic assays. In parallel with PRRS marker vaccine development, it has also been explored whether differential diagnostic assays can differentiate vaccinated from naturally infected animals. These studies represent our continued effort to apply basic knowledge of the biological function of PRRSV nsps to develop a new generation of PRRS vaccines and diagnostic assays.

6
Principles of controlling PCV2 – The importance of controlling viremia

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Porcine Circovirus type 2 (PCV-2) has been associated with different disease syndromes including Postweaning Multisystemic Wasting Syndrome (PMWS), Porcine Dermatitis and Nephropathy Syndrome (PDNS), Porcine Respiratory Disease Complex (PRDC) and certain reproductive disorders since it was first isolated in wasting pigs from Canada in 1997. Many farms globally were found positive for PCV-2, but its impact has been very varied. It was later understood that such variance depended on the presence (or absence) of many other co-factors that influence the course of PCV-2 infection including immune suppression, immune stimulation and the presence of other infectious agents including Porcine Parvo Virus (PPV), Porcine Reproductive and Respiratory Syndrome (PRRS) and *Mycoplasma hyopneumoniae*.

Before the launch of PCV-2 vaccines, the control of impact of PCV-2 was through improvements in management and reducing the impact of co-infections. Madec's principles became widely known and applied. These principles when applied concurrently with effective control programs for Mycoplasma (vaccination and medication) and PRRS (vaccination and management – replacement breeder acclimatization and herd closure) yielded good results but not to the performance levels prior to the clinical outbreak of PCV-2.

Vaccines became available starting year 2006. Response to vaccination has been very good leading to significant improvements in mortality and growth rates. Now, several years later, PMWS is rarely seen in farms that vaccinate against PCV-2. However, there are still cases of PRDC later in the grower phase and occasional cases of PDNS where PCV-2 has been implicated even though those farms continuously use PCV-2 vaccines.

Controlled comparative studies among piglet vaccines often yielded similar productive performance results. A more consistent difference among those tested was in their ability to control PCV-2 viremia. In one of the controlled comparative studies conducted recently, significant difference in both viremia and nasal shedding among the different piglet PCV vaccine groups was observed. The importance of viremia or the level of PCV-2 virus in the blood can be directly correlated to the amount of PCV-2 virus shed through oral and nasal secretions and feces. The virus shed though these routes can potentially infect immune suppressed or non-immune pigs in the same pen.

Depending on farm circumstances, differences in terms of the ability of vaccines to control viremia may not always be correlated to improvements in productive performance Vaccines that control viremia better, however, will also cause much less viral shedding in the environment and such can lead to better control of PCV-2 circulation in the herd in the long term.

7
Reflection of sow serology in piglet litters oral fluids – a sow farm surveillance field study

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PRRSV surveillance can be done easily, effectively, and inexpensively using pre-weaning oral fluid samples from litters of piglets. Hypothetically, the anti-PRRSV antibodies and their concentrations in oral fluid samples from litters of pigs reflect the PRRSV exposure of both the litter and their dam. That is, IgG in oral fluids from the pigs represents maternal antibody obtained from colostrum, whereas either IgM or IgA reflect PRRSV infection in the pigs. Similarly, PRRSV viremia detected from oral fluid of the piglets reflects the status of PRRSV circulation in the sow herd. The objective of this proposed research was to provide producers an easier, cheaper, and more effective method to detect and monitor PRRSV circulation.

This study was performed in four ~12,500 sow breeding herds. All four herds were considered endemically infected with PRRSV on the basis of diagnostic history from routine growing pig rRT-PCR and PRRS antibody ELISA monitoring. Commercial PRRSV vaccines were used intermittently to control clinical losses over the 12 months prior to sampling, with some differences in the vaccination protocols among sites. Gilts entering the sow farms were routinely vaccinated with either PRRS MLV or ATP commercial vaccines. Oral fluid samples were collected from a total of 600 litters of pigs prior to weaning. Serum samples from their dams were collected 3 days after weaning. Samples were frozen at -80°C, randomized and submitted collectively for PRRSV (qRT-PCR and sequencing) and PRRSV antibodies (IgM, IgA, and IgG). Results were analyzed for associations with sow parity, litter size, and farm by analysis of variance (ANOVA) and correlation analyses.

Eight of 600 piglet oral fluid samples were confirmed positive by rRT-PCR at two laboratories, representing 3 of the 4 sow farms. Two of these were sequenced (Orf 5) and reported as wild type PRRSV. No sow serum samples were positive for PRRSV by rRT-PCR. Of the 8 litter oral fluids, IgM and IgA antibody S/P ratios were <0.39. From those same samples, IgG oral fluid antibody S/P ratios ranged from 1.26 to 5.97. Likewise, S/P ratios using the IDEXX PRRS Oral Fluids antibody ELISA kit ranged from 1.92 to 8.83. Respective dam sera results for IgM and IgA of those 8 litters ranged from 0.0 to 1.54, and IgG, 0.85 to 2.36. Similarly, sow sera S/P ratio results from the IDEXX X3 Ab test ranged from 0.92 to 2.91.

Isotype results from both sera and oral fluids sample types showed both the PRRS X3 Ab Test and PRRS Oral Fluids Ab Test corresponded well with the IgG isotype. Mean IgM or IgA S/P ratios for all farms (600 samples each) was <0.25 (sera) and ≤0.05 (oral fluids) respectively. Over all farms and samples, mean sera ELISA S/P ratios were approximately 1.00, while corresponding mean litter oral fluid ELISA S/P ratios were approximately 4.62.

Herd PRRSV monitoring programs can be improved by evaluating the dynamics of PRRSV transmission using oral fluid samples collected from litters of pigs prior to weaning. In endemically infected and/or vaccinated herds, piglet oral fluids are a useful tool for surveillance of both PRRSV exposure and shedding in sow herds.

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Genetics of host resistance to PRRSV infection: Progress of the PRRS Host Genetics Consortium

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The most important infectious disease threatening pig production worldwide is porcine reproductive and respiratory syndrome (PRRS). The PRRS Host Genetics Consortium (PHGC) was established to probe the role of host genetics in resistance to PRRSV infection and related growth effects. Using a nursery pig model, 14 groups of 200 commercial crossbred pigs were infected with PRRS virus and followed for 42 days post infection (dpi). Blood serum and Tempus (RNA) samples were collected at 9 timepoints and weekly weights recorded. All pigs were viremic, peaking at 4-14 dpi. Genomic DNA was genotyped with the Porcine SNP60 SNPchip for genome wide association studies (GWAS) that identified a genomic region on swine chromosome 4 (SSC4) with significant impact on viral load and growth response following viral challenge. Using Bayes-B analyses for 5 trials, 8 1-Mb regions accounted for 35.7% of the genetic variance for viral load from 0-21 dpi with the SSC4 region explaining 17.7% of the genetic variance. For weight gain, 10 1-Mb regions accounted for 31.2% of the genetic variance, with SSC4 accounting for 14.3%. Sera and RNA are now being analyzed using the Pigoligoarray, next-generation sequencing (RNA-seq), QPCR and multiplex immunoassays to elucidate factors involved in viral replication and recovery from infection, including speed and levels of immune cytokine expression. For RNA transcriptomic analyses, we developed a porcine specific globin reduction protocol so that we could perform RNAseq on blood RNA samples collected at numerous dpi from PHGC pigs with different SSC4 genotypes and post-peak anti-viral responses. Serum cytokine levels were evaluated using FMIA (Fluorescent Microsphere Immunoassay) and affirmed statistical differences due to dpi and specific associations with early innate cytokines, interleukin-8 (IL-8) and interferon–alpha (IFNa), as well as with the chemokine CCL2. Combined with the genetic data, these analyses should help to unravel the complexities of pig responses to PRRSV infections and identify biomarkers that further distinguish PRRS resistant/maximal growth pigs from PRRS susceptible/reduced growth pigs. PHGC funding: US National Pork Board, USDA ARS and NIFA, NRSP8 Swine Genome and Bioinformatics Coordinators, Genome Alberta/ALMA, Genome Canada, and private companies.

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An update on PRRS immunology

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Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most important causes of disease in pig operations worldwide. The control of the infection has proven to be difficult among other reasons because of the lack of fully efficacious and universal vaccines. In fact, the features of the immune response against PRRSV reveal a complex interaction in which the virus interfere with the immune system and use escape mechanisms. Early studies on the adaptive immune response against PRRSV evidenced that infected animals rapidly (7-14 days) developed antibodies against the virus but those antibodies where devoid of neutralizing capabilities. Actually, neutralizing antibodies appear much later and some animals do not even develop a neutralizing response. Similarly, the cell-mediated immune response -measured as the development of virus-specific interferon-gamma producing cells- develops slowly and suffers ups and downs for several weeks until acquiring a steady state. Moreover, the development of neutralizing antibodies or of a cell-mediated response against a given strain does not grant complete immunity, particularly when the animal faces a heterologous challenge. Several papers have shown that is very difficult to correlate quantitatively the humoral or cell-mediated responses with protection and thus the exact role of these two compartments of the specific immunity is still unclear when talking about PRRSV. Why the adaptive response against PRRSV is so uncommon. There is no a single answer to this question although several elements can be cited. Firstly, the ability of the virus to inhibit or suppress important elements of the innate immune response. Current knowledge indicates that PRRSV interferes with type I interferon (IFN) responses of macrophages and dendritic cells. This inhibition is mainly attributable to several non-structural proteins (nsp) of the virus, particularly nsp1, nsp2, nsp4 and nsp11. However, the virus may inhibit the IFN-α responses of plasmacytoid dendritic cells (the specialized antiviral dendritic cells) without replicating in them indicating again that PRRSV probably has several mechanisms for inhibiting type I IFN. Besides this, the virus may also regulate tumor necrosis factor-alpha (TNF-α) and Interleukin-10 (IL-10) responses, two crucial cytokines with different effects. While TNF-α is essentially pro-inflammatory and antiviral, IL-10 is anti-inflammatory and an excess of the cytokine may help to the persistence of the infection. Several reports indicated that IL-10 maybe involved in T-cell regulatory responses against genotype 2 PRRSV. The high virulence of some isolates has been related either to higher inflammatory responses or to higher viral replication, indicating a role of the immune system in both cases. In summary, there is still a need for more research and a better understanding of the immune response against PRRSV.

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Breeding for PRRS resistance

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There is a distinction to be made between resilience or tolerance to a disease, and resistance to that disease. Tolerance refers to a reduced impact of an infection on the host. Resistance, in contrast, refers to the host's ability to control the infection and reduce its transmission, thereby reducing the prevalence of the disease.

There is considerable evidence for genetic variation in pigs' response to various diseases, including PRRS. The pig breeding company PIC has selected indirectly for tolerance to disease since 2005, when it began performance testing progeny of genetic nucleus (GN) herd sires in PRRS virus positive commercial environments. Today, just over half the data used in livability trait genetic evaluations comes from testing pedigreed crossbred pigs in commercial farms, the other half coming from the GN farms.

With the advent of genomic selection, where information from tens of thousands of genetic markers throughout the genome is used to improve the accuracy of genetic evaluations, the opportunity exists to select for traits not directly measured in testing programs, such as resilience to the PRRS virus. Using challenge experiments, field data sets, and genome wide association studies (GWAS), PIC is currently developing and field testing genomic breeding values (gEBVs) for resilience / tolerance to the PRRS virus.

Genus-PIC also has an extensive collaborations with academic and industry partners in the US (e.g., the PRRS Host Genetic Consortium), Canada (PigGen Canada), and the UK (Roslin Institute). Projects range from studying host macrophage responses to PRRS virus infection, through methods to genetically improve resistance both in sows and growing pigs using natural and engineered variation, to developing mathematical models to describe the impacts of infection within the pig, and the transmission of infection between pigs.

Achieving genetic resistance to all strains of PRRS virus is, of course, the Holy Grail. Increased understanding of how the virus attaches to, is enveloped by, and replicates in porcine alveolar macrophages provides possible strain independent targets. Concurrently, genome editing technologies such as transcription activator-like effector nucleases (TALENs) capable of altering gene expression with precision are beginning to be applied in pigs. Research supported by Genus on modifying pig genes critical to all strains of the PRRS virus has the goal of one day not only being able to reduce the impact of the virus on pork producers, but enabling creation of genetic lines resistant to the virus.

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Control of PRRS on farm and regional level

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Porcine reproductive and respiratory syndrome virus (PRRSv) can have a significant economic impact on swine herds due to reproductive failure, preweaning mortality and reduced performance in growing pigs. Control at the farm level is pursued through different management procedures (e.g. pig flow, gilt acclimation, vaccination, air filtration). PRRSv is commonly eliminated from sow herds by a procedure called herd closure whereby the herd is closed to new introductions for a period of time during which resident virus dies out. However, despite thorough application of biosecurity procedures, many herds become re-infected from virus that is present in the area. Consequently, some producers and veterinarians are considering a voluntary regional program to involve all herds present within an area. The concept of voluntary, producer-led, coordinated regional PRRS control has spread and there are now approximately 20 such programs in North America. In pig-dense regions of United States, an increasing number of sow farms are modifying air intake such that all air is filtered. Reports suggest an 85% reduction in the rate of outbreaks of farms after filtration when compared to the 5 years prior to filtration. Analysis of rate of return on filtration suggests that this is an attractive investment option for sow farms situated in pig-dense regions. Taken together, cooperative control in low-medium dense regions, and air filtration in high dense regions, we are well positioned for effective PRRS control and eventual elimination.

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Global Research on PRRS – development of STAR-IDAZ PRRS network

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STAR-IDAZ (Global **St**rategic **A**lliances for the Coordination of **R**esearch on the Major **I**nfectious **D**iseases of **A**nimals and **Z**oonoses) is an EU funded global initiative to address the coordination of research programmes at international level in the area of animal health and in particular infectious animal diseases including zoonoses. It aims to strengthen the linkages between and reduce the duplication of global research effort on high priority infectious diseases of animals (including zoonoses), maximise the efficient use of expertise and resources and accelerate coordinated development of control methods. PRRS has been identified as one of the top priority diseases and proposals are being developed for the establishment of a PRRS research network involving both the research community and the research funders/programme owners. Improved coordination of, and international collaboration on PRRS research is needed to ensure the rapid development of effective control tools. We hope to explore the interest in such a network and the research gaps needing to be addressed.

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Immunological interactions of PRRSV and pigs

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Vaccination is the principal tool used to control and treat porcine reproductive and respiratory syndrome virus (PRRSV) infection. However, despite extensive efforts, little progress has been made to improve the protective efficacy PRRS vaccines since the first introduction of a live, attenuated product in 1994 in the USA. Here, we describe recent progress to characterize antigen-specific immune responses, especially those involving PRRSV and antibodies, since advances in vaccine development are likely to be based on a better understanding of fundamental immunological interactions, and since antibodies are expected to play a key memory role in protection against future infection. We have discovered that sows in herds suffering outbreaks of type 2 PRRSV have high titers of neutralizing antibodies. Notably, neutralization is cross-protective since sows were exposed to highly virulent field isolates, and viral neutralization was carried out with VR2332, which is genetically dissimilar to current virulent field isolates. We hypothesize that the cross-neutralizing antibodies are directed to highly conserved epitopes that are critical for the infection process. Detailed studies of porcine immunoglobulins, including genomic organization of light and heavy chain loci, patterns of gene rearrangements used to express the antibody repertoire, and the effect of PRRSV infection on antibody diversity were conducted. The total heavy chain repertoire diversity in pigs is comprised of approximately 260,000 independent sequences; lambda and kappa light chain repertoire diversities are approximately 260,000 and 150,000 independent sequences, respectively. The abundance distributions follow the power law in all three cases. Following PRRSV infection there are dramatic increases in abundance of a small subset of light and heavy chain fragments that is most apparent in the CDR3 regions which comprise the primary sites of antigen binding. We expect that screening of recombinant antibody expression libraries will result in identification of porcine immunoglobulin molecules with specific affinities for individual PRRSV proteins, including targets of neutralizing antibodies. Biological variation in the virus also is a key limitation to achievement of broadly cross-protective vaccines. Association of genetic variation in specific regions of the virus with variation in biological characteristics related to virulence, pathogenesis and immunity has been frustrating. Alternatively, it is possible that quasispecies variation in viral populations might contribute to biological variation independently of specific sequence differences in defined genetic loci. To address this question, massively parallel sequencing was performed on viral isolates of high and low virulence. With few exceptions, minimal genetic diversity was observed across the entire genome. Initial findings indicate that quasispecies complexity may not make a significant contribution to PRRSV fitness and virulence in swine.

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The pathogenesis of porcine reproductive and respiratory syndrome (PRRS) is fully determined by the intriguing interplay of PRRSV with monocytic cells

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Porcine reproductive and respiratory syndrome virus (PRRSV) is infecting monocytic cells, leading to lysis and functional disturbances. In-depth analysis of its replication in different monocytic cells leads to better insights in the virological, immunological, pathological and clinical outcome. Old-type PRRSV (LV and VR2332) targets differentiated macrophages that are spread all over the body but with higher concentrations in lungs (alveolar, interstitial and intravascular macrophages), endometrium/placenta and lymphoid tissues. This tropism is the result of several viral-cellular protein interactions. Sialoadhesin acts as a binding and internalization receptor. It interacts with sialic acids, present on the glycans of GP5. Upon entry, PRRSV becomes disassembled via a complex cascade of events. First, viral-containing endosomes fuse with other endosomes and proteases become activated by a pH drop. CD163 is crucial during this stage of the replication cycle. Primary replication occurs in tonsils and deeper parts of the respiratory tract. Afterwards, it replicates in internal lymphoid tissues. Because cells die by apoptosis, inflammation is restricted and pathology and clinical signs are moderate (American type>European type). Monocytic cells play a central role in the innate immune response and their destruction during a PRRSV infection may lead to catastrophic clinical signs upon co-infections with pathogens or exposure to toxins. In addition, interaction of PRRSV with sialoadhesin has recently been shown to block phagocytosis. PRRSV only crosses the placenta after 70 days of gestation. The reason for the absence of transplacental spread during earlier gestation can be attributed to (1) the absence of sialoadhesin on placental macrophages in the allantochorion and (2) the presence of efficient defense against PRRSV-infected cells. Microchimerism may be involved in the transplacental crossing of PRRSV during late gestation. Emerging Eastern European (Lena) and Asian (high fever disease) PRRSV strains are behaving differently. They replicate to higher titers, spread more easily, cause more pathology (vascular damage) and disease (fever during 10-14 days upon single infection; fever during 21-28 days with high mortality upon co-infection). With the Lena strain, new insights in the cellular pathogenesis were already obtained. PRRSV(Lena) replicates in hundred times more cells in the respiratory tract. In contrast with old-type PRRSV, it has gained access to a population of large sialoadhesin-negative epithelial and subepithelial monocytic cells with long and multiple cellular filopodia. Morphologically, they resemble mucosal dendritic cells. This new target explains a lot of the changed pathogenesis. High virus titers are produced in the nasal mucosa, facilitating horizontal transmission. A network of connected dendritic cells is as a fisher's net filtering out attacking pathogens. Destruction of these cells by PRRSV(Lena) opens the fence for invading pathogens. The latter may explain why sepsis is regularly observed in PRRSV(Lena) infected animals.

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Luminex® application for PCV2 and PRRSV diagnosis

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Porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) are among the most globally and economically important swine pathogens today. PRRSV can be divided into two major genotypes: type 1 (European strains) and type 2 (North American strains). PRRSV is typically associated with reproductive failure (sick dams, abortions, stillborn and weakborn fetuses) in the breeding herd and respiratory disease in growing pigs. PCV2 can be further divided into three genotypes, PCV2a, PCV2b, and PCV2c. While PCV2a and PCV2b occur worldwide, the presence of PCV2c is limited to certain areas such as Denmark. PCV2 is commonly associated with porcine circovirus associated disease (PCVAD), a combination of several disease manifestations such as postweaning multisystemic wasting syndrome, respiratory disease, enteric disease and reproductive failure. Several very efficacious vaccines are available for PCV2; however, PRRSV control remains a challenge and eradication is one way to attempt to control this virus. Diagnosis of PRRSV or PCV2 infection is typically done on serum samples (individual samples or pools of up to 5 samples) from live pigs or lung and lymphoid tissues from dead pigs. Within the last 1-2 years, oral fluids are becoming the sample type of choice for PRRSV surveillance and particularly for monitoring expected-negative flows. While much effort has focused on demonstration of RNA or DNA in tissues and fluids, detection of antibodies remains an inexpensive alternative to screen herds for presence and movement of PRRSV and PCV2. Anti-PRRSV antibodies are frequently detected by commercial assays and especially the IDEXX PRRSV ELISA is widely used in Veterinary Diagnostic Laboratories (VDLs) in the USA. Although some commercial serology assays are available for PCV2 antibody detection, most VDLs utilize in-house assays. In recent years, multiplex platforms which allow detection of more than one analyte in the same sample are gaining importance in human medicine and now also in veterinary medicine. Recently, a fluorescent microbead-based immunoassay (FMIA) using the N protein and the nonstructural protein 7 as capture antigens for detection of PRRSV antibodies was developed and evaluated by a research group in South Dakota. In general, the FMIA technology is based on xMAP (multi-analyte profiling; Luminex®) which combines flow cytometry, fluorescent microspheres (beads), lasers, and digital signal processing. Therefore FMIA is capable of simultaneously measuring up to 100 different analytes in a single sample. Some additional benefits of this technology include rapid data acquisition and excellent sensitivity and specificity. Our group has been utilizing the FMIA platform to analyze and characterize a variety of samples from pigs with different disease presentations for detection of antibodies against PCV2 and PRRSV using several markers. In general, we have found that FMIA allows simultaneous detection of PRRSV type 1 and type 2 and PCV2 antibodies (including several important markers such as baculovirus for vaccine compliance and PCV1) in a serum samples but also in oral fluids. Indications are that this platform will provide a very promising tool for characterization of outbreaks or evaluation of vaccination protocols. Data to support our observations will be presented.

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The role of host genetics in vaccine development

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PRRSV employs an array of host evasion strategies that subvert innate and adaptive immune responses. The virus can evade antibody by generating a high degree of genetic diversity within surface proteins. In addition, many of the virion surface proteins are heavily glycosylated: a camouflage mechanism that shields neutralizing epitopes from antibody. And finally, PRRSV blocks early innate immune events, such as the induction of interferon. These and other evasion strategies contribute to disease progression and hamper the development of the next generation of PRRS vaccines, which must possess the capacity to induce a strong and early viral immune response that is protective against a wide range of field isolates. The PRRS Host Genetics Consortium (PHGC) was formed to identify genomic markers and pathways that relate to PRRS resistance and susceptibility. One outcome is the generation of a large number of well characterized serum samples, which possess unique virus neutralization properties. The experimental approach is to perform virus neutralization assays on sera collected at 42 days after infection with North American PRRSV isolates, NVSL or KS06. Virus neutralization assays are performed using four different viruses, the homologous virus used for infection as well as three heterologous isolates located on different branches of an ORF5 phylogenetic tree (for details see Abstract and Poster No. 88, Trible et al.). In summary, the results show that all virus neutralization responses can be placed into distinct categories or groups: Grp 1) no nAb; Grp 2) homologous nAb; Grp 3) heterologous nAb, which neutralize 1 or 2 other isolates; and Grp 4) broadly neutralizing Ab (bnAb), which show neutralizing activity against all viruses. Both total antibody and virus neutralizing activities possess a heritable component. The current hypothesis is that the different neutralization groups reflect different epitope specificities. Chimeric and mosaic viruses are being constructed to map the different epitope groups. Analysis of the targets of broadly neutralizing antibody creates the opportunity to identify common epitopes, which can be used to construct vaccines that induce broad protection.

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Molecular evolution of PRRSV in Europe

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a recently emerged pathogen, with the earliest serological evidence from 1979 in Canada, in the 1980's in other regions of North America, Europe, and east Asia, and in China in the mid-1990's. Today PRRSV is a major threat to swine production globally. The existence of extensive genetic variation in endemic strains and the presence of highly virulent strains in different geographic regions pose the threat of devastating epidemic outbreaks. In Europe Type 1 (European genotype) dominates and three genetic subtypes are recognized and a fourth subtype appears to be present. In Type 1 PRRSV, only subtype 1 (Lelystad virus-like) circulates in Central and Western Europe and also globally. In Eastern Europe, all subtypes are present. The two strains from subtype 3 were proved to be significantly more virulent than any of the subtype 1 strains analysed before. The subtypes of Type 1 PRRSV also exhibit length differences in the nucleocapsid protein, ranging in size from 124 to 132 amino acids depending on subtype. Surprisingly, it affects the C-terminus, otherwise thought to be under strong structural constraints. Divergent subtypes of Type 1 PRRSV have produced high rates of false-negative RT-PCR results in diagnostic tests, and may also reduce the reliability of serological assays using the nucleocapsid protein antigen. Type 2 PRRSV was considered to be genetically homogenous in Europe due to a unique presence of an introduced vaccine strain, but independent introductions of virulent Type 2 field viruses are now evident. Moreover, the Type 2 strains circulating in Slovakia and Hungary belong to the same genetic cluster as virulent MN184 from North America. The broad genetic diversity of Type 1 PRRSV in Europe, and the presence of diverse Type 2 PRRSV strains, emphasize the importance of more extensive and systematic molecular phylogeny studies to fully understand PRRSV epidemiology in Europe, to provide swine producers with reliable diagnostics, and to better assess the potential consequences of endemic spread and exotic introductions.

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Development and application of a dual-marker vaccine for highly pathogenic Porcine Reproductive and Respiratory Syndrome

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Introduction

Vaccination has been wildly used as the most effective strategy of control of porcine reproductive and respiratory syndrome (PRRS). However, there is no serological method to differentiate the field infected animals from the vaccinated, which make extremely difficult of PRRS eradication. In the study, we first established an infectious molecular clone of HuN4-F112, a vaccine strain derived from a highly pathogenic PRRS virus HuN4, and then deleted 75 nucleotides in *nsp2* region followed by inserting an antigenic domain of NP gene of Newcastle disease virus (NDV) into the deletion region. Immune efficacy of the recombinant vaccine was evaluated in piglets, and two ELISAs detecting antibodies to the deleted and insert were established using synthesized peptides as antigen.

Materials and Methods

HuN4-F112 is an attenuated vaccine derived from highly pathogenic PRRSV HuN4; rHuN4-F112 is an infectious molecular clone of HuN4-F112; rHuN4-F112-Δ25 is an infectious molecular clone of rHuN4-F112 with 25aa deletion in *nsp2* region; rHuN4-F112-Δ25+NP49 is a rescued recombinant virus from rHuN4-F112-Δ25 with insertion of NP49 from NDV in *nsp2* region. 25aa from nsp2 region of PRRSV and 49aa from NP protein of NDV were artificially synthesized as antigen for detecting corresponding antibodies in swine sera by ELISA.

40-day-old piglets free of PRRSV and PRRSV antibody were selected for evaluating immune efficacy of the recombinant vaccine and sera were collected before and after immunization and challenge for detecting antibodies against PRRSV, 25aa peptide or NP49, respectively.

Results

1. Recombinant rHuN4-F112-Δ25+ NP49 showed similar growth kinetics to its original parental virus in Marc-145 cells and expression of insert NP49 could be consistently detected up to 20^{th} passage of the virus in cell culture by IFA with MAb against NP49. Sequence determination of insert NP49 from every fifth passage showed that the insert is stable and no single point mutation done to 20^{th} passage.

2. Piglets immunized with the recombinant virus developed detectable antibodies by days 14, and were challenged with HP-PRRSV HuN4 at 28 days after immunization. After challenge, all immunized piglets were survived without clinical signs or death, while piglets in control group developed long lasting high fever and high mortality (80%) by days 28 post challenge.

3. Sera collected from immunized piglets at different time, were detected antibodies positive to NP49 and negative to 25aa peptide. Detectable antibodies to NP49 could last for over three months. In control, antibodies to 25aa peptide could be detected in sera from pigs infected with HP-PRRSV or classical PRRSV.

Conclusions

Recombinant rHuN4-F112-Δ25+NP49 with a deletion (serve as negative marker) and an insert (serve as positive marker) was developed and preliminarily used in piglets. The immune efficacy of the dual-marker vaccine has been indicated as good as its parental vaccine HuN4-F112. The vaccine immunized pigs could be differentiated from naturally infected ones by two ELISAs detecting antibodies against NP49 or 25aa peptide, respectively.

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Porcine reproductive and respiratory syndrome situation in China: the past, present and future

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Porcine reproductive and respiratory syndrome (PRRS) is an economically important viral disease for the pig industry worldwide. The initial outbreak of PRRS was documented in an intensive pig farm of North China at the end of 1995. During the following 2-3 years, majority of pig farms suffered an abortion catastrophe in pregnant sows, like the situation in North America and Europe. Since then, the disease has been accompanying the Chinese pig production, and brought considerable losses to the swine industry in China each year although no exact amount was estimated. Clinically, the disease was mainly characterized by reproductive failure including late abortion, elevated fetal losses (mummies, stillbirths and neonatal death), and reduced growth performance and elevated mortality in weaning and nursery pigs, as well as respiratory disorders along with secondary bacterial infections in pigs of all ages.

In May of 2006, clinical outbreaks of so-called "swine high fever disease" occurred in pig-producing areas of South of China, and then spread to over the country during one year, which were characterized by high fever (41-42°C), rubefaction on the skin, respiratory disorders and gastrointestinal signs (diarrhea) and neurological disorders of the affected pigs. The suffered pig farms exhibited high morbidity (50%-100%) and mortality (20%-100%) in pigs of all ages, especially in suckling piglets and nursery pigs, and severe reproductive failures (abortion, over 40%) in pregnant sows. The affected pigs presented severe bacterial secondary infection (mainly *Haemophilus parasuis, E.coli, Streptococcus Suis Serotype 2).* The epidemic of the outbreaks resulted in a dramatic decline of pig amount, especially in severe epidemic areas, and bankrupt of large amount of small- and middle-size pig farms, and brought huge economic losses to Chinese swine industry. Etiological studies indicated that a highly pathogenic PRRSV is responsible for the unparalleled large-scale clinical outbreaks. The virus has unique genomic molecular characteristic, namely 90 nt (30 amino acids) deletion in its nonstructural protein 2 (Nsp2)-coding region (consecutive deletion of 29 amino acids at position 533-561 and one amino acid deletion at position 481). Afterwards, the HP-PRRSV became the dominating virus causing clinical outbreaks of PRRS in major pig-producing areas of China. According to our molecular epidemiological monitoring of PRRSV, 78% of viruses belonged to HP-PRRSV in 2006-2008, 86% in 2009, 88.9% in 2010-2011. During the epidemic of the virus, its genome has exhibited minor change in partial coding regions, especially in Nsp2-coding region, while its pathogenicity and virulence showed no marked change.

Porcine reproductive and respiratory syndrome (PRRS) has become the first ranking disease that impacts Chinese swine production among the viral diseases, since the emergence of highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV). Nowadays, PRRS caused by HP-PRRSV is sporadic and endemic, and a number of clinical outbreaks remained to be recorded in pig farms. Majority of PRRSV strains including HP-PRRSV belong to of genotype 2 in China. However, the genetic diversity of PRRSV exists in the field and the virus can be divided into three subgroups. Some strains might be evolved directly from the vaccine virus. Also the recombinant viruses including the recombination between the vaccine virus and field virus have been monitored. Additionally, the viruses of genotype 1 have been isolated recently and characterized from the samples of pigs with clinical diseases.

Vaccination is the primary measure for preventing and controlling PRRS in China. After the emergence of HP-PRRSV, the inactivated vaccine derived from HP-PRRSV could not provide effective protection, while commercial attenuated live vaccine was extensively applied in pig farms. To date, several attenuated vaccines derived from HP-PRRSV strains by passage on MARC-145 have been used in practice. Mass vaccination using these live vaccines is routinely performed in sows and growing pig herds. However, the safety of the vaccine derived from HP-PRRSV needs to be concerned. And much attention should be paid to its reversion to virulent virus besides its pathogenicity to pregnant sows and piglets. Clinically, a number of outbreak cases (including diseased piglets and sows' abortion) directly associated with the vaccination have been observed. Undoubtedly, the continued spread of the HP-PRRSV and currently excessive use of HP-PRRSV-derived live vaccine in pig farms will enhance the genetic diversity of PRRSV and further aggravate the complicated situation of PRRS in China. Therefore, it is necessary to emphasize the role of biosecurity measures and reasonable use of attenuated live vaccine in controlling PRRS. In the future, PRRS will be a more complicated swine disease in China. A lot of works for completely controlling PRRS need to be done in China, particularly in the prevention of PRRSV spread among different areas, implement of properly regional control and eradication programs for PRRS.

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Scale-up and transformation of Chinese swine production in the next decade and analysis of high-efficacy hog production strategies

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With the globalization of world economy, the supply and demand change of world feedstuff and food supply, shift of world economic zone , improvement of science and technology, labor cost increase, and rapid Chinese economic development, all these factors will help accelerate the development and transformation of Chinese live animal production in the direction of scale-up, integration, industrialization and automation.

The vertical integration and value chain add-up of Chinese swine industry will encounter dramatic opportunities, in which the traditional mainstream of small-scale and backyard producers will transform into vertical integrator or specialized hog producer in the next decade.

The major hog producers will be classified into three major categories, including the following: the first category will be large-scale vertical integrator who will raise 200,000 to 1 million sows (producing 3 to 20 million commercial hogs per year), which will be 20 to 30 companies across Chinese; the second category will be medium-size specialized hog producers who will raise 300 to 2,000 sows (producing 5,000 to 40,000 commercial hogs per year), which will have about 20,000 producers in China; the third category will be small-scale specialized producer that own 30 to 300 sows (producing 500 to 5,000 marketed hogs per year), which will have around 1 million producers altogether.

The overall hog industry will be transforming from segmented business structure into more vertically integrated business model, which will cover hog breeding and feed production, live hog production and slaughtering, meat processing and food branding. Contracted hog production and farmer cooperatives will be dominant business model, which need to be based on the mechanism of commercial trust and fairness and share of growth and profit.

Six core motifs during the scale-up transformation of Chinese swine industry will be how to become superior organizer and leader of high-efficacy hog producer, how to be overall nutritional program suppliers, how to become comprehensive hog production solutions provider, how to promote safe and healthy animal production and practitioner, how to offer series of value-added service throughout the vertical integration from hog production to food service, and how to help advancement of wise hog production and sustainable growth and development of the industry.

In terms of the systemic strategy for the high-efficacy pig production technology, there will be six major areas for improvement, including the following: 1. Supply high-efficacious genetics, with the aim to reach high prolificacy and good genetic potential; 2. Design environmental-friendly production facilities and system in order to control internal and external environment and save energy expenditure; 3. Guarantee comprehensive and precise nutritional program so as to generate optimal output and improve production efficiency; 4. Establish animal health management procedure to assure the standard operation principle and justify disease prevention and control program; 5. Apply wise systemic management program, which means the combination of hardware and software application so as to optimize the overall systemic competency; 6. Develop comprehensive service program aiming to generate added value and improve the profitability of hog producers.

Therefore, if we strive to improve the production efficiency, increase the growth performance and raise the profitability of scale-up and specialized hog producers, the whole swine industry and integration system will be vitally growing into more value-added industry and offer more safe, healthy, nutritious and tasty pork to the consumers in the next decade.

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Structure and function of PRRSV proteins

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The PRRSV genome contains at least 11 open reading frames (ORFs) coding for a total of 15 non-structural proteins (nsp's) and 8 structural proteins. ORF1a and ORF1b code for two large polyproteins via -1 ribosomal frameshifting, and these proteins are processed to yield 14 nsp's. A novel -2 ribosomal frameshifting has been identified in the nsp2-coding region, and nsp2TF (trans-frame fusion) is produced in the +1 frame that overlaps the nsp2 sequence. During replication, 6 different subgenomic mRNA species are transcribed from the genomic RNA, and 6 structural proteins corresponding to ORF2 through ORF7 are translated. In addition, two small membrane proteins are produced from ORF2b and ORF5a. ORF2b resides within ORF2 and codes for the E protein. E is a myristoylated membrane protein and is postulated to function as an ion-channel protein facilitating the uncoating process during entry. ORF5a overlaps ORF5, and its translation product is a small membrane protein with an unknown function. Both the E and ORF5a proteins are essential for PRRSV infectivity. Accumulating evidence shows that some nsp's function as a viral antagonist and modulate host innate immunity. Nsp1-alpha degrades CREB-binding protein in the nucleus while nsp1-beta blocks the nuclear translocation of interferon stimulated gene factor 3, thus both nsp1-alpha and nsp1-beta downregulate the type I interferon (IFN) response. Nsp2 is a viral protease and possesses ovarian tumor domain-containing deubiquitinase activity. It deconjugates ubiquitin and a ubiquitin-like modifier ISG15 from cellular targets to modify the host's antiviral response. Nsp11 is a viral endoribonuclease and has been shown to degrade the mRNA of the mitochondria adaptor protein MAVS, resulting in the suppression of IFN signaling. Such findings highlight that PRRSV has evolved the general plasticity of RNA virus genomes capable of coding for various multifunctional proteins providing selective advantages to evade the rigorous host innate immune defense.

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Towards a better PRRS vaccine: what are the missing links?

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Porcine reproductive and respiratory syndrome virus (PRRSV) causes what was initially coined as Swine Mystery Diseases. After two decades of intensive studies, many puzzles remain and become obstacle to rationally design the next-generation of vaccine. In this presentation, I will summarize 1) current situations of PRRS vaccine development and application: 2) the mystery of the PRRSV viruses.

Arteriviruses are enveloped positive-strand RNA viruses for which the attachment proteins and cellular receptors have remained largely controversial. Arterivirus particles contain at least eight envelope proteins, an unusually large number among RNA viruses.

These appear to segregate into three groups: major structural components (major glycoprotein GP5 and membrane protein [M]), minor glycoproteins (GP2a, GP3, and GP4), and small hydrophobic proteins (E and the recently discovered ORF5a protein). Biochemical studies previously suggested that the GP5-M heterodimer of PRRSV interacts with porcine sialoadhesin (pSn) in porcine alveolar macrophages (PAM). However, another study proposed that minor protein GP4, along with GP2a, interacts with CD163, another reported cellular receptor for PRRSV. In this study, we provide genetic evidence that the minor envelope proteins are the major determinant of arterivirus entry into cultured cells. A PRRSV infectious cDNA clone was equipped with open reading frames (ORFs) encoding minor envelope and Eproteins of equine arteritis virus (EAV), the only known arterivirus displaying a broad tropism in cultured cells. Although PRRSV and EAV are only distantly related and utilize diversified transcription-regulating sequences (TRSs), a viable chimeric progeny virus was rescued. Strikingly, this chimeric virus (vAPRRS-EAV2ab34) acquired the broad in vitro cell tropism of EAV, demonstrating that the minor envelope proteins play a critical role as viral attachment proteins. We believe that chimeric arteriviruses of this kind will be a powerful tool for further dissection of the arterivirus replicative cycle, including virus entry, subgenomic RNA synthesis, and virion assembly.

It has been proposed that the N-linked glycan addition at certain sites in GP5 protein of PRRSV is important for infectious virus production and virus infectivity. However, some strains of PRRSV have no such specific glycosylation site, which implies that the existence of GP5-associated glycan per se is not vital to the virus life cycle. In this study, we showed that mutation of individual glycosylation sites at N30, N35, N44, and N51 in GP5 does not affect infectious virus recovery and virus infectivity. Mutants carrying multiple glycosylation sites mutation in GP5 are viable but exhibit a significantly reduced infectivity compared with the wild-type (wt) virus. No viremia and antibody response were detected in pigs infected with a mutant with deletion of all N-glycans in GP5, which suggests that N-glycosylation of GP5 is critically important for virus replication in vivo. The study also showed that deletion of the N44-glycan in GP5 affects the sensitivity of the virion to antibody neutralization, but does not affect the immunogenicity of the neutralization epitope or the specificity of the antibody raised to wt PRRSV. These results made significant contributions to better understand the importance of glycosylation of GP5 in the biology of PRRSV. Codon-optimization of GP5 was attemepted to increase the previous believed main neutrolizing target protein, which was failed because of the sophosticated subgenomic mRNA regulatory network and a crptic gene. The ORF5a protein is a newly identified structure protein of PRRSV encoded by an alternative ORF5 present in all Arteriviruses. Recently, it has been shown that ORF5a protein in EAV is not essential but important for virus infectivity. In this study, we found that codon changes in the overlapping region (1-104 nucleotide, nt) between ORF5 and ORF5a introduced by codon optimized GP5 have a vital effect on virus viability, suggesting that the nucleotide changes or amino acid mutations in GP5 or ORF5a protein are not tolerant for the production of infectious virus. Furthermore, inactivation of ORF5a expression in the context of type 1(pSHE) and type 2(pAJXM and pAPRRS) full-length PRRSV cDNA clones is lethal for the production of infectious virus, and a viable PRRSV could be recovered by expressing ORF5a protein in trans, suggesting that ORF5a protein is essential for virus viability. Finally, ORF5a protein could be extended to putative 63 amino acids by inactivation of the downstream stop codon candidates, demonstrating that the C terminal of ORF5a could be variable.

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Feed addition with Tilmicosin reduce PRRSV viral loads in pigs in vivo

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Introduction

Porcine respiratory and reproductive syndrome virus (PRRSV) is an important porcine pathogen that causing huge economic impact in swine industry worldwide. Since the genetic instability of PRRSV and antibody dependent enhancement phenomenon induced after PRRSV infection had been proved, to find a better way for control PRRSV infection is an urgent demand beyond develop effective vaccines. Previous studies reveal that the macrolide antibiotics can inhibit replication of various viruses in vitro. Tilmicosin is a new semi-synthetic macrolide antibiotic developed from Tylosin. It can enter pulmonary alveolar macrophages (PAM) and inhibit replication of PRRSV in PAM in vitro. The aim of this study was to evaluate the ability of Tilmicosin in control replication of PRRSV in vivo.

Materials and Methods

Forty 4-week-old weaned piglets were randomly chosen and ear-tagged from a PRRSV-contaminated herd. Piglets were equally divided into two groups and housing in a same pen but separated into individual space. Control group was given regular feed throughout experimental period. Another group was provided with regular feed contained Tilmovet® 20% premix from 4 to 7 weeks of age and following by change to give regular feed with no Tilmovet® 20% premix until the end of this field trial. Serum samples were collected from each animal every 2 weeks from aged 4-12 weeks and quantified the PRRSV load by zip nucleic acid probes based real-time PCR.

Results

During the experiment period, 5 and 1 piglet were died in control and experiment group, respectively. PRRSV viremia was detected in both groups throughout the trial except 4 weeks of age. The mean PRRSV nucleic acid copy number of experiment group was lower than control group at each time-point and statistical significance were found at 8 ($P < 0.05$) and 10 weeks of age ($P < 0.01$). The highest detection rate of both control and experiment groups during PRRSV viremia period were all detected at 8 week of age with 95 and 89.5%, respectively. Besides, although the average weight of experiment group in the beginning of experiment was lower than control group, the average daily weight gain of experiment group was higher than control group.

Conclusions and Discussions

The results of this study indicate that Tilmicosin can decrease PRRSV viral load in serum and promote pig growth.

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Operating system for gene surveillance of porcine reproductive and respiratory syndrome virus (PRRSV) in Korea

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Porcine reproductive and respiratory syndrome (PRRS) has been reported in pig producing regions throughout the world. The PRRS is caused by PRRS virus (PRRSV) and the viruses easily undergo gene mutation. Since the efficacy of diagnostic kits or vaccines for the virus is affected by mutation, the variation of virus genes should be continuously monitored. Thus, the gene surveillance system (GSS) has been operated since 2010 in the web based Korea Animal Health Integrated System (KAHIS). The GSS is necessary to collect and provide genetic information on the major mutation of PRRSV. The present study is intended to advertise the GSS in the KAHIS so that information on the genes of PRRSV currently spread in fields can be periodically and continuously accumulated and utilized. The KAHIS system is operated by individual regional veterinary service laboratories based on surveillance and diagnostic work. When any PRRSV positive reactions are identified during surveillance or diagnoses, the results are uploaded to the KAHIS and are immediately linked to the gene surveillance system to be automatically received and registered. Thereafter, the gene positive specimens are sent to the Viral Disease Division (VDD) of the Animal, Plant and Fisheries Quarantine and Inspection Agency (QIA). Then, the VDD extracts the RNA of the specimens, amplifies individual necessary genes and request for the analysis of the gene base sequences of them to a sequence analyzing company. The genes of PRRSV are detected and then open reading frame (ORF) 5, 7 and nsp2 regions are identified depending on the virus genotypes. In the GSS, genetic information is automatically analyzed based on the reference sequences and the gene mutation is checked based on the analysis. The analysis of virus mutation types will become possible if a DB of genetic information on the viruses that are currently spreading is established through the analysis of the sequences of PRRSV. The results of the analysis will enable the prediction of an emerging virus, can be utilized in establishing suitable national measures for quarantine and will consequently reduce economic damage to farmers by PRRS. The gene information collected through the activation of the KAHIS system will become genetic resources and thus the efficiency of disease control work can be improved through sharing the information on the results of analysis. Therefore, virus mutation surveillance functions will be activated through the establishment of DBs of information on the base sequences of viruses prevailing in Korea, the prediction of the genotypes and mutation types of new and mutant viruses so that great effects on the establishment of national measures for quarantine can be expected.

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Development of Real Time RT-PCR (RRT-PCR) commercial kit for reliable detection and typing of PPRSV

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most significant diseases of swine as it produces great economic losses. Its causative agent, PRRSV is a highly variable virus from a genetic point of view. Isolates have been divided into two different genotypes: type 1, which comprises European isolates, and type 2, which includes American ones.

Early diagnosis is crucial in the control of this infection. RT-PCR has been used extensively as the earliest way to detect positive animals. In this way, RRT-PCR has become a powerful tool, as it has proved to be faster and more sensitive, compared to conventional RT-PCR.

Our main object was to develop a Real Time one-step RT-PCR commercial kit for detection and typing of PRRSV.

As probe-based typing assays had demonstrated to be insufficient in order to differentiate, even detect, newly isolated strains of the virus, a SYBR Green detection RRT-PCR was designed. This way, no false negative results occur when virus mutates, due to the failure of probe hybridization.

Materials and methods

Optimal conditions for the RRT-PCR were established as follows: specific primers for PRRSV universal detection were selected. One step RRT-PCR consisted of a 30 minutes incubation at 50ºC and a denaturation step of 10 min at 95°C, followed by 40 cycles consisting of 15s at 95°C and 60s at 60°C.

After that, the mix was subjected to a high resolution melting curve of 60s at 95ºC, 60s at 40ºC, 60s at 65ºC and a continuous lecture of fluorescence (25 acquisitions per minute) until a temperature of 95ºC was reached.

Melting temperature analysis was performed for every amplified product, to confirm their specificity, and two differentiated groups were detected. A Tm-based cut off could be established, being European (type 1) isolates Tm´s superior to 84ºC, while Tm from American (type 2) isolates remained below that value.

Results

A collection of 40 typified PRRSV RNAs was tested. The collection comprised isolates from 15 different strains including both types, in several concentrations. All strains were detected and classified into type 1 or 2, by means of their Tm values, corresponding to the expected results.

The presented kit gives promising results and could be of great value for rapid and reliable detection and typing of PRRSV, without the need of redesigning the assay every time a new isolate appears.

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PRRSV elimination in a 600 sow one-site system in China

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Introduction

Elimination of porcine reproductive and respiratory syndrome virus (PRRSV) was conducted in a 600 sow one-site farrow-to-finish system. Several elements were important to the successful elimination; stabilization of sow herd's PRRSV immunity, cessation of introductions into the herd, complete depopulation/repopulation of nursery and finisher unit, one-directional pig flow, and strict control of people traffic. No PRRSV vaccine was used at any time in any phase of production in this system.

Materials and Methods

A 600 sow breeding herd, one-site system (including breeding/gestation/farrowing [BGF] unit, isolation unit, nursery unit and finisher unit) was located in JiangXi province of China. An acute outbreak of PRRS was confirmed by serology, polymerase chain reaction (PCR) and occurred in late August 2010. It appeared initially in farrowing unit, then spread to breeding unit, and finally to the whole herd. The affected pig herd displayed high morbidity and mortality. Over 40% of pregnant females suffered abortion. Mortality was variable but very high: 69% in sucking piglets, 59% in nursery pigs, 33% in finisher pigs, 56% in sows.

The BGF unit was filled to capacity by adding multi-weight, multi-aged females (6 month supply). All gilts and sows were acclimatized to stabilize PRRSV immunity. To stabilize herd health, several action plans were implemented. Morbid pigs in all ages were culled immediately. Doxycycline (50ppm) and Tiamulin (100ppm) were added to the feed for three weeks. The isolation unit was used to hold overflow replacement gilts. Introduction of replacements was halted.

A seven-week period with no gilt or sow breeding was initiated in September 2010. This placed a void between litters potentially infected or shedding, and naïve litters from PRRSV stable dams. Aggressive culling practices for any morbid females were implemented during this seven week period.

Each nursery and finisher unit was completely emptied, cleaned, disinfected and allowed to dry between the removal of PRRSV positive and entry of PRRSV (non-shedding) negative pigs. Each nursery room had more than two weeks of downtime, though the nursery as a whole had only two weeks without pigs. Each finisher room had 6-7 weeks of downtime, though the finisher as a whole had only four days without pigs. Close attention was given to sanitation and drying.

PRRS status was monitored by PCR and enzyme-linked immunosorbent assay (ELISA) testing of serum samples. After the repopulation of the nursery (April 2011) and until September 2012, serum samples were gathered from growing pigs and the sow herd to determine PRRS status on a frequent but irregular basis. Since September 2012, 15 random individuals in the breeding herd and 15 random growers were sampled each month. Serum samples are tested both by PCR (pools of five) and ELISA since September 2012.

Results and Discussion

Between July 2011 and April 2013, all (100%) of 554 serum samples have tested negative by PCR (44 individual test, 90 pools of 3 samples, 48 pools of 5 samples). There have also been infrequent PCR test of lung samples (n = 14) and semen (n = 3) also yielding negative results.

Although sows that entered the breeding herd as PRRSV positive still exist in the breeding herd (currently 8.5% of the sow population), shedding of PRRSV has apparently not occurred, based on continued sampling of sows and pig flow.

This process involved the cessation of matings for seven weeks. In retrospect, that component may not be necessary to the successful elimination of PRRS. Numerous successful elimination processes have not required the cessation of matings. However, the combination of high mortality and cessation of matings allowed for greater physical distance between infected/shedding animals and naïve/negative animals in this single-site production flow.

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Evidence that Torque teno sus virus 1 (TTSuV1) is not associated with porcine circovirus associated diseases (PCVAD)

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Porcine Torque teno sus virus (TTSuV) with a circular DNA genome of ~2.8 kb resembles the genomic organization of human Torque teno virus (TTV)-related viruses that are currently classified into a newly-established family *Anelloviridae*. TTSuV comprises two genera, TTSuV1 and TTSuV2, which have a high prevalence in both healthy and diseased pigs worldwide. TTSuV has been shown to partially contribute to a few porcine diseases when pigs were co-infected with other porcine viral pathogens such as PRRSV and PCV2. In particular, in a gnotobiotic pig model, co-infections of PCV2 and TTSuV1-containing tissue homogenates induced postweaning multisystemic wasting syndrome (PMWS) or porcine circovirus associated diseases (PCVAD). However, whether TTSuV is indeed associated with a swine disease is still debatable. In this study, using a TTSuV genotype 1a (TTSuV1a) or TTSuV1b enzyme-linked immunosorbent assay (ELISA) based on the respective putative ORF1 capsid antigen and TTSuV1-specific real-time PCR, the combined serological and virological profile of TTSuV1 infection in pigs was determined for the first time. The results revealed a high rate of seropositivity to TTSuV1 in most pigs from different sources. Pigs that had decreasing TTSuV1a or 1b viral loads were associated with elevated anti-TTSuV1 ORF1 IgG antibody levels over a 2-month period. Furthermore, statistical analyses demonstrated that, TTSuV1 is likely not associated with porcine PCVAD, since both the viral loads and antibody levels were not different between affected and unaffected pigs, and since there was no synergistic effect of concurrent PCV2/TTSuV1 infections. Our study established essential serodiagnostic tools for the investigation of TTSuV1 infection, and provided new information that will help elucidate the potential pathogenicity of TTSuV in pigs.

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The establishment of the PRRS detection liquid blocking ELISA method

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Background: Comparing with the North American isolate (VR-2332), the HP-PRRSV outbreak in China in recent years has a 30-amino-acid (30-aa in 481 and 533–561) deletion in its Nsp2-coding region, which can be as a main marker to distinguish the two. We preparation the monoclonal antibody 1E9 against PRRSV Nsp2-deletion by using the synthetic 30-amino-acid polypeptide, and then establish the PRRS detection liquid blocking ELISA method by using 1E9. The corresponding antibody of PRRSV infection cases in the serum can be detected and results positive, but the HP-PRRSV infection cases can't. This method can be applied to epidemiological investigation in the pig industry, and provide the basis for the purification of pig farms.
Materials and methods: Align the Nsp2-protein sequence of VR-2332 and several other HP-PRRSV separated from our country, and choose the conserved 30-amino-acid deletion as a template to synthesize polypeptide. We prepared the monoclonal antibody 1E9 against PRRSV Nsp2-deletion by using the 30-amino-acid polypeptide. The liquid blocking ELISA used the 30-amino-acid polypeptide as the envelope antigen, then to join the sample serum, monoclonal antibody 1E9 and goat - anti -mouse second antibody, finally joining the OPD substrate to react and determine the ODp values.
Results: The monoclonal antibody 1E9 has high specificity. The liquid blocking ELISA method has a coincidence rate of 97% which had compared with commercial kits and PCR results.
Discussion: There had researches shown that the Nsp2-coding region is genetically the most variable area and crucial for viral replication due to its protease activity. We align the Nsp2-protein sequence of VR-2332 and several other HP-PRRSV and choosing the conserved 30-amino-acid deletion, which can insure a widely applied, meanwhile, studies shown that there has one linear epitope at least in the 30-amino-acid deletion. We use the 30-amino-acid polypeptide as the envelope antigen, the combined with monoclonal antibody 1E9 can be block up if there has corresponding antibody in the sample serum, and last shows the lower of ODp values and prove the exist of PRRSV infection. If there's no corresponding antibody in HP-PRRSV infection cases so that there can't block up the combine of 1E9. Therefore, PRRSV or HP-PRRSV infection can be distinguished.

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Seroprevalence of PRRSV, swine influenza (SIV), and PCV2 in feral pigs

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Feral pigs, also known as wild hogs, wild boar, or feral swine, have been reported in at least 45 of the 50 United States. In addition to being immensely destructive, feral pigs can carry and transmit numerous diseases that are threats to commercial swine production systems. As part of a swine disease surveillance program, serum samples were collected from feral pigs on the islands of Hawaii and in the state of Texas, which borders Mexico. Sera were assayed for antibodies to PRRSV, PCV2, and SIV using a multiplex serological test based on Luminex technology. Antigenic proteins from each virus – PRRSV N, SIV NP, and PCV2 CP – were expressed in *E. coli* as recombinant proteins fused with ubiquitin using the pHUE expression vector. Purified proteins were coupled to sets of fluorescent magnetic microspheres, each with a unique spectral address. After incubation with serum and secondary antibody, beads were analyzed on a MagPIX magnetic particle analyzer. From the fluorescent signature, the MagPIX determines the bead address (corresponding to the capture antigen) and whether the target has bound; with this technology it is possible to measure up to 50 different analytes in a given sample. Additionally, depending on the secondary antibody used, it is possible to differentiate IgM (acute infection) vs. IgG (convalescence). In this study, serum samples were analyzed from 345 feral pigs in Hawaii and from 230 feral pigs in Texas; all collected over a 4 year period (2007-2010). In both states, antibodies to PCV2 were by far the most prevalent with some subpopulations being nearly 90% seropositive, while antibodies to SIV and PRRSV were present at much lower rates. On Hawaii, the rate of PCV2 seropositive pigs stayed relatively constant over time, ranging from 51% to 75% in different years, with an average prevalence of 61%. The average SIV seroprevalence on Hawaii was 7%, starting from 0% in 2007 and increasing to 19% by 2010, while the number of PRRSV seropositive samples remained fairly constant at a rate of 1% to 5% in each year. In Texas, the rate of PCV2 seropositive pigs increased from 28% in 2008 to 72% in 2010, whereas SIV and PRRSV seropositive sera were only observed in 2010 (4% and 1.5%, respectively). DNA in PCV2 IgM-positive sera was amplified using PCV2-specific primers. DNA sequence analysis showed that the pigs were infected with PCV2b. Overall the results demonstrate the utility of Luminex-based multiplex serological assays for disease surveillance in feral pig populations. Additionally, the results to date indicate that feral pigs may not represent a significant reservoir for new PRRSV infections.

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Protective effects against PRRSV infection and boosting effects on PRRSV vaccine of immunostimulator(Barodon®) in pigs

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is the most significant infectious disease currently affecting the swine industry worldwide and is characterized by the reproductive losses of sows and respiratory disorders in piglets[1]. Barodon®(Barodon-S.F, Korea) has been introduced for its effectiveness as a nonspecific immunostimulator in pigs. The purpose of this study is to evaluate protective effects against PRRSV infection in pigs.

Materials and methods

Forty weaning pigs were divided into non-vaccine groups and vaccine groups largely. Groups excepted control were fed with Barodon® according to concentration of Barodon®. Non-vaccine groups were divide into NV-A(control), NV-B(0.025%), NV-C(0.05%) and NV-D(0.1%). Vaccine groups were divide into V-A(control), V-B(0.025%), V-C(0.05%) and V-D(0.1%). Total experimental period was 8weeks. Vaccine groups were inoculated PRRSV vaccine(Behringer Ingelheim, Germany)at 4weeks. The pathogenic NA PRRSV($10^{5.5}TCID_{50}$/ml) was inoculated via nasal cavity(2ml) and trachea(2ml) at 7weeks. Clinical sign and average daily gain(ADG) were checked for the experimental period. Blood samples were collected at 4, 7, 8, 9, 10 and 11 weeks for ELISA(Idexx, USA). Nasal swap was conducted at 7, 8, 9, 10 and 11 weeks to detect excretion of PRRSV. After necropsy, lungs were observed to check gross lung lesion[2]. Respiratory organ samples(tonsil, hilar lymph node and lung) were collected for detection of PRRSV and quantitative analysis by real-time PCR. H&E stain was used in the lungs for measuring microscopic interstitial lung lesion[3].

Results

In ADG, the results of NV-C and NV-D were higher than those of NV-A, significantly(Fig 1A). In quantitative analysis of PRRSV, the results of NV-C and NV-D were lower than those of NV-A in hilar lymph node, significantly(Fig 1B). Antibody titers of NV-C and NV-D increase rapidly after PRRSV inoculation significantly(Fig 2A). Antibody responses of V-C and V-D fed Barodon® were boosted at 4-7weeks after PRRSV vaccination significantly(Fig 2B). In clinical sign, gross lung lesion, excretion of PRRSV via nasal cavity and microscopic interstitial lung lesion, groups fed Barodon® showed results improved comparing with control, although there were no significant difference in experimental groups.

Discussion

In this experiment, feeding supplemented with Barodon®had an effect to improve ADG and antibody response and reduce respiratory clinical signs, interstitial lung lesion, detection of PRRSV in respiratory organs.

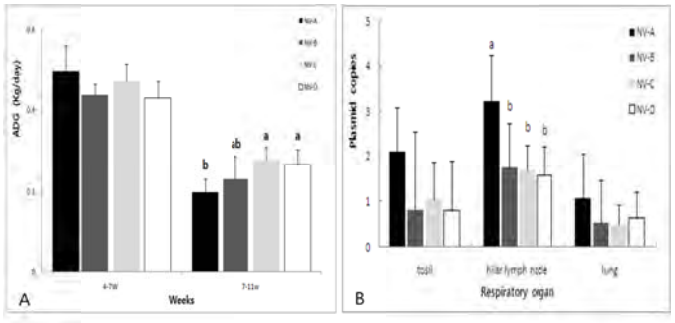

Fig. 1. ADG(Average daily gain) of non-vaccine groups before and after PRRSV inoculation(A). PRRSV quantification from respiratory organs after inoculation(B).

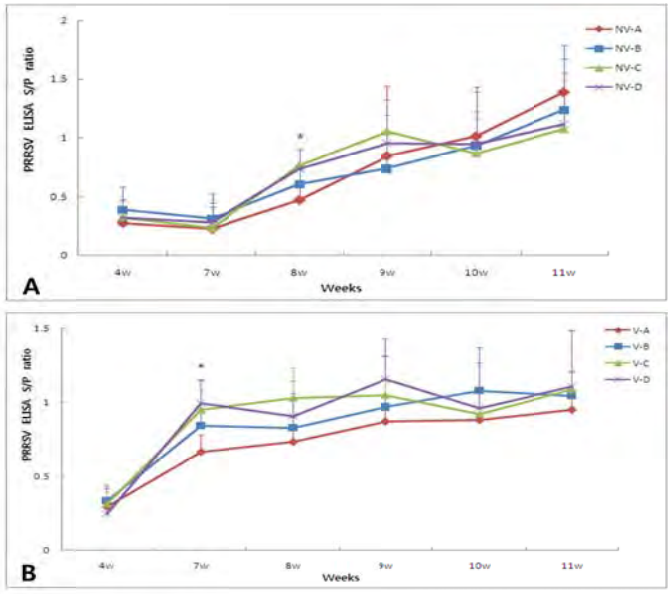

Fig. 2. Antibody titer(S/P ratio) of non-vaccine groups(A) and vaccine groups(B) after inoculation.

References

1. Kang *et al*. 2004, Virus Res 104: 165-179.
2. Straw B. 1996, Vet Med 81: 747-756.
3.Halbur *et al*. 1995, Vet Pathol 32:648-660.

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Application of high molecular poly-gamma-glutamic acid on pigs infected with PRRS virus

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Poly-gamma-glutamic acid (gamma-PGA) is a natural polymer produced by *Bacillus subtilis. g*amma-PGA has features of nontoxic, edible, water soluble and biodegradable. Various studies using gamma-PGA have been reported since it was known that it induces immune stimulation and shows antitumor effects on mouse models. In this study, high molecular (> 2,000 kDa) gamma-PGA produced by *Bacillus subtilis sups. Chungkookjang* was evaluated for antiviral effects against Porcine Reproductive and Respiratory Syndrome virus (PRRSV) and immune enhancement on PRRSV- infected pigs. Three animal experiments were conducted to evaluate immune modulation induced by gamma-PGA with different doses (1, 3, and 5ml, each ml contains gamma-PGA 20mg) and antiviral effect of gamma-PGA on pigs challenged with two genetically diverse strains of PRRSV (MN184 and JA142). All treated groups (2 separate injections at a one-week interval) with each different dose of gamma-PGA showed significantly higher average daily weight gain (ADWG) than that of control group. High levels of cytokines, IFN-beta in particular, were also observed in the pig groups treated with 3 and 5ml gamma-PGA. The pigs treated with gamma-PGA (5ml) at 7 days after challenged with MN184 showed significantly higher ADWG compared with control group and all groups treated with gamma-PGA at 0, 3, and 7 days after MN184 challenge showed lower levels of viremia than control group. Similarly, the pigs treated gamma-PGA before and after JA142 challenge also showed higher levels of ADWG and lower levels of viremia as compared with control group though the differences were not statistically significant. Interestingly, significantly high levels of serum virus neutralizing (SVN) antibodies were observed in the pig groups injected with gamma-PGA after challenge with either MN184 or JA142. No adverse effects were observed in all of the pigs treated with gamma-PGA throughout the studies according to cytological and pathological examinations. In conclusion, the current study demonstrated that pigs injected with high molecular gamma-PGA showed significant increases in growth performance, induced higher levels of cytokine and SVN antibodies, and resulted in lower levels of viremia upon PRRSV challenges. These results indicate that high molecular gamma-PGA could be beneficial in alleviating clinical symptoms and enhancing growth performance of pigs infected with PRRSV.

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Genetic analysis and phylogenetic tree of porcine circovirus type 2 field strains isolated from slaughtered pigs in Korea

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Introduction

PCVD or PCVAD is one of the most important disease and causes considerable economic losses in the pig industry. The majority of PCV2 isolates can be divided into one of two genotypes, known as PCV2a and PCV2b. The genotypic classification of PCV2 is complicated by field isolates composed of PCV2a and PCV2b sequences. The PCV2 genome is dominated by three open reading frames (ORFs): ORF1, ORF2, ORF3. The 233-amino-acid capsid protein (CP), coded for by ORF2, is the main structural and major immunogenic protein of PCV2. This study was conducted genetic analysis of PCV2 based on the ORF2 gene to evaluate genetic characterization of Korean isolation from the slaughters.

Materials and Methods

One hundred fifty kidneys were collected randomly from slaughter house in Korea. Ten PCV2 were detected from kidney samples by PCR. A full-length ORF2 gene of PCV2 was amplified by PCR with forward primer, PCV2-f1(5'-CCA TGC CCT GAA TTT CCA TA-3') and reverse primer, PCV2-r1(5'-ACA GCG CAC TTC TTT CGT TT-3'). The PCR was performed in 50 ㎕ mixtures(10 ㎕ of DNA extracted, 2 ㎕ of each primer, 25 ㎕ of pre-mix, 11 ㎕ of free water). The amplification reaction was performed with an initial step at 94℃ for 2min, followed by 35 cycles of denaturation at 94℃ for 30sec, annealing at 60℃ for 30sec and extension step at 72℃ for 7min. The PCV2 positive samples of 702bp were used for DNA sequencing. DNA sequencing was carried out by Solgent co.(Korea) with primers used in the previous PCR. The 10 PCV2 sequences were analyzed together with 7 representative ORF2 sequences reported in GenBank including the strain of PCV2a (AY256455, AY322004, AF264042) and PCV2b (AY484409, AY864814, EU302140, AB072302), the former Korea isolate in 2009 (FJ905468), PCV2c (EU148503) and PCV1 (AY193712) shown in Table 1. A figure showing alignment of amino acid and a phylogenetic tree was constructed by MEGA 4 software.

Table 1. 10 PCV reference strains used for the comparison in the same group by nucleotide and deduced amino-acid sequence of ORF2

Accession number	Nucleotide sequence	Amino acid sequence	Genotype	Subgroup	Country
FJ905468	CCCCGC	PR	PCV2b	1A/B	Korea
AY484409	CCCCGC	PR	PCV2b	1A/B	Netherland
AY864814	CCCCTC	PL	PCV2b	1C	Thailand
EU302140	CCCCTC	PL	PCV2b	1C	Indonesia
AB072302	AAAATC	KI	PCV2b	1C	Japan
AY256455	AAAATC	KI	PCV2a	2C	Hungary
AY322004	AAAATC	KI	PCV2a	2D	France
AF264042	AAAATC	KI	PCV2a	2D	USA
EU148503	CCCCTC	PL	PCV2c	-	Denmark
AY193712	ATCTTC	IF	PCV1	-	China

Results

The results of sequencing analysis were shown as Fig 1., Fig 2. and Table 2.

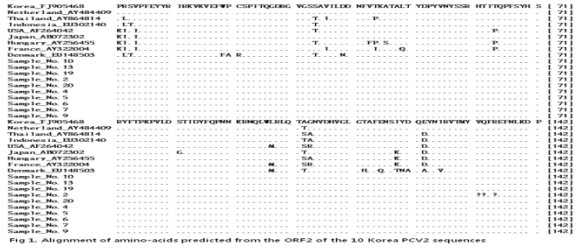
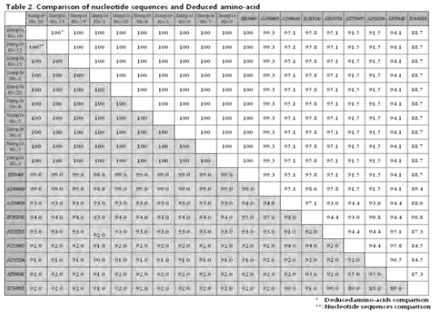


Fig 1. Alignment of amino-acids predicted from the ORF2 of the 10 Korea PCV2 sequences

Table 2. Comparison of nucleotide sequences and Deduced amino-acid



*: Deduced amino-acids comparison
**: Nucleotide sequences comparison

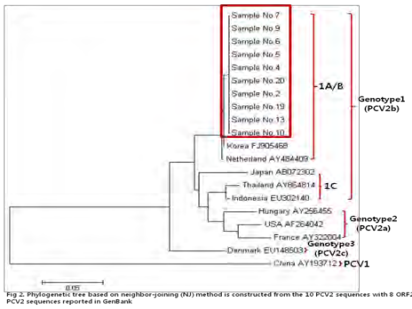


Fig 2. Phylogenetic tree based on neighbor-joining (NJ) method is constructed from the 10 PCV2 sequences with 8 ORF2 of PCV2 sequences reported in GenBank

Discussion

The results of sequencing analysis of PCV2 indicate that there are no differences with FJ905468 isolated in Korea. The genotype of 10 samples used in this study was PCV2b and the subgroup was 1A/B. All 10 ORF2 of PCV2 sequence had a genome length of 702 nt and revealed nucleotide identities ranged between 98~99% compared with the strain FJ905468 isolated in Korea. This results also indicate that these 10 sequences are more similar to AY484409 strain isolated in Netherland than the other strains (AY864814, EU302140, AB072302, AY256455, AY322004, AF264042, EU148503). Overall, PCV2 in Korea scarcely occurred genomic mutation.

References

1. Kim J et al.; 2002, *J Vet Med Sci* 64: 57-62.
2. Cheung AK; 2003, Virology 310: 41–49.
3. Hesse R et al.; 2008. Virus Res 132: 201–207.
4. Nawagitgul P et al.; 2000, J Gen Virol 81: 2281–2287.

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Recombination leads to the re-emergence of highly pathogenic PRRSV in China

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Recombination is an important evolutionary mechanism for PRRSV. However, despite of growing evidence of recombination from analyzing viral sequences, its evolutionary significance (e.g. increase of virulence, resistance to antivirals) and epidemiological impact (e.g. circulation of recombination progenies in the field) remains unclear. In this study, we analyzed 163 complete genome sequences of PRRSV sampled in China, among which 67 (38.7%) were confirmed to have recombined. More importantly, we discovered one group of "circulating recombinants" (n=34, 2008-2011) that originated from single recombination event and had been expanding in diversity and geographic range since 2008. Three breakpoints (positions 4264, 7896, and 13000 relative to VR2332) were identified in the recombinant genomes, which split the full genome into 2 major parental regions closely related to Strain HLJHL and 2 minor parental regions closely related to Strains 09HUB1&2. And based on the divergence from minor parental strains, we estimated the emergence of this recombination group to be between 2007 and 2008 under a relaxed molecular clock model. Moreover, it is important to notice that majority of the viruses in this group, originally reported by Zhou et al (2011) and Yu et al (2012), were the leading cause of high morbidity and mortality rate in the years 2009 and 2010. Indeed, as shown in our demographic reconstruction, this group has underwent exponential population growth that accounts for the second wave of highly pathogenic PRRSV population expansion in China, which also bears very similar demographic feature to the initial outbreak in 2006. In summary, we detected a recombination event which seeded and potentially dominated a new wave of high pathogenic PRRSV infections in China.

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Comparison of viremia of type II porcine reproductive and respiratory syndrome virus in natural PRRSV infected pigs by zip nucleic acid probes based real-time PCR

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a RNA virus with high genetic variation. This virus cause significant economic losses in most pig-producing countries. Clinical presentation of PRRSV can be asymptomatic to devastating. In this study, we developed a sensitive and specific zip nucleic acid probe based real-time PCR assay to evaluate the viremia of natural PRRSV infected pigs in Taiwan. Serum samples were collected from 547 pigs. These include 424 clinical health pigs together with 123 symptomatic pigs that with porcine respiratory disease complex (PRDC). Viremia was quantified in 76 of 424 (17.9%) clinical health pigs and in 102 of 123 (82.9%) PRDC cases. Significant highest viremias in pigs with PRDC compare with clinical health pigs (P <0.0001). These results suggest that high viral load is a major feature of PRRSV affected pigs.

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Comparison and application of several porcine circovirus type 2 antibody detection kits

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Background: Porcine circovirus type 2 (PCV2) infection alone can't cause the herd serious clinical symptoms usually, however, when it co-infection with other causative agents will trigger post-weaning multisystemic wasting syndrome. Vaccination is one of the effective measures to control the disease, and this strategy has been widely adopted and achieved successfully. Next, we meet the problem that how to evaluate the immune efficiency PCV2 vaccine. Several commercial PCV2-ELISA antibody detection kits are available, but the difference in brand leads to the diversity of quality possibly.
Methods: The immunoperoxidase monolayer assay (IPMA) was used as the gold criterion for comparison of five PCV2-ELISA antibody detection kits. In order to achieve their data of sensitivity, specificity and consistency, 35 samples of PCV2-positiv sera in different antibody titer, and 15 samples of PCV2-negative sera had been calibrated by IPMA. The known sera were used to evaluate the five ELISA kits. With IPMA, a kind of PCV2-ELISA kit was chosen to detect pig serum antibody of PCV2, which prepared from PCV2 vaccine immunized pigs (10 samples) and experimentally infected pigs (10 samples) as well as 159 samples of clinical serum without immunization.
Results: For comparison of five ELISA kits, their sensitivity was from 71.4% to 97.1%, specificity was 80.0% - 100%, coincidence rate was 74.0% - 98.0%. The blocking ELISA and IPMA was used for detection antibody of PCV2-vaccinination pigs. The seroconversion appeared at 21 days post-vaccination (dpv) and its positive rate was 50%. The antibody positive rate was 100% at 28 dpv. The antibody titers reached 1:800 at 35 dpv. The test of PCV2-infected pig serum showed that the seroconversion appeared at 28 days post-inoculation (dpi) and its positive rate was 30%. The antibody positive rate reached 100% at 42 dpi. The antibody titer was from 200 to 800 at 56 dpi. The clinical samples test showed that the antibody positive rate was from 45.0% to 76.5%. PCV2-postive rate and antibody titer were increasing distinctly with day of age.
Discussion: The coating antigen is the pivotal factor to the performance of ELISA kit. Such as the synthetic peptide endows with ideal purity, but its antigen reactivity is relatively poor. The antigen based on baculovirus expression can be processed and modified after expression; therefore its antigenicity is superior to the recombinant protein expressed by the prokaryotic system. The recombinant protein readily mixes with the E. coli host cell component, due to there was the antibody against E.coli in pig serum, so it probably leads false-positive.
Conclusion: This study suggests that the blocking ELISA based on monoclonal antibody is the best way to evaluate PCV2 antibody level for vaccination and natural infection of the virus.

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Prevalence of porcine hokovirus and its co-infection with porcine circovirus 2 in China

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Background: A broad spectrum of parvoviruses circulates worldwide in different species and cause disease in animals and humans. One of the several novel animal parvoviruses is porcine hokovirus (PHoV), which was first discovered in Hong Kong in 2008. Postweaning multisystemic wasting syndrome (PMWS) has caused great economic losses to the swine industry worldwide. Several groups have demonstrated that pigs dually-inoculated with PCV2 and porcine parvovirus (PPV) develop more severe disease and lesions than pigs infected with PCV2 alone. Therefore, it is necessary to investigate the prevalence of PHoV and its co-infection with PCV2.
Methods: A total of 485 specimens from 15 provinces in China from 2005 to 2012 were sampled and grouped according to their geographical origin and the date of collection. DNA extracted from all samples was used for the detection of PHoV and PCV2 by polymerase chain reaction (PCR). NS1 gene from eleven PHoV strains was amplified and sequenced to conduct phylogenetic analysis.
Results: Our data demonstrated that the prevalence of PHoV and PCV2 were 51.3% and 36.3% respectively. The positive rate of co-infection with PHoV and PCV2 was 20.21%. Among all the tissue samples, the positive rates of PHoV in lymphoid nodes (79.1%) and spleen (72.1%) were the highest. Similarly, positive rate of PCV2 was 47.8% in lymphoid nodes and 46.5% in spleens, which were the highest as well. The PHoVs from domestic pigs in China showed a close relationship to those isolated from Hong Kong.
Discussion and conclusion: In the present study, the results showed that PHoV-positive rate in China (51.3%) was significantly higher than that in German (32.7%) and Romanian wild boars (35.0%), and in domestic pigs in Hong Kong (45.2%). The high prevalence of PHoV in the samples collected from 2005 to 2007 （57.9%）showed that PHoV was already widely disseminated throughout the Chinese mainland before it was first reported in Hong Kong in 2008. Lymphoid nodes and spleens were the best sources for both PHoV and PCV2. In addition, co-infection with PHoV and PCV2 was common in the pig populations of China, which further suggested that PHoV may contribute to the induction of postweaning multisystemic wasting syndrome (PMWS). According to the phylogenetic analysis, PHoV isolates from domestic pigs on the Chinese mainland and Hong Kong, and those detected in Romanian wild boars in 2010–2011 formed a separate cluster. Thus, these viruses likely circulate in both domestic and wild pig populations.

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Three new emerging subgroups of torque teno sus virus (TTSuVs) and co-infection of TTSuV with porcine circovirus type 2 in China

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Background: Torque teno sus virus (TTSuVs) is a non-enveloped virus and has a single-stranded, negative sense circular DNA genome and is widely distributed in pigs. But till now, the prevalence of TTSuVs with porcine circovirus type 2 in pig herds of China is not very clear; and the genetic variation among different TTSuVs isolate is very large and need to divide the subgroups. In this study, the co-infection with TTSuVs and porcine circovrius (PCV) in the pig population of China was investigated and the subgroups of all TTSuVs genomes in Genbank were divided.
Methods: A total of 280 swine inguinal lymph node samples collected from pigs in 14 provinces were tested by PCR for the presence TTSuVs and PCV DNA. For the both PCV2 and TTSuVs-positive samples, the full-length genomes of TTSuV1 and TTSuV2 were amplified using proofreading DNA polymerase and the specific primers. The amplified DNA was inserted into the pMD18-T vector. Plasmid DNA was extracted and sequenced. Sequence analysis was carried out on the genomes of all the TTSuV1 and TTSuV2 isolates in GenBank using MEGA 4 software.
Results: Among the 280 samples, 176 samples were PCV-positive, 105 samples were PCR-positive for PCV2 and in the 176 PCV-positive samples, 115 of these samples were also PCR-positive for TTSuV1; Fifty-five among all 280 samples were positive for both PCV1 and PCV2. Results showed that the rate of co-infection with TTSuVs reached 75% in PCV2-positive samples. Also six TTSuV1 and five TTSuV2 isolates genome sequences were obtained, and the similarity of all TTSuV1 and TTSuV2 genomic sequences in GenBank were compared. Phylogenetic trees indicated that both the TTSuV1 and TTSuV2 sequences could be divided into four genotypes. Within the TTSuV1a group, the similarity was between 87.5% and 95.2%, while in the TTSuV1b group it was 83.8%–99.9% and in the TTSuV1c group 80.3% – 97.6%; but only two sequences in TTSuV1d group which only isolated in China. The similarity of the sequences of TTSuV2a ranges between 86.7% and 99.7%, which is similar to TTSuV2b and TTSuV2d, while only two strains obtained in China within TTSuV2c. Interestingly, the sub-genotypes TTSuV1d, TTSuV2c and TTSuV2d exist only in the pig population of China.
Conclusions: This study demonstrates that co-infection with TTSuVs and PCV is very common in the pig population of China, in which the viruses contribute to clinical diseases cooperatively. In addition, three new subgroups of TTSuVs emerged in China for the first time and a high level of variation among different isolates of TTSuV1 and TTSuV2 was indicated by their genetic diversity.

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Quantification of PRRSV transmission: effect of pig vaccination

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The use of vaccination for controlling PRRS at a regional level would be greatly facilitated if vaccination reduced significantly the probability of infection or transmission of PRRSV among vaccinated pigs. With the objective of quantifying transmission of PRRSV under quasi-natural conditions and estimating how the basic reproduction ratio (R_0) can be modified by piglet vaccination, a trial of heterologous challenge was conducted. Initially, 98 three-week-old PRRSV-naïve piglets were divided in two groups: vaccinated animals (V, n=40) that received an intramuscular dose of a commercial modified live vaccine (MLV) and unvaccinated animals (NV, n=58) which did not receive any treatment. At 35 days post-vaccination, 14 NV pigs were separated and inoculated intranasally with 2 ml of a genotype 1 PRRSV inoculum (strain 3267, 93.4% of global nucleotide similarity to the vaccine) containing $10^{5.5}$ $TCID_{50}$ ml^{-1}. Those animals were designated as "seeder" pigs (S). In parallel, the remaining V and NV were distributed in groups of 5 pigs allocated in separated pens (8 pens for V and 6 pens for NV). After 48 h, one S pig was introduced into each pen to expose uninfected pigs to PRRSV (contact phase). Additionally, 2 sentinel groups of 5 NV pigs each were allocated in pens contiguous to NV+S groups and a third sentinel group of 4 NV animals was placed adjacent to a V+S pen. Blood samples, nasal and fecal swabs were collected at 0, 3, 7, 10, 14, 17 and 21 days post-contact (dpc). Quantification of PRRSV was done by means of one step Taqman RT-PCR (ORF7) and the R_0 was calculated from PCR results. All S pigs became viremic after inoculation and remained so at least the following 17 days; the 64% of them were still viremic at day 21 post-inoculation. Regarding viral transmission, 100% (30/30) of NV pigs and 55% (22/40) of V animals became infected by contact with S ($p<0.05$), corresponding to R_0 values of 4.46 vs. 0.43, respectively ($p<0.05$). On average, 2 NV pigs/pen became infected directly from the S pig on the first cycle of infection (3 or 7 dpc) compared to 1.5 for V pigs. Considering the proportion of new infected pigs in subsequent cycles of infection, the differences between treatment groups were higher. Thus, the estimated 50% survival time for a NV pig was 7 days ($CI_{95\%}$: 5.2-8.7) compared to 21 days for V pigs ($CI_{95\%}$: 14.1-27.9). The average duration of the viremia was 12.2±3.8 vs. 5.5±5.0 days for NV and V, respectively ($p<0.01$). Regarding viral shedding, NV pigs had a longer nasal shedding compared to V animals (6.5±4.4 vs. 2.2±2.6 days; $p<0.05$). Fecal shedding was erratic and only 40% of NV pigs had at least one fecal swab positive compared to 20% of infected V animals. Finally, all sentinel pigs (10/10) placed adjacent to NV+S groups became infected whereas for sentinel pigs lodged contiguous to V+S group only 1/4 pigs became infected. In conclusion, piglet vaccination did not completely avoid infection but significantly reduced transmission of PRRSV probably by reducing the duration of viremia as well as the nasal shedding.

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The isolation and identification of new subtype of PCV2a

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Seven PCV2 strains were isolated from 16 swine farms in Eastern China between 2009 and 2011, and the full genome of these strains was sequenced. To analyse their subtypes, the phylogenetic tree of the 7 PCV2 ORF2 sequences with 381 PCV2 ORF2 sequences collected from NCBI Genebank was constructed based on the Neighbor-Joining method proposed by the EU consortium on PCVD. The analysis showed that five of seven PCV2 isolates (YHang09, TZhou11, HNing09, YWu09, XShan09) belong to PCV2b/1C subtype. Two of seven PCV2 isolates (HYan09 and NBiao10) contained PCV2b genotype tag motif, but the phylogenetic tree showed that they belonged to PCV2a, similar to 10 PCV2 isolates (10AH, 10GX, 09HaiN1, 09HaiN2, AY035820, INDON07-P09-07-07Lg2, INDON01-P009-10-01, THKUL16, THKUF49, 01NP1) reported previously. However, the genetic distance did not reveal that these 12 PCV2 strains was a new genotype, but these PCV2 strains exceeded the genetic distance between any PCV2 subtypes. To verify these data, total 45 PCV2 ORF2 sequences clearly defined subtypes were collected based on the isolation time, regions, genetics distance from NCBI. The optimal evolution model and evolution parameters of these PCV2 ORF2 sequences were obtained after calculated by PAUP, MrMTgui and MODELTEST soft stack based on AIC(Akaike Information Criterion) and used for the phylogenetic tree construction of these sequences with the Neighbor-Joining method, the Maximum Parsimony method, the Maximum Likelihood method, the Bayesian method, respectively. The constructed trees with different methods show the same results as the very first analysis. The aligned analysis of the ORF2 nucleic acid sequences showed the 12 PCV2 srains have specific nucleotides AA at 175-176, corresponding to a specific amino acid lysine, as well as the specific nucleotide T at 411 and 417. In addition, recombinant analysis showed that the 12 PCV2 strains are not recombinant virus. Overall, these 12 PCV2 strains were classified as a new subtype belong to PCV2a and named as PCV2a/2F. The identification of PCV2a/2F enriched PCV2 subtypes.

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Development of best practices to diagnose Porcine Reproductive and Respiratory Syndrome (PRRS) virus subtypes with high sensitivity in swine

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Introduction: Due to its potentially high economic impact, Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most significant diseases in the swine industry. The two PRRS virus genotypes (type 1/European and type 2/North American) display profound variation in their genomic sequence, and recombination between distinct PRRS viruses has been reported, making simultaneous detection and differentiation of each genotype difficult. This difficulty is compounded by the requirement to analyze multiple specimen types including oral fluids, serum, and semen with simple and rapid sample preparation, while maintaining high sensitivity and specificity. Here, the development and validation of an improved PRRS virus real-time PCR detection assay, meeting all of these stringent requirements, is reported.
Experimental Design: Tetracore's existing NextGen Real-Time RT-PCR detection assay was modified to include 1) additional multiplexed primers and probes targeting unique regions in type 1 and type 2 genotypes; 2) newly available reagents to improve speed and inhibition tolerance; and 3) increased sample volume. This novel assay was validated using multiple PRRS type 1 and type 2 viral strains obtained through international collaborations. Samples were extracted in-house using routine extraction methods. The assay was also externally tested by two separate diagnostic laboratories.
Results: The newly developed assay was found to have greater sensitivity and specificity, and successfully distinguished both PRRS viral genotypes. Results from two diagnostic laboratories showed the assay also displayed increased sensitivity. Importantly, data from these diagnostic labs further demonstrated successful detection in oral fluid specimens as well as differentiation of type 1 and type 2 genotypes. In serum specimens, the assay detected type 1 PRRS virus (n = 30, comprised of 4 unique subtypes and 10 genetic lineages) with 97% sensitivity, and type 2 PRRS virus (n = 90) with 98.8% sensitivity. In 150 oral fluid specimens, 100% sensitivity was achieved in a sampling taken from a pen of 25 pigs which included 3 pigs vaccinated with PRRS virus.
Conclusions: Both in-house and international diagnostic lab testing validates the newly improved PRRS virus real-time PCR detection assay to have high sensitivity and specificity. International lab testing verified the ability of using this improved assay for universal detection of currently circulating PRRS viral strains. Increased cooperation between both public and private diagnostic laboratories and manufacturers of PRRS virus commercial assays is a critical path forward to ensure the PRRS virus does not escape detection. Tetracore will take these results and apply to the development of a direct qRT-PCR detection method that precludes the need for extraction. This new strategy could be used in conjugation with the new portable real-time PCR Instrument from Tetracore T-COR8 which would allow rapid and portable sensitive detection at the pen-side.

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Discrimination of Porcine Reproductive and Respiratory Syndrome Virus Type I and Type II by an indirect ELISA

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Porcine reproductive and respiratory syndrome (PRRS) is one of the most devastating viral diseases causing significant economic losses to the swine industry. The agent responsible for this endemic disease is the PRRS virus (PRRSV) which is easily transmitted via infected pigs through urine, semen and fomites, infecting almost exclusively pig monocytes and macrophages. Based on the genetic differences of the virus, it is possible to discriminate between two main types: European genotype (Lelystad virus or Type I) and American genotype (VR-2332 virus or Type II). These PRRSV genotypes have a nucleotide identity between 55% and 70% when both genomes are compared. Due to the challenging PRRSV situation swine producers have to face worldwide, there is a need for new control and prevention strategies. Glycoprotein 4 (GP4) of PRRSV is very well conserved between genotypes and it can be used as a target for extremely specific serological detection by indirect Enzyme-Linked Immunosorbent Assay (ELISA), due to its capacity to produce neutralizing antibodies. Towards the goal of obtaining a novel control method that would detect the presence of the virus at herd level, we developed a double-well indirect ELISA using specific epitopes in the GP4 directed against capturing Type I (GP4_VL4) and Type II (GP4_VR-2332) neutralizing antibodies. The aim of this indirect ELISA is to provide swine producers with the exact information of the status of the PRRSV infection in a single test. This ELISA demonstrates a very high diagnostic specificity for Type I and Type II (99.41%/98.91%) due to the use of GP4 epitopes that capture specifically neutralizing antibodies. Even on individual animal level, the relative diagnostic sensitivity for each genotype is suitable (55.80%/45%) to use the ELISA on herd level basis to provide information about the PRRSV status of the herd. As a result, swine producers can improve their vaccine sanitary strategy depending on the predominance of neutralizing antibodies which are directed either against Type 1 or Type 2 PRRSV.

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Detection of porcine reproductive and respiratory syndrome virus (PRRSV) using the light scattering assay (LSA) based on the microfluidic chip

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Porcine reproductive and respiratory syndrome (PRRS) is considered as an economically important swine disease because this disease causes serious reproductive failure and respiratory problem leading to the lost productivity in pig industry. PRRS virus (PRRSV) is a causative agent of PRRS and its presence has typically identified using virus isolation, reverse transcription-polymerase chain reaction (RT-PCR) or real time RT-PCR. But, virus isolation or PCR was required a considerable amount of time, labor for a skilled technician and good facilities. Light scattering assay (LSA) has been considered alternative diagnostic methods to PCR due to rapid and high specificity of antibody assay. In the present study, LSA was applied using the microfluidic chip to detect of PRRSV, to simplify the sample preparation and to reduce kinetics assay times.

VR2332 was used as a PRRSV strain and the virus titer was 104.0 TCID50/ml. Coupling antibody (4A5) against PRRSVs was purchased from Median Diagnostics (Chuncheon, Korea). The antibody and nanoparticles (Bio-probe) were mixed using the EDC coupling and the completed bio-probe was stored at 4℃ until use. For long term storage, bio-probe was dried on the glass fiber (Millipore, USA). The glass fiber pads were stored at room temperature until use. LSA was applied using a microfluidic chip and 380nm light source with a 377nm light-emitting diode. Spectra suite software (Ocean Optics, USA) was used to measure the signal intensities at 377nm and intensity time was set to 100ms. To compare with LSA and RT-PCR results, positive and negative samples were spiked in the PBS (pH 7.4, Sigma Aldrich, USA) and confirmed using the PRRSV (NP) RT-PCR kit (Median Diagnostics, Korea). PCR products were electrophoresis with 1% agarose gel.

The LOD for PRRSVs was 0.1 TCID50/ml in LSA. The specificity was excellent as no agents relevant for differential diagnosis yielded a positive reaction. In order to compare RT-PCR and LSA results, the spiked samples were tested using the RT-PCR and identified 5 of positive panels and 45 of negative panels. The RT-PCR positive panels were shown 3 of positive results in LSA and negative panels were shown 45 of negative results in LSA. Sensitivity and specificity compared with RT-PCR were 93% and 100%, respectively. The results showed that microfluidic chip is suitable to detect for PRRSV and can be estimated as a potential application of diagnostic methods for PRRS.

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Serosurvey and molecular detection of PRRSV-1 in European wild boars (*Sus scrofa*) in Lithuania

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The role of wild boar in the transmission of PRRSV to domestic pigs is unclear. Antibodies to PRRSV have been detected in wild boar in some countries, although others failed to detect PRRSV antibodies in wild boars. Therefore, the objective of this study was to investigate the epidemiological PRRSV situation in European wild boar and to characterize genetically the detected strains.

A total of 1511 serum samples from wild boars from 61 locations throughout Lithuania were collected during autumn–winter hunting seasons 2008–2012. The wild boars sera were analyzed via different producers ELISA test systems according to manufacturer's instructions. For genetic characterisation samples of blood sera and lungs of 195 wild boars were collected from 23 hunting grounds situated in 10 regions of Lithuania and investigated by RT nested PCR specific for ORF5 of genotypes 1 and 2 PRRSV. ORF5 PCR products were sequenced and used for phylogenetic characterisation.

From 1511 examined wild boar sera, 90 (5.95 %) were positive to PRRSV antibodies. The number of seropositive animals over the 5 year period decreased from 9.1% in 2008 to 5.1% in 2012 however such differences were statistically not significant. Antibodies to PRRSV were detected in all age groups, however seroprevalence was significant higher in adults ($P<0.05$) than in juveiniles, subadults and unknown age. Samples of 19 (9.8 %) wild boars tested PRRSV-positive in genotype 1 specific ORF5 nPCR. No positive results were obtained by PCR amplifying ORF5 sequences of genotype 2 PRRSV strains. None of wild boar samples were positive with both 1 and 2 genotypes ORF5 primers. The sequencing and genetic comparison of the selected ORF5 amplicons revealed that these wild boar sequences belonged to two genetic subtypes 3 and 4 which were never found in domestic pigs in Lithuania.

This is the first report of serological evidence of PRRSV infection in wild boar population in Eastern Europe. Interestingly, in neighboring countries such as Russia or Poland PRRSV antibodies in feral pigs were not detected. The results of our studies in wild boars showed relatively higher PRRSV seroprevalence that it was estimated in Lithuanian pigs (4.29%). In this study we have also for the firt time succesfuly amplified sequnces and estimated genotype 1 PRRSV prevalence in wild boar lungs and serum samples. This study has for the first time demonstrated that wild boar population can harbour different genetic lineages of PRRSV strains than those found in domestic pigs in Lithuania. Altogether, these findings are strongly supporting the role of wild boars as a possible natural reservoir for PRRSV.This work was funded by Projects No MIP-065/2012 of Research Council of Lithuania.

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Genetic analysis of porcine reproductive and respiratory syndrome virus strains in Japan from 1992 to 2011

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Genetic analysis of porcine reproductive and respiratory syndrome virus (PRRSV) field strains is necessary to control the disease. We analyzed the nucleotide sequence variation of North American genotype (type 2) PRRSV strains in Japan and compared them with those of PRRSVs from other countries. In addition, the genome of European genotype (type 1) PRRSV isolated for the first time in Japan in 2008 was analyzed. The ORF5 genes of 57 type 2 PRRSVs isolated from lung samples of pigs with respiratory signs in the various areas of Japan in 2009-2011, were sequenced. The published ORF5 sequences of 48 Japanese strains from 1992 to 2008 and those of 46 distinctive viruses from other countries were also used for phylogenetic analysis. A type 1 PRRSV was isolated from lung of a 70-day-old pig using porcine alveolus macrophages, and its genome was sequenced. In phylogenetic analysis, ORF5 sequences of type 2 PRRSVs were classified into six clusters in Japan. Clusters I, III, IV, V and VI consisted of 10, 77, 2, 2 and 1 Japanese PRRSVs, respectively, and 13 viruses in Japan belonged to Cluster II constructed with viruses from other countries and RespPRRS/Repro vaccine strain. The cluster III PRRSVs were Japan-specific viruses and major in Japan, and the genomes of PRRSVs classified in clusters I and III have become highly diversified. On the other hand, ORFs5, 6 and 7 gene sequences of a type 1 Japanese isolate showed 96.0 to 97.5% identities with those of the strain SD01-08 isolated in the US. These results indicate that type 2 PRRSVs classified in cluster III have been prevalent since the virus was first isolated in Japan in 1992, and type 1 Japanese PRRSV might be introduced from the US recently.

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Vaccination against PRRS and PCV2 reduced the loss in nursery: a case report

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Introduction: PRRS and PCV2 are two of the most widely spread pathogens for swine in China [1] [2]. These two viruses can cause severe losses in the nursery. Moreover, secondary bacterial infections like H. parasuis (HP) and Strep. suis can make the disease situation on the farm even more complex. This is a field observation from a farm in Jiangsu province in China on the reduction of losses and the use of antibiotics in the nursery after implementation of PRRS and PCV2 vaccination.

Materials and methods: Data was collected from a 1500-sow farrow-to-finish farm in Jiangsu province. The farm was built in 2001 with a continuous flow management. Piglets are weaned at 21 days and transferred to the nursery, where they are kept until 80 days of age. Half of the pigs are sold after the nursery. Pigs in nursery suffered from high losses from 2008 onwards. HP was considered the main pathogen according to clinical signs and necropsy findings. The farm started to use HP vaccination in 2009, with sows being injected twice (5 weeks and 2 weeks before farrowing) and piglets at 14 days of age. No PRRS and PCV2 vaccine was used at that time. After implementing HP vaccination the health situation did not change much. Therefore further samples were taken and PCR testing revealed a high level of PCV2 and PRRSv infection in the nursery. Following that, vaccination with Ingelvac® PRRS MLV was implemented in January 2010, with sows being mass vaccinated, 4 times a year, and piglets at the same time as HP vaccine (14 days of age). In February 2011, Ingelvac CircoFLEX® was introduced to the farm with mass vaccination of sows, 3 times a year, and piglets vaccinated at 14 days of age (the same time as PRRS MLV). At the same time, the farm stopped to vaccinate piglets against HP. Since then the farm continued to use this vaccination program. In addition, the breeding herd was vaccinated against PRV, CSFV, PPV, JEV, while piglets were vaccinated against CSFV, PRV, and M. hyopneumoniae. Mortality and culling rate, as well as average medication costs per pig, were recorded on a quarterly and yearly basis from 2008 to 2012.

Result: The introduction of Ingelvac® PRRS MLV and Ingelvac CircoFLEX® successfully reduced the losses in the nursery (Tab. 1). The yearly mortality and culling rate in the nursery decreased from 18.8% in 2008 to 4.5% in 2012. Medication costs per pig were reduced from 16 RMB in 2008 to 6 RMB in 2012.

Table1 Mortality and culling rate and medications costs from 2008-2012.

	Q1 (%)	Q2 (%)	Q3 (%)	Q4 (%)	Yearly (%)	Med. costs (RMB)
2008	21.3	18.5	15.8	19.4	18.8	16
2009	18.7	16.2	14.3	17.1	16.6	13
2010	13.6	11.4	9.5	10.8	11.3	9
2011	8.1	6.6	5.9	6.2	6.7	7
2012	6.7	4.3	3.6	3.4	4.5	6

Q=Quarter

Conclusion and discussion: Like many pig farms in China, this farm had experienced high losses in the nursery. Bacterial infection was found and the farm tried to resolve the problem by introducing HP vaccines and antibiotics. However, HP vaccine and antibiotics did not work well since PRRSv and PCV2 were other two major pathogens which contributed the high losses in this farm. In this case, Ingelvac® PRRS MLV and Ingelvac CricoFLEX® vaccination have both shown (on top of the regular vaccination program) to be effective tools to successfully reduce losses and antibiotic costs in the nursery.

References

[1] Tian et al. (2007). PloS ONE. 2(6): e526.
[2] Ge et al. (2012) Virus Res.164 (1-2):100-6.

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PRRSV and other co-infecting viruses in PMWS affected pigs

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Introduction: Pigs affected with postweaning multisystemic wasting syndrome (PMWS) are infected not only with PCV2 but sometime with PRRSV and other viruses. In this work, we compared co-infection of PMWS, PMWS+PRRS and healthy groups of pigs with porcine teschovirus (PTV), porcine bocavirus (PBoV), torque teno sus virus type 1 and type 2 (TTSuV1 and TTSuV2).

Materials and Methods: A collection of 24 lymph nodes of pigs affected with PMWS originating from Slovakia was used in this study. PMWS was confirmed by observation of clinical status of animals typical for this syndrome, observation of lesions on organs, confirmation of PCV2 by immunohistochemistry and PCR. The group of clinical samples was further divided into two groups: Group I consisting 12 pigs with PMWS and group II with 12 animals showing PMWS and PRRSV infection detected by RT-PCR. Most animals in the group II had also symptoms typical for PRRS (respiratory problems). In addition, the group III of 24 healthy pigs was also involved in the experiment. All animals were examined for the presence of following viruses: PCV2 (single PCR), PRRSV (nested RT-PCR with ORF5 primers), PTV (nested RT-PCR), PBoV (nested PCR), TTSuV1 and TTSuV2 (specific real-time PCR).

Results: Differences in the detection of PTV (5 versus 4 animals of 12 analyzed) and PBoV (3 versus 2 animals) were not significant in group I and II. Thus co-infection of PMWS animals with PRRSV had no strong effect on the occurence of PTV and PBoV. However, TTSuV1 was more often detected in group I than in group II (75% versus 42%) while TTSuV2 was more prevalent in group II than in group I (58% versus 25%). In group III, e.g. healthy animals, in which 80% of animals were positive for the presence of PCV2, three viruses as PRRSV, PTV and PBoV were not detected. On the other hand TTSuV1 was more often detected than TTSuV2 (46% versus 29%).

	Group I (PMWS)	Group II (PMWS+PRRS)	Group I + II	Group III (healthy animals)
0 viruse	0	0	0	5
1 virus	0	2	2	4
2 viruses	3	2	5	12
3 viruses	4	7	11	3
4 viruses	2	0	2	0
5 viruses	2	1	3	0
6 viruses	1	0	1	0

More viruses were simultaneously found in the groups of clinically sick (PMWS and PMWS+PRRS) pigs than in healthy animals (see Table)

Conclusion: In general, our observation confirmed that there is not big difference in the detection of viruses analysed in PMWS and PMWS+PRRS animals, except TTSu1 and TTSuV2. These viruses were also found in healthy animals together with PCV2. However, simultaneous detection of more viruses were found in sick than in healthy animals. What the role is of PTV, PBoV, TTSu1 and TTSuV2 in the development of PMWS is still not clear.

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The first detection of PRRSV in wild boars hunted in Central European region, Slovakia

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is the etiological agent of porcine reproductive and respiratory syndrome which is characterized by reproductive failure in sows and respiratory problems in piglets. Although PRRSV is widely spread in domestic pigs the finding of this virus in wild boars is relatively rare. There are selorogical evidences on PRRSV specific antibodies in wild boars but virus or viral RNA has been found only in several European countries as Italy, Germany and Lithuania. Our group also search for the presence of PRRSV RNA in organs of wild boars in Slovakia.
Material and Methods: A collection of 115 suspensions prepared from kidney, tonsils and spleen as well as 14 lymph (popliteales) nodes of wild boars killed in hunting period 2011-2012 were used for the detection of PRRSV genome. Viral RNA was isolated by Trizol reagent and virus was detected by RT-PCR using ORF7 primers. PCR products were sequenced in both directions with PCR primers using the Sanger method employing fluorescently labelled ddNTPs. Nucleotide sequences were analyzed by BLAST and phylogenetic analysis using Neighbor joining method.
Results: All samples but two were negative for viral RNA. However, two samples of lymph nodes collected from during hunting in December 2011 in Eastern Slovakia (region of Slanske vrchy, 40 – 60 km to Hungarian and Ukraine border) were strongly positive in RT-PCR. Sequencing of PCR amplicons reveled that both nucleotide sequences were 100% identical. BLAST analysis, nucleotide sequence identity and phylogenetic tree confirmed that sequences of ORF7 were identical with the Lelystad and Porcilis PRRS vaccine strains (Acc. No. M96262 and DQ324710). The length of ORF7 was 128 amino acids.
Discussion: The amplification of viral RNA genome in two wild boars indicates that these animals may be a potential reservoir of the virus transmission as it is in case of CSFV, Aujezsky disease virus and other viruses. Our previous genetic analysis of PRRSV isolates from domestic pigs in Slovakia did not confirm circulation of the Lelystad group viruses in the country. Laboratory contamination is eliminated by fact that Lelystad or similar isolates have never been analyzed in our laboratory. The pig farm management in this region of Slovakia excluded possibility for direct contact of domestic and wild pigs. Potential migration of wild boars between Slovakia and Hungary may happen but migration to Ukraine is excluded due to the mountain barrier. The origin of PRRSV of the Lelystad group in wild boars in Eastern Slovakia is not clear at present.
Conclusion: This work describes the detection of PRRSV in wild boars in Central European region, namely in Slovakia, for the first time. Epidemiological link and significance for the transmission of virus to domestic pigs remains to be elucidated.

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Porcine Reproductive and Respiratory Syndrome in hybrid wild boars, China

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This study was aimed at determining whether the epidemiology of PRRSV involved the hybrid wild boars. We conducted a serologic investigation of porcine reproductive and respiratory syndrome virus (PRRSV) in hybrid wild boar herds in China during 2008–2009. 613 blood samples from Shandong Province were obtained from hybrid wild boars that had not been vaccinated against PRRSV. 167 (27.2%) of 613 samples were seropositive for PRRSV, indicating that the prevalence of PRRSV differed between domestic pigs and hybrid wild boars in this area, and 2 PRRSV isolates (TAYZ and ZCYZ) were plaque-purified once in MARC-145 cells and amplified for sequencing and infection of other pigs. The result showed TAYZ and ZCYZ had higher nucleotide homologies in ORF5 with highly pathogenic PRRSV (prototype virus JXA1). For NSP2 of TAYZ, 2 deletions were identified: a 1-aa deletion at position 482, and a 29-aa deletion at positions 533–561. These deletions were similar to those in JXA1, HUB1, and SD-JN isolates associated with porcine high fever disease reported in China. For NSP2 of ZCYZ, 2 deletions were identified: a novel 25-aa deletion at positions 476–500, and a 29-aa deletion at positions 533–561. In hybrid wild boars injected intramuscularly with 1mL of ZCYZ isolate (103 50% tissue culture infectious doses/mL), high fever >40.8°C and respiratory disorders were observed at 5–6 days postinfection (dpi). Two boars died at 8 dpi and 1 boar died at 9 dpi. Two pigs were killed at 21 dpi and pulmonary hyperplasia and consolidation and cardiac hemorrhage and edema were observed. PRRSV-specific antibodies were detectable by 7 dpi, had increased by 14 dpi, and remained high until autopsy at 21 dpi. For the infected domestic Duroc crossbred pigs, high fever >41°C and respiratory problems were observed at 4-5 dpi, and red discoloration was observed in the ears. Two pigs in this group died at 6 dpi; the other 3 pigs in this group died at 7 dpi. The dead pigs all had multiple lesions in various organs, such as edema and hemorrhage in the lung and hemorrhagic spots in the heart. Duroc crossbred pigs and hybrid wild boars in the control group injected with Dulbecco minimal essential medium had a normal appearance, appetite, and rectal temperature during the experiment, and no obvious pathologic changes were observed in the lungs and heart. In conclusion, experimental infection of pigs indicated that hybrid wild boars play an important role in the epidemiology of PRRSV.

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Exploration of large breeding herd with PRRSV serum acclimation

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Introduction: The number of return and abortion sows of ZhongXuZhan(the pig farm scale is 1200) significantly increased from January to February in 2011. The number of abortion is 9 and 15, respectively; the return number is 11 and 10, respectively. We detected PRRSV from abortion sow serum. According with clinical signs, we decided inoculate serum to sows and gilts, and then closed the herd.
Materials and methods: The PRRSV positive serum from ZhongXuZhan, was detected no classical swine fever and pseudorabies virus. The herd was inoculated (10μl serum/head) by intramuscular injection on March 8 and 10, respectively.
Results: There is little effect on the breeding performance during the acclimation period. The number of abortion and return is 6 and 18, respectively in March. The herd returned to normal in April. We sample the serum and test the PRRS antibody every six months. We find the PRRS antibody positive rate of breeding herd gradually decreased when the time passed. The S/P ratio of PRRS antibody levels also gradually decreased ,the rate of PRRS negative pigs risen, the nursery pigs become PRRS negative (Table 1). The performance of sow herd improved (see Table 2).

Table 1 ZhongXuZhan PRRS antibody test results

Time	S/P ratio	boar	sow	nursery
201012	>0.4	-	82%	60%
	>2.0	-	28%	11%
201105	>0.4	-	73%	80%
	>2.0	-	28%	53%
201111	>0.4	67%	61%	0%
	>2.0	0%	7%	0%
201205	>0.4	57%	46%	0%
	>2.0	14%	2%	0%
201211	>0.4	20%	59%	0%
	>2.0	0%	2%	0%

Table 2 ZhongXuZhan breeding performance compared before and after the serum accumulation

Year	Breeding number	Pregnancy rate	Farrowing rate	Born alive	Total litter size
2010	2407	94.68	85.25	10.25	11.52
2011	4530	96.29	86.78	9.97	11.39
2012	3314	98.07	86	10.38	11.6

Discussion**:** The timing of serum acclimation is critical, usually we should observe the abnormal signs of the sows, such as sow fever, abortion or return etc. abnormal appearance, combined with laboratory diagnosis. When we diagnose the abortion caused by a new PRRSV strains, we will determine whether serum inoculate or not. The serum must be free of PRV and CSFV, or maybe spread the other virus and cause greater loss. When discovered sow farm instability caused by new PRRSV strain more than 2 month later, the loss will be much more. Serum acclimation and herd closure is not always successful, there are many unpredictable factors. Sometime it is a dilemma when we decide whether serum acclimation for the herd or not. However, as long as the full understanding of the health status of the pig farm and a well-planned, we have a very high success rate of serum acclimation.
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A potential novel Porcine Reproductive and Respiratory Syndrome virus subgroup emerged in South China based on the genetic analysis of the ORF5 gene from 2007 to 2011

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Aims: To study the molecular epidemiology and etiological characteristics of porcine reproductive and respiratory syndrome virus (PRRSV) in Guangdong, China, a total of 3199 clinical samples were collected from 267 pig farms suspected of PRRSV infection between 2007 and 2011. The ORF5 genes of 51 PRRSV isolates were sequenced and analysis.

Methods and Material: Sample collection, RNA extraction and RT-PCR, nucleotide sequencing, ClustalX 2.0 program (DNASTAR Inc., Madison, WI, USA) were used for homology analysis. A phylogenetic tree was constructed using MEGA 5, which is also used for amino acid analysis of the GP5.

Results and analysis: Nucleotide homology analysis revealed that most of the isolates were closely related to the highly pathogenic PRRSV, which shared a nucleotide identity with the representative strain JXA1, ranging from 84.1 to 99.7%. When compared with the other representative strains, LV for the European genotype, VR2332 for the North American genotype, and CH1-a for Chinese isolates, it was clear that they shared a nucleotide identity ranging from 62.8-64.8%, 82.1-97.8%, 85.6-95.7% respectively, and share an identity of 82.6-100.0% among isolated strains from different years. Phylogenetic analysis showed that the 51 isolates were mainly divided into three subgenotypes. Forty-seven of the 51 isolates belonged to subgenotype I, along with the representative strains of highly pathogenic PRRSV (HP-PRRSV), which share an amino acid identity of 97.0-99.5% and nucleotide identity of 98.2-99.7%. Furthermore, the prevalence of HP-PRRSV in different years presented with a certain regularity, while only one of the isolates belongs to subgenotype II together with the classical North American type VR2332. More importantly, subgenotype III occupied a separate branch. Five of them are closely related to the recently isolated novel field strains QYYZ and GM2, the potential recombinant strain between the field and the RespPRRS vaccine strain. The widespread use of modified live-attenuated vaccines (MLV) might help to reduce the severity of the disease, but there is also a great risk that an MLV might exhibit the potential to revert to virulence, causing the recombination of the field and vaccine strains. The recently reported novel variant strain GM2 has proved to be a potential recombinant between an MLV strain and the field strain QYYZ.

The potential N-linked glycosylation sites (NGS), primary neutralizing epitope (PNE) residues and signal peptides, extravirion domains, transmembrane regions (TM), and intraviron domains were analysed. The glycosylation of the enveloped protein is related to immune evasion. Differences in the N-glycosylation sites were also observed in different groups and subgenotypes. The critical glycosylation sites N41 and N51 are well conserved among all of the isolates, There are four potential NGSs (N30, N35, N44, and N51) in group I and part of 2011's isolates in group II (SZ2-GD-11, HZ20110010, NZ-11, and MM-11) of subgenotype I. There are five potential NGSs (N30, N34, N35, N44, and N51) in groups III and IV. However, in subgenotype III, there are only three potential NGSs (N34, N44, and N51). This could explain the extensive variation in subgenotype III ,for the lack of N-Glycan Shielding protection effect, leading to the amount mutations ,especially in the region of PNE. Extensive mutations were observed in subgenotype III. Observed amino acid mutations in a signal peptide (9C→9G, 24Y→24C, 25L→25S, 26A→26I), the PNE of the extravirion region (27V→27A, 30N→30S, 32S→32N, 34S→34N, 35N→35S, 38H→38Y, and 39I→39S), the turn membrane (TM) area (66T→66C, 92G→92S, 117L→117F), and the intavirion area (128A→128V, 151R→151K, 152L→152I, 161V→161I, 189L→189V, 191R→191K, 192V→192I, 196Q→196R, 199R→199H, 200L→200P). R13Q and R151G are considered to be involved in the attenuation of the virus In subgenotype III, one mutation (R151K) was observed in HZ20110032, QY20110041, and FS134-GD-11, while the GZ1-09 and YJ1-10 included both of the mutations (R13Q and R151K). Whether the virus was attenuated or in a transition state of revert to virulence needed to be identified in a further step.

Conclusions: All of the 51 GP5 sequences of PRRSV isolated from 2007 to 2011 in South China belonged to the NA type. Three subgenotypes were be classified based on the NA-type, and most of the sequences belonged to HP-PRRSV, and even presented a certain regularity. Only one strain grouped together with the classical NA-type strains. Interestingly, the third subgenotype was found to be highly variable and was also closely related to the recently identified novel strain with the potential for recombinant events between field and vaccine strains. This revealed that the novel strain might be widespread in South China. More importantly, the diverse genetic mutations of these novel strains are of great concern. The data presented in this paper is useful for understanding the epidemic situation of PRRS in South China, and provide reference statistics for further surveillance of PRRS.

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Study on antiviral activity of four Chinese herbs to Porcine Reproductive and Respiratory Syndrome virus

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To study the antiviral activity of natural herbs to porcine reproductive and respiratory syndrome virus (PRRV), anti-viral effect of Indigo Naturalis, Sichuan pepper, euonymus and aloes were analyzed by using in vitro cell culture system model. Based on detection of median toxic concentration (TC50) and maximal nontoxic concentration (TC0) of four herbal extracts by CCK-8 method, the cytopathic effect was observed and virus nucleoprotein gene (ORF7) mRNA expression determined real-time quantitative RT-PCR. Results of cytotoxicity and virus proliferation inhibition analysis shows that antiviral selection index (SI) of Indigo Naturalis, Sichuan pepper, Euonymus and Indigo naturalis were respectively 10.3, 5.2, 12.7 and 6. Besides, mRNA expression level of viral NP protein gene (ORF7) determined by real-time PCR were significantly inhibited within certain range of concentration. Results of this study confirm that extracts of four herbals have obvious anti-PRRSV effects, the best effect found in extract of Euonymus.

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Effects of ozone, ultraviolet and disinfectant against Porcine Reproductive and Respiratory Syndrome virus

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A good level of biosecurity practice is most important for efficient PRRS control. The antimicrobial effects of ozone against various viruses and bacteria have been well demonstrated previously. In the current study, disinfecting ability of ozone against PRRS virus was evaluated in comparison with that of ultraviolet and a disinfectant, considering the possible use of ozone for disinfecting farm vehicles, equipments, and materials to reduce the risk of PRRS virus introduction to farms. Two consecutive experiments were conducted to evaluate disinfectant efficiency of ozone against PRRS virus. In the first experiment, 4 rooms that have independent ventilation system were contaminated with VR2332, prepared in 100 ml of 0.01 M PBS at 10^5 $TCID_{50}$/ml per each room. After virus contamination, ozone or UV light was operated in room 1 or 2, respectively, and 10 swabs were collected from each room at 0, 10, 20, and 30 min. In room 3, 10 swabs were collected from the floor right after virus contamination (0 min) then 10, 20, and 30 min after 1L of disinfectant was sprayed onto the floor to cover all floor surfaces contaminated with virus. In room 4, 10 swabs were collected from the floor at 0, 10, 20, and 30 min after virus contamination without any treatment. For *in vivo* evaluation, 3, 4-week old, PRRS-free pigs were introduced into each room right after the last swab collection. In addition, 10 swab samples collected at the last time point (30 min) were pooled per each room and injected intramuscularly in the pigs housed in each room. The pigs were bled before housed in the rooms (0 wk) and at 1 and 2 weeks after housing and injection. Serum samples were subjected to PRRS real-time RT PCR and ELISA to detect evidence of PRRS virus replication. In the second experiment, both *in vitro* and *in vivo* tests were also conducted to evaluate disinfectant efficiency of ozone with shorter exposure times (0, 5, 10 and 20 min), following the same procedures described in the first experiment. While the virus collected after treated with UV and the disinfectant still replicated in pigs, the virus collected after at least 10 min exposure to ozone failed to replicate in pigs. Based on the results, it was concluded that ozone is much more effective in inactivating PRRS virus as compared with UV and disinfectant.

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Preparation and immune activities of specific transfer factor against porcine reproductive and respiratory syndrome

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Objectives
Porcine reproductive and respiratory syndrome (PRRS) is one of immunosuppressive diseases seriously endangering the world's pig production. Transfer factor (TF), a potential immunoenhancer stimulating immune cell activity, regulating immune reponse, enhancing cellular immunity, can transfer cellular immune function from the donor to the receptor. Specific transfer factor (STF) can transfer specific immune information to the receptor and motivate receptor's specific immunity. To improve immune function of pigs infected by PRRSV and increase immune effect of PRRSV vaccine, this study prepared a specific transfer factor against PRRS (PRRS-STF) and analyzed its amino acid composition and immune activities.
Methods
PRRS-STF was prepared from spleens of pigs immunized with PRRS attenuated vaccine by dialysis and its kinds and contents of amino acids were determined using high performance liquid chromatograph (Agilent 1200). STF was injected five times into three BALB/c mice via intraperitoneal route, and phagocytotic percentages in 200 peritoneal macrophages calculated. The proliferative activities of peripheral blood lymphocytes (PBLC) from mice and from four-week healthy piglets inoculated intramuscularly with PRRS attenuated vaccine and STF simultaneously or with the mixture of PRRS attenuated vaccine and STF were detected with MTT, respectively.
Results
PRRS-STF consisted of 21.5μg/ mL of nucleic acid and 3.479 mg/ mL of eighteen amino acids including seventeen essential amino-acids and one taurine. The phagocytotic percentages of peritoneal macrophages from the mice injected with STF and the blank control mice were 54.48% and 39.72%, respectively; and the difference between two groups was significant ($P < 0.01$). The stimulating index (SI) of mouse PBLC transformation with STF was markedly higher than that of mice with ConA or that of blank control ($P < 0.05$). On the 20th day after inoculation, the PBLC transformation SI of piglets inoculated simultaneously with PRRS attenuated vaccine and STF or with their mixture was 2.83±0.32 or 2.96±0.38, which were significantly higher than that of piglets inoculated with vaccine alone ($P < 0.05$). There were no significant differences ($P > 0.05$) between the two groups of injection STF.
Discussion
PRRSV-STF consisted of a variety of essential amino acids for protein synthesis and one taurine closely related to cystine and cysteine metabolisms. It could not only enhance peritoneal macrophage phagocytosis and PBLC proliferative capacities of mice but also significantly increase proliferation capacities of PBLC from porcine vaccination with PRRS attenuated vaccine. PRRSV-STF can be expected as one immune agent to improve immune function of pigs infected with PRRSV and immune effects of PRRS vaccine.

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Possible cross-species infection of porcine circoviruses and its control measures

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The controversy on whether PCV infected humans and other animals existed in academic circles. However, recently, some literatures reported PCV2 was detected in human stools, cattle, beef, flies and mosquitoes. The present paper mainly reviewed possible cross-species infection of porcine circoviruses including foodborne infection (to ingest or contact fresh pork, to ingest undressed beef or fresh milk and to drink raw water), vaccination infection, xenotransplantation infection, airborne infection, vector infection, leech therapy and its control measures including (1. To reduce ingestion or contaction with fresh pork products, install different cutting boards used for different food; 2. To eat cooked beef/not to drink raw milk; 3. To drink safe water; 4. To use safe vaccines or porcine-derived commercial products; 5. To reduce or prevent the bites of flies and mosquitos; 6. To treat patients using non-PCV infection leeches; 7. To take bio-security measures in pig farms, such as wearing masks, etc.) for human and (1. To take bio-security measures in pig farms, such as kill mice, flies and mosquitos; 2. To use safe vaccines, especially live vaccines, such as CSFV, PRRSV, PRV; 3. To provide safe and clean water for pigs; 4. To strengthen PCV2 antigen detection and eliminate PCV2-positive pigs; 5. To improve nutrition and conditions, animal welfare, and non-specific immunity for pigs) for pigs and other animals in order to attract a great deal of attention in public health and cross-species transmission.

To summarise, PCV, especially PCV2, brought about more and more serious diseases (PCVAD) for swine herds, so its control also became more and more difficult for veterinarians. However, PCV1 and PCV2 were also detected in human stools, vaccines of human use, beef, pepsin, rodents. What about its pathogenicity for humans? Whether horizontal transmission or vertical transmission caused by PCV could occur among human populations? Whether PCV2c, PCV2d, PCV2e or PCV2f exist in humans? Whether pigs were infected through infected humans again? In future, through more studies from scientific researchers, we can get some reasonable answers to those puzzled questions.

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Preparation of monoclonal antibody against PRRSV Nsp9 gene and establishment of block-ELISA method

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Background: The RRSSV Nsp9 gene coding RNA dependent RNA polymerase of the virus, which taking part in process of the duplication is very stable. The bodies do not have immunoreaction if no prevalent field infect. Therefore we can distinguish prevalent field and inactivated vaccine. This research make up a method of ELISA that can distinguish the PRRS prevalent field and inactivated vaccine using the rebuilding Nsp9 protein take the monoclonal antibody (2D6). So we can take a Serological surveillance in the pig farm to make there is no swine been infected.

Materials and Methods: Prokaryotic express and purify the Nsp9. Using the protein (Nsp9) vaccine the Balb/c, we successfully take 3 monoclonal antibody (2D6, 2C11, 4E6). We find the 2D6 have higher immunogenic activity than others we take by Western blot. We set up a method of ELISA to detect the swine which are infected by PRRSV using 2D6 monoclonal antibody.

Results: Our lab constructed the prokaryotic expression vector that can express the gene of PRRSV Nsp9 and take the Nsp9 protein. By Cell fusion experiment, we find the hybridoma cell lines (2D6, 2C11, 4E6) which can product monoclonal antibodies. We set up a method of ELISA to detect the swine which are infected by PRRSV using 2D6 monoclonal antibody. The result is very sensitive though we use the method to detected 170 serum samples.

Discussion: The PRRSV Nsp9 gene which code RNA-dependent RNA polymerase (RdRp) participate the replication of viral. In order to avoid the virulence return strong, attenuated vaccine can’t be used in breeding pig farm, in view of the inactivated virus can not copy, the body cannot produce Nsp9 antibodies. We use the Nsp9 protein as the envelope antigen, the combined with monoclonal antibody 2D6 can be block up if there has corresponding antibody in the sample serum, and last shows the lower of ODp values and prove the exist of PRRSV infection.

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The analysis of genetic variation among 19 Nsp2 genes of PRRSV isolated from southwestern China in 2009-2012

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Objectives: Porcine reproductive and respiratory syndrome virus (PRRSV) is an endemic virus in pig farms worldwide and causes persistent or acute infection depending on the viruses and other unknown conditions. Previous study showed that PRRSVs isolated over the world are antigenically and pathologically heterogenic and a fruitful genetic diversity was detected in viral genome, especially in viral Nsp2 gene, in which a remarkable sequence variation, including the nucleotide substitution, deletion and insertion, was widely reported(Zhou, et al 2009,Tian,K. et al, 2007). Nsp2 has become the most sensitive region to choose for veterinary clinical diagnostics and the research on PRRSV evolution. In the present study, 19 Nsp2 cDNAs of PRRSV isolated in southwestern China were sequenced and analyzed.

Brief methods: Tissue samples suspected to PRRSV infection were collected from large scale farms in southwestern China during 2009-2012. Viral RNA was extracted from each sample, followed by RT-PCR amplification of the complete Nsp2 gene. The Nsp2 cDNAs were then cloned and sequenced for phylogenetic analysis as described previously (Zhou, Y et al,2012 ,) .

Results: Total nineteen PRRSVs were isolated from the suspected samples collected from different pig farms during 2009-2012. Twelve Nsp2 genes out of the 19 viruses were sequenced in 2009-2011 (access number: JN836554-JN836565) and the other seven were sequenced in 2012. Four different sizes of Nsp2 cDNA were detected among the 19 viruses, including two with 2940bp, 15 with 2850bp, one with 2847bp and one with 2841bp. The obvious size diversity of Nsp2 indicates a complicated population of PRRSV existing in the region. In addition, there are a lot of nucleotide mutants in the 19 Nsp2 genes. When compared with the Nsp2 of VR2332, 379-500 nucleotide mutants were found in each Nsp2 sequence and it seems more point mutations existed in the seven viruses isolated in 2012. The phylogenetic analysis revealed that two viruses are belonged to the classic-PRRSV, located in the VR-2332 and CH-1a subcluster, and all the other 17 viruses were located in one subcluster, grouped with the HP-PRRSV subcluster, such as Em2007 and JXA1, indicating that the majority of PRRSV sporadic outbreak during 2009-2012 in southwestern China may be derived from the HP-PRRSV outbreak during 2006-2007 and they are continuously evolving.

Conclusion: The current study demonstrated that HP-PRRSV and C-PRRSV were co-circulating in local pig farms and HP-PRRSV was a predominant virus in southwestern China. Based on the analysis of Nsp2 variation, we found that the HP-PRRSV is undergoing genomic divergence, and novel mutants might be continuously emerging. The present data provide a deep insight into endemic PRRSV and also suggest the necessity for continuous surveillance of PRRSV in the region.

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Autophagy is involved in genetic resistance to PRRSV infection

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The porcine reproductive and respiratory syndrome virus (PRRSV) is a major threat to the swine industry. In the United States alone, revenue totaling more than $600 million per year is lost due to PRRSV. Autophagy is a highly conserved catabolic process that involves degradation of intracellular components via the lysosome in mammalian cells. Thus, autophagy can serve as an antiviral defense mechanism. The objective of the present study was to investigate an autophagy-related gene that is involved in autophagosome formation and determine its role in genetic resistance to PRRSV infection. Reverse-transcription PCR analysis revealed that this gene was highly expressed in pituitary, lymph node, and placenta of healthy animals, besides, it was also expressed in lung, ovary, and skin and we got the same result from real-time PCR that this gene has a low Ct value in placenta, lymph node, pituitary ovary but almost nothing in lung, and vagina. Primers were designed to target different regions of the gene and direct sequencing of PCR products revealed 24 single nucleotide polymorphisms (SNPs). Five of these SNPs were genotyped on more than 600 pig samples collected through the National Pork Board funded PRRS Host Genomic Consortium (PHGC) that viral load and pig body weight were collected at 10 different time points. Further analysis with Haploview identified three tagged SNPs. Association analysis using PHGC1-3 trial animals showed that this gene is significantly associated with both virus levels and pig body weights at different time points post-infection with PRRSV. The electrophoretic mobility shift assay was used to detect the functional variants as regulatory elements among three SNPs in the promoter region. For each variant, two 31 bp oligo pairs containing non-risk and risk alleles were used as probes to detect nuclear-binding protein. We discovered that one SNP showed differential binding affinity to the nuclear protein from lymph node, placenta and ovary tissues. Characterization of the regulatory protein is underway. In brief, our study provides evidence that autophagy is involved in genetic resistance to PRRSV infection.

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Cloning and functional analysis of 5′regulatory region of porcine CYP3A88 gene

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Introduction: Cytochrome P450 is a set of structures and functions related heme isozyme, participating in the transformation process of many exogenous compounds and playing a very important role for the organism's metabolism. CYP have three families including CYP1, CYP2 and CYP3, among whom CYP3A subfamily is the main member. CYP3A Four subtypes (CYP3A22, CYP3A29, CYP3A39, CYP3A88) were found in CYP3A family. By microarray approach we compared the changes in gene transcription in lungs of Dapulian pigs (DPL) and Duroc ×Yorkshire × Landrace commercial pigs (DYL) that occur after infection with PRRSV in vitro, to screen differentially expressed genes between these two pig breeds. The results of microarray and real-time qPCR showed that differences in the mRNA expression of CYP3A88 gene exist between DPL and DYL pigs, the mRNA expression of CYP3A88 gene is significantly lower in DPL compared to DYL pigs, suggesting that CYP3A88 may be involved in PRRSV. Studies on regulation and the role of CYP3A88 gene in causing disease is unknown, in this study, we carried out a preliminary study to characterize the regulation and function of porcine CYP3A88 gene as is related to the resistance to PRRSV.

Materials and methods: In this study we obtained the 5 ' regulatory region of porcine CYP3A88 gene by the Genome walker methods. Vectors varying in promoter size and with mutation were constructed by restriction enzyme digestion methods and site-directed mutagenesis, respectively. To analyze the promoter of CYPA88 gene, dual-luciferase reporter assay system was used to detect luciferase activities of different vectors through cell culture and transfection approach.

Results and discussion: In this study, based on previous study of microarray and fluorescence quantitative PCR experiments, we analyzed the function of the regulatory region of porcine CYP3A88 gene. Deletion experiment revealed that deletion from −694bp to −282 bp of the 5′-upstream region of the promoter increased transcriptional activity markedly ($P<0.05$), suggesting the existence of the cis-acting elements that reduce the expression of porcine CYP3A88 gene. Further deletion from −282bp to −95bp caused significant decrease in luciferase activity ($P<0.05$), suggesting the presence of the cis-acting elements that enhance the expression of porcine CYP3A88 gene. When the fragment was deleted to −27 bp , the promoter activity was nearly lost, suggesting that the region between −95 bp and −27 bp in the CYP3A88 gene promoter region contains the core promoter elements. The −78 T >A mutation in the important regulatory region likely caused the binding of transcription factor YY1 as predicted with TESS software. When YY1 binding site is mutated the promoter activity in driving transcription was reduced significantly ($P<0.05$), suggesting that YY1 could stimulate transcription of CYP3A88 gene, and is consistent with the fact that the expression level of CYP3A88 was higher in PRRSV susceptible pigs. By molecular marker selection on pigs with lower expression of CYP3A88 gene to form a basis group for breeding, we may improve the resistance of pigs to PRRSV.

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Generation of an infectious clone of SDA2, a highly virulent North American-type isolate of porcine reproductive and respiratory syndrome virus

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Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically significant swine diseases. The porcine reproductive and respiratory syndrome virus (PRRSV) causes reproductive failure in sows and respiratory disease in pigs of all ages. Recently, full-length infectious cDNA clones have been developed to identify the virulence factors and to study pathogenic mechanisms associated with the virus. Here, a field strain of PRRSV was isolated and identified as a highly virulent PRRSV variant named SDA2. The complete genome of SDA2 was sequenced and deposited in GenBank under accession no. JX878379. Here, we reported that the genome of a highly pathogenic PRRSV isolate SDA2 was assembled into the pBAC vector (to generate pBAC-SDA2), which carries the CMV promoter. pBAC-SDA2 contains a unique Sma I site in the ORF7 gene, which serves as genetic marker. pBAC-SDA2 was transfected into MARC-145 cells, which then produced infectious SDA2 progeny. The rescued virus could be stably passaged in MARC-145 cells, and showed similar growth patterns and viral plaque formation to those of the parental virus. Compared with other infectious cDNA clones constructed with T7 prometer, pBAC-SDA2 is easier to be rescued using the pBAC system under CMV promoter and it will be useful tool to study the molecular mechanism underlying PRRSV replication and virus-host cell interactions.

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Cloning and functional analysis of 5′ regulatory region of porcine USP18 gene

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Introduction

USP18 is a member of ubiquitin-specific proteases (UBP) family and encodes deubiquitinating enzymes(DUBs).Previous studied indicated that deubiquitylation has an important role in the regulation of both innate and adaptive immune responses. Overexpression of the porcine USP18 leads to reduced replication of PRRSV(Ait-Ali et al., 2009). Zhang et al. (1999) found that USP18 is specifically up-regulated in porcine lung and tonsil tissues after PRRSV infection, implying that the USP18 gene is involved in resistance to PRRSV. In order to study the relationship of expression level of USP18 and the resistance to porcine reproductive and respiratory syndrome, we used a porcine microarray to investigate changes in gene transcription in lungs of Dapulian pigs and Duroc ×Yorkshire × Landrace commercial pigs that occur after infection with PRRSV in vitro, to screen differentially expressed genes between these two pig breeds. The results of microarrya and real-time PCR showed that difference in the mRNA expression of USP18 gene in tissues exist between DPL and DYL pigs, the mRNA expression of USP18 gene is significantly higher in DPL compared to DYL pigs, suggesting that USP18 may be involved in PRRSV. The objective of this study was to explore possible mechanisms of USP18 restriction of PRRSV infection in DPL pigs by analysis of promoter region of porcine USP18 gene, to provide markers for genetic selection against PRRSV susceptibility in further pig breeds.

Materials and methods

The vectors varying in promoter size and mutation vectors were constructed by PCR and restriction enzyme digestion methods, respectively. To analyze the promoter of USP18 gene, dual-luciferase reporter assay system was used to detect luciferase activities of different vectors through cell culture and transfection technology.

Results and discussion

Cloning a 2481bp DNA fragment of 5′-regulatory region of USP18 gene of DPL and DYL commercial pigs was carried out by PCR and were introduced into pGL3-Basic vector. The luciferase activities were determined by dual-luceferase assay system kit after transfection of MARC-145 cells. Statistical analysis showed that luciferase activities was obviously higher with promoter cloned from DPL than from DYL pigs ($p<0.001$). Deletion experiment found that deletions from-1812bp to-1336 bp of the 5'-upstream region of the promoter decreased transcriptional activity markedly($P<0.05$), suggesting the existence of potential positive-regulatory elements which enhances USP18 transcription from–1812 bp to –1336bp; when the fragment from -1336bp to – 836bp was deleted, a significant increase in luciferase activity ($P<0.05$) was observed, suggesting the presence of potential transcriptional inhibitory elements decreasing USP18 transcription between -1336bp and – 836bp. Analysis of USP18 promoter by direct sequencing showed that there was a mutation site(-1533 A>G) in the fragment varying from –1812bp to –1336bp which caused the binding of transcription factor FOXI1a analyzed by polymorphism analysis combined with TESS software. Site-directed mutagenesis of FOXI1a site caused a significant decrease in promoter activity. We hypothesize that FOXI1a is a transcription factor that upregulates USP18 promoter transcriptional activity, and the mutation in it leads to the significantly higher expression levels of USP18 mRNA in DPL pigs compared to DYL pigs. Based on the marked differences between these two breeds in regard to the resistance to PRRSV, further study of the molecular mechanisms of resistance PRRSV is required by functional analysis of 5′-regulatory region of porcine USP18 gene.

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Porcine circovirus type 2 (PCV2): genetic variation and newly emerging genotypes in China

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Background: Porcine circovirus type 2 (PCV2), the causative agent of postweaning multisystemic wasting syndrome (PMWS), is a serious economic problem for the swine industry in China. In this study, we investigated the genetic variation of PCV2 in China using strains isolated from 2004–2008. Viruses were isolated from samples collected from pigs with multi-systemic lesions and clinical signs of PMWS from different regions of China, and the genomes of these viruses were sequenced. The assembled sequences were used to define the genotypes of these strains; PCR-RFLP methodology was used to distinguish isolates and capture ELISA was used to demonstrate the antigenic changes resulted from ORF2 gene mutation of the isolates.
Results: We identified 19 PCV2 isolates, including four newly emerging PCV2 mutant strains. The 19 isolates were designated into three genotypes (PCV2a, PCV2b and PCV2d). PCV2d represented a novel genotype and a shift from PCV2a to PCV2b as the predominant genotype in China was identified. This is the first report of 1766 nt PCV2 harboring a base deletion at other new different positions. Amino acid sequence analysis identified two novel ORF2 mutations (resulting in ORF2 sequences 705 and 708 nt in length) in three deletion strains (1766 nt) and one strain with a genome 1767 nt in length. Finding of two amino acids elongation of the ORF2-encoded Cap protein is firstly observed among PCV2 strains all over the world. The isolates were distinguished into different genotypes by PCR-RFLP methodology and antigenic changes were present in Cap protein of mutation isolates by capture ELISA.
Conclusions: The results of this study provide evidence that PCV2 is undergoing constant genetic variation and that the predominant strain in China as well as the antigenic situation has changed in recent years. Furthermore, the PCR-RFLP method presented here may be useful for the differential identification of PCV2 strains in future studies.

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Analysis of synonymous codon usage in Porcine Reproductive and Respiratory Syndrome virus

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It is well known that the genetic code chooses 64 codons to represent 20 standard amino acids and stop signals. These alternative codons for the same amino acid are termed as synonymous codons. Although synonymous mutations tend to occur in the third base position, the cases can be interchanged without altering the primary sequence of the protein product. Some reports indicate that synonymous codons are not chosen equally and randomly both within and between genomes. Generally, translation selection in nature and compositional constraints under the mutational pressure are thought to be the two major factors accounting for codon usage variation. In some RNA viruses, compared with translation selection in nature, mutation pressure plays an important role in synonymous codon usage pattern. PRRSV is an enveloped, single-stranded positive-sense RNA virus. There are Northern American isolate (NA) and the European isolate (EU). PRRSV genome contains ORF1a, encoding papain-like cysteine protease, ORF1b, encoding RNA dependent RNA polymerase, ORF2-6, encoding envelop proteins, and ORF7, encoding the nucleocapsid protein. Little information about codon usage pattern of PRRSV genome including the relative synonymous codon usage (RSCU) and codon usage bias (CUB) in the process of their evolution are available. In this study, we calculated RSCU values and CUB values to implement a comparative analysis of codon usage pattern of open reading frames (ORFs) which belong to the two main genotypes of PRRSV. By analysis of synonymous codon usage values in each ORF, the optimal codons for most amino acids were all C or G-ended codons except GAU for Asp, CAU for His, UUU for Phe and CCU for Pro. The synonymous codon usage patterns in different ORFs of PRRSV were different and genetically conserved. Among them, ORF1a, ORF4, ORF5 and ORF7 could cluster these strains into the two main serotypes (EU and US). Due to mutational pressure, compositional constraint played an important role in shaping the synonymous codon usage pattern in different ORFs, and the synonymous codon usage diversity in ORFs was correlated with gene function. The degree of CUB for some particular amino acids under strong selection pressure probably served as a potential genetic marker for each ORF in PRRSV. However, gene length and translational selection in nature had no effect on the synonymous codon usage pattern in PRRSV. These conclusions could not only offer an insight into the synonymous codon usage pattern and differentiation of gene function, but also assist in understanding the discrepancy of evolution among ORFs in PRRSV.

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Antiviral activities of the porcine IFIT family genes against PRRSV

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Porcine reproductive and respiratory syndrome (PPRS), caused by a small RNA virus (PRRSV) has devastated the pig industry worldwide for many years. This disease is characterized by severe reproductive failure in sows and respiratory disease and high mortality in piglets, causing $644 million in losses annually. Vaccination for protection of pigs against PRRSV seems ineffective due to its high genetic diversity. As such, improved genetic resistance to PRRSV should be a powerful option for the industry to efficiently combat the mysterious disease: PRRS. The Interferon-induced protein with tetratricopeptide repeats (IFIT) family is a prominent family of interferon stimulated genes (ISGs) that possess strong antiviral function. Our previous microarray data and serial analysis of gene expression (SAGE) database showed that porcine *IFIT1*, *IFIT2* and *IFIT3* were up-regulated in porcine alveolar macrophages (PAM) during PRRSV infection both in vivo or in vitro. The objective of this study was therefore to determine if porcine IFIT family genes are involved in the anti-PRRSV process. To this end, , we annotated the porcine IFIT family genes (*IFIT1*, *IFIT2*, *IFIT3* and *IFIT5*) based on ESTs and current genome assembly. We observed that *IFIT1*, IFIT3 and *IFIT5* have only one transcript, whereas *IFIT2* might possess two transcripts. Reverse-transcription PCR revealed that IFIT family genes were expressed in nine tissues, but only abundantly in lung, spleen and heart. Three immune-related tissues (lung, spleen, lymph node) were selected to pursue real time PCR analysis. In addition, IFIT family genes were dramatically up-regulated in these three tissues after challenge with PRRSV. To date, 30 single nucleotide polymorphisms (SNPs) have been discovered in IFIT family and 16 of them were genotyped on more than 600 samples, provided by the PRRS host genetics consortium. Statistical analysis showed that those SNPs were significantly associated with virus load at different time points post-infection. The SNPs in the promoter regions of IFIT family genes may affect transcriptional factors binding. Therefore, our current findings showed that IFIT family plays an important role in genetic resistance to PRRSV, and may help the pig industry to control PRRS disease.

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PRRS modified live vaccine adjuvanted with Montanide™ Gel01 ST provides enhanced protection against homologous PRRSV infection in swine

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Porcine reproductive and respiratory syndrome (PRRS) is a devastating disease caused by the PRRS virus. The Montanide™ class of flexible polymeric adjuvants has recently been shown to enhance protective immunity against PRRSV infection in piglets when used in combination with PRRS modified live vaccines (MLV). In this study, we explored the efficacy and immunological mechanisms of protection of Montanide™ Gel01 ST (Gel01) adjuvanted modified live PRRSV vaccine in pigs challenged with two genetically distinct strains of PRRSV. Gel01-MLV reduced lymph node pathology scores in pigs challenged with VR-2332 (parental strain of MLV vaccine) but not that in pigs challenged with MN184A (heterologous strain), when compared to that in pigs vaccinated with un-adjuvanted MLV. Pigs vaccinated with Gel01-MLV had higher levels of PRRS-specific antibodies, as measured by IDEXX ELISA and virus neutralizing antibodies, after vaccination and VR-2332 challenge. In addition, pigs vaccinated with Gel01-MLV had decreased levels of IFN-gamma, IL-10, and T-regulatory lymphocytes in the blood as compared to that in pigs vaccinated with MLV alone. Interestingly, we found that addition of Gel01 did not change the profile of other T lymphocyte populations after PRRSV challenge. These results demonstrate that the MLV adjuvanted with Gel01 provides enhanced protection against homologous PRRSV infection, possibly by regulating the production of PRRSV-specific antibodies and cytokines involved in the development of T-regulatory cells. Thus, Gel01 ST is a promising adjuvant that can be formulated with PRRSV MLV vaccines to reduce disease severity and tissue damage caused by PRRSV infection in pigs.

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Distribution of PRRSV and PCV2 in sera and tissues and pulmonary findings after PCV2 vaccination in field pigs

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Specific objective: It is well known that porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) are the main causes of porcine respiratory disease complex (PRDC) and the most important pathogens in Taiwan pig farms. The Boehringer Ingelheim CircoFLEX ® was the only PCV2 vaccine marketed in Taiwan when this study was conducted at that time. The purpose of this study was to evaluate its influence on distribution of PRRSV and PCV2 in sera and tissues as well as the pulmonary changes after PCV2 vaccination in the field pigs.
Methods: In this field study, 300 4-week-old pigs were divided into vaccinated and control groups, with 150 pigs each in three pens respectively in the same house. The pigs in the vaccinated group were intramuscularly injected with one dose of BI CircoFLEX® vaccine and the pigs in the control group with one ml of saline. Twenty pigs in each group were randomly selected and tagged. Among them, four pigs in each group were humanely euthanized at days 0, 7, 21, 35 and 49 after vaccination. The lung lesions were macroscopically examined, scored and collected. The sera, lymph nodes and spleens were also collected at same time to quantify the amounts of PRRSV RNAs by reat-time PCR. ANOVA analysis was performed to compare significant difference between groups.
Results and conclusions: Significant difference on the scores of lung lesions was seen in pigs between vaccinated and control groups at day 21. It was also found that the pigs in the vaccinated group had lower quantities of PRRSV RNAs than those in control group, as significant difference seen in lung, lymph node and spleen at day 49 ($p<0.05$). Taken together, the usage of BI CircoFLEX® could reduce the lung lesions and tissues' PRRSV RNAs in vaccinated pigs and so that could provide them at certain level of protection from PRRSV infection.

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The effects on distribution of pathogens, reproductive performance of sows and growth performance of weaned pigs after attenuated live PRRSV vaccination in sows

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Specific objective: Porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases in Taiwan pig farms. Vaccination with modified live vaccine is one of the solutions to control PRRS. The objective of this study was to investigate the effect of sow vaccinated with an attenuated live vaccine under different levels of antibody titers in field condition.
Methods: This trial was conducted in a farrow to finish pig farm, with 1400 sows in a single site. A total of sows (N=84) in a batch during their 75th -84th days of pregnancy were selected and their antibody titers were tested by a commercial ELISA kit (IDEXX herd check). Animals with either negative (S/P<0.4) or medium level of titer (S/P values between 1.2-1.5) were excluded in this trial. The rest of the sows (n=60) were then assigned into low and high titer groups (L: S/P in between 0.4-1.2, n=30; H: between 1.5-3.3, n=30). Then, these 30 sows in each group were further divided into two treatment groups of vaccination and non-vaccination control groups (LV, n=15; HV, n=15; LC, n=15, HC, n=15). The sows in the vaccination groups were injected with one dose of Ingelvac PRRS® MLV. All of the piglets farrowed from those sows were weaned at 4 weeks of age, ear tagged and mixed into 4 nursery barns with the same feed and water supply. Reproductive parameters including farrowing rates, total pigs born, pigs born alive, average piglet bodyweight, number of stillborns, pigs weaned, average weaning weight, and pre-weaning mortality and growth performance including average daily gains, feed conversion rates, and mortalities were collected. Positive rates of PRRSV and PCV2 in the serum and tonsil scrapings of all tested pigs were also examined and detected by RT-PCR and PCR. Statistical significance between and among groups was evaluated by t-test.
Results and conclusions: A significant difference on the lower PRRSV-positive rates was seen in HV treated pigs when compared with those in other groups, leading to a better performance on the average weaning weight and pre-weaning mortality in the suckling pigs and average daily gain in weaned pigs in HV group. Based on the results, we conclude that keeping higher immune status with Ingelvac PRRS® MLV boosting vaccination against PRRV in the sows might provide better protective maternal antibodies for their farrowing pigs till their early weaning period.

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Evaluation of a DNA vaccine candidate co-expressing GP3 and GP5 of PRRSV with interferon alpha/gamma in immediate and long-lasting protection against highly pathogenic PRRSV challenge

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PRRSV has become one of the most economically important diseases to the global pork industry. Current vaccination strategies only provide a limited protective efficacy. In this study, a DNA vaccine pVAX1©-α-γ-GP35 co-expressing GP3 and GP5 of PRRSV with interferon alpha/gamma was constructed and the immediate and long-lasting protection against highly pathogenic PRRSV (HP-PRRSV) challenge were examined in pigs. For immediate protection, the results showed that pVAX1©-α-γ-GP35 could provide partially protective efficacy, which was similar with the pVAX1©-α-γ (expressing interferon alpha/gamma). For long-lasting protection, pigs inoculated with pVAX1©-α-γ-GP35 developed significant higher PRRSV-specific antibody response, T cell proliferation, IFN-γ and IL-4 than those vaccinated with pVAX1©-GP35 (expressing GP3 and GP5 of PRRSV). Following homologous challenge with HP-PRRSV strain SD-JN, pigs inoculated with pVAX1©-α-γ-GP35 showed almost no clinical signs, no lung lesions and significantly lower viremia, as compared to those in pVAX1©-GP35 group. It indicated that pVAX1©-α-γ-GP35 could induce enhanced immune responses and provide both immediate and long-lasting protection against HP-PRRSV challenge in pigs. The DNA vaccine pVAX1©-α-γ-GP35 might be an attractive candidate vaccine for the prevention and control of HP-PRRSV infections.

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The immunization of pigs with a Gp3 subunit vaccine elicits specific antibody response but does not protect against PRRSV infection after challenge

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Porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases in pig industry, causing high economical losses worldwide. Since the emergence of the disease more than 20 years ago, a lot of efforts have been focused on the development of efficacious vaccines. Live-attenuated viruses are the most commonly used PRRSV vaccines, but they are not fully protective. Several subunit vaccines were designed based on different PRRSV proteins. Gp3 was reported to carry antigenic regions (AR) generating neutralizing antibodies (NAbs). For this reason, a fragment of the Gp3 protein (Gp3.14) from the Olot/91 PRRSV strain carrying one AR and lacking the signal sequence and the endodomain was cloned and expressed using the baculovirus system. The recombinant GP3.14 protein was purified by Immobilized metal ion affinity chromatography and used to immunize pigs. The vaccine dose consisted of 100 μg protein (GP3.14 or an unrelated FMDV peptide as control) mixed 1:1 with Montanide ISA 206 VG adjuvant (SEPPIC SA). Two groups of five 20-week-old pigs were vaccinated twice with 31 days interval by intramuscular injection of the GP3.14 vaccine formulation or with the control peptide formulation, respectively. In order to determine the protective efficacy of the vaccine, the pigs were challenged intranasally with 10^6 PFU/pig of Olot/91 virus 25 days after the 2nd vaccination. Blood was collected at regular intervals. The animals were euthanized 28 days after challenge infection. Viremia was determined by quantitative RT-PCR. GP3-specific antibodies were detected by immunofluorescence assays and by ELISA using Gp3 polypeptides as coating antigens. All pigs seroconverted against GP3 before challenge, but no signs of protection from viremia were observed after homologous challenge infection with PRRSV Olot/91. Accordingly, there were no detectable NAbs in the vaccinated animals.

There are several reports suggesting that Gp3 elicits neutralizing antibodies and is involved in protection from virus infection. The present study shows that the Gp3.14 protein can induce a specific antibody response. However, the immune response against GP3.14 is not sufficient to protect pigs from viremia with the immunization protocol used, probably due to the lack of sufficient NAbs. With subunit vaccines it was reported that the immunization protocol can influence the production of NAbs. Thus, although Gp3.14 alone did not generate NAbs, combination with other PRRSV vaccine formulations in a prime boost protocol may be evaluated.

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Development and validation of porcine reproductive and respiratory syndrome virus specific neutralizing antibody detection assay in pig oral fluid samples

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PRRS virus (PRRSV) neutralizing antibodies (NA) are important to control the disease. Pen-based pig oral fluid sample submissions for disease surveillance and diagnosis have increased recently due to ease of collection and cost-effectiveness. Detection of virus NA in oral fluid samples may be a good indicator of immunological resistance to certain viral infections. Objective of this study was to develop and validate the virus neutralizing test (VNT) to identify NA titers against PRRSV in pen-based pig oral fluid samples. At first, we standardized PRRS VNT using pooled pen-based oral fluid samples (n=37) collected 11 times over a period of three months from a commercial swineherd vaccinated with PRRSV modified live vaccine (PRRS-MLV). We also tested 200 oral fluid and serum samples collected from individual boars at day 21 post-vaccination with PRRS-MLV or infected with a Type 2 PRRSV (MN184 strain). Our results suggested that PRRSV NA titer of greater than 1:8 in oral fluid samples is virus specific, but it could be detected from post-vaccination or infection day 28 onwards. Further, to validate this assay, we tested 104 pen-based oral fluid and representative five serum samples from each pen collected from a wean-finish-swineherd. In addition, 100 oral fluid samples of litters from four breeding farms (25 samples each) collected immediately after weaning, and serum samples of sows of respective litters vaccinated repeatedly (4-5 times using PRRS-MLV and PRRS-ATP) were analyzed. Our results suggested that, NA titers in pig oral fluid samples were associated with the respective serum titers. We could detect high levels of PRRSV specific NA titers in litters' oral fluid samples, suggesting the maternal transfer of NA. Analysis of our VNT data has indicated the diagnostic specificity of 94.3% and repeatability of 90.5%. We are in the process of detecting diagnostic sensitivity of the assay using large number of individual serum and oral fluid samples of pigs with the proper history of vaccination or infection with PRRSV. In conclusion, we have standardized and validated an assay to detect PRRS NA titers in pen-based pig oral fluid samples, and it has the potential to monitor PRRS herd immunity in infected and/or vaccinated swineherds. KO and BB contributed equally to this research. This project was supported by USDA-NIFA PRRS CAP2 and OARDC, The Ohio State University to RJG. KO is a recipient of China Scholarship Council (CSC#2011666005) fellowship.

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Characterisation of the T cell response induced by infection with subtype 1 or 3 European porcine reproductive and respiratory syndrome viruses

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The porcine reproductive and respiratory syndrome virus (PRRSV) is a rapidly evolving and diversifying pathogen for which there is a need for the development of improved vaccines. Immunity to PRRSV is not well understood although there are data suggesting that virus-specific T cell IFN-gamma responses play an important role. We therefore aimed to better characterise the T cell response to European genotype PRRSV, with an emphasis on identifying and characterising the major T cell antigens. Groups of pigs (n=5) were experimentally infected with an attenuated subtype-1 strain (Olot91) or with a pathogenic subtype-3 virus (SU1-bel) and then challenged with the homologous viruses after 35 days. PBMC were stimulated with viruses and IFN-gamma responses assessed by ELISpot assay or multi-parameter flow cytometry. Virus-specific CD4 and CD8 T cell IFN-gamma responses were detected from day 14 post-infection. Responses increased significantly from day 30-35 and there was no evidence of boosting upon secondary infection. Twenty-one days post-primary and secondary infection, PBMC were stimulated with pools of synthetic peptides representing the 19 PRRSV proteins. While T cell reactivity was observed against a number of PRRSV proteins, significant responses were observed in both groups against the structural M protein and the non-structural protein 5 (NSP5), which were particularly dominant after the second infection. CD4 T cell responses were identified in 3/10 animals and all were directed against a single epitope on the M protein. In total, CD8 T cell responses were directed against three epitopes on NSP5 and five epitopes on the M protein, with individual animals showing distinct reactivity profiles which associated with specific MHC haplotypes. Further characterisation of the M and NSP5-specific CD8 T cells showed them to possess the phenotype $CD3^{+}CD4^{-}CD8^{high}CD62L^{-/low}CD44^{high}CD25^{-}CD27^{+/-}$ which suggests a mixed population of effector and effector memory T cells. Significantly, these cells appear to have a cytotoxic function as assessed by surface mobilisation of the degranulation marker CD107a and direct lytic activity against NSP5 peptide pulsed cells. Overall, this study has shown that M and NSP5 proteins represent well-conserved targets of cytotoxic T cell responses from PRRSV-immune pigs and warrants their further evaluation as vaccine candidate antigens.

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Development of a multiplex fluorescent microsphere immunoassay for diagnosis of porcine respiratory disease complex

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Porcine Respiratory Disease Complex (PRDC) is a significant economic problem for swine producers. Development of diagnostic tests that are able to rapidly and simultaneously detect multiple pathogens offer an important tool for disease surveillance and control measurements. In this study, we have developed a multiplexed fluorescent microsphere immunoassay (FMIA) for the simultaneous detection of specific antibodies in oral fluid and serum samples from animals infected with porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SwIV), and porcine circovirus (PCV2). Recombinant nucleocapsid proteins of PRRSV, SwIV, PCV2 were generated and used as antigens that were covalently coupled to Luminex fluorescent microspheres with a distinct spectral address. The FMIA was developed based on testing experimentally derived standard positive and negative control sera or oral fluid samples, and the diagnostic specificity and sensitivity were compared to that generated from the classical enzyme-linked immunosorbent assay (ELISA) or hemagglutination inhibition (HI) test. Based on an evaluation of 1444 oral fluid samples with known serostatus, the oral fluid-based multiplex FMIA achieved greater than 92.0% sensitivity and 80.4% specificity. In serum samples (n = 2484), the multiplex FMIAs reached greater than 98.2% sensitivity and 98.3% specificity. To test the robustness of the multiplex FMIA, we obtained 32 field serum samples that were seropositive for all three PRRSV, PCV2 and SIV. Results showed that multiplexing did not affect the diagnostic sensitivity or specificity of each individual assay. Time course studies showed that the assay can detect antibody responses to PRRSV, SIV and PCV2 as early as 14 days post infection and for greater than 90 days post infection in oral fluid and serum. This study provides a platform for the development of multiplex assays for detecting various swine pathogens simultaneously in preventing and controlling of PRDC. The assay is feasible for large-scale field application on porcine respiratory disease surveillance and epidemiology studies.

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Evaluation of the efficacy of an attenuated live vaccine against highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) in young pigs

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The highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) is characterized by a high fever and a high mortality in pigs of all ages and has severely affected the pork industry of China in the last few years. An attenuated HP-PRRSV strain, TJM, was obtained by passaging the HP-PRRSV TJ strain on MARC-145 cells for 92 passages. Porcine reproductive and respiratory syndrome virus (PRRSV) and antibody-free pigs were inoculated intramuscularly with TJM (105.0 TCID50) and challenged on 28, 60, 120, and 180 days post-immunization (DPI). Results showed that 5/5, 5/5, 5/5, and 4/5 immunized pigs were protected from the lethal challenge and did not develop fever and clinical diseases at each challenge, respectively. Compared to control pigs, vaccinated pigs showed much milder pathological lesions and significantly gained more weight ($P < 0.01$). Sequence analysis of different passages of TJ strain showed that the attenuation resulted in a deletion of continuous 120-aa, additional to the discontinuous 30-aa deletion in the nsp2 region. Analysis also demonstrated that the 120-aa deletion was genetically stable in vivo. These results suggested that the HP-PRRSV TJM was efficacious against a lethal challenge of virulent HP-PRRSV strain, and the effective protection could last at least four months. Therefore, the TJM strain is a good candidate for an efficacious modified-live virus vaccine development, as well as a useful molecular marker vaccine against the HP-PRRSV.

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Virulence evaluation of a modified live highly pathogenic porcine reproductive and respiratory syndrome virus vaccine by serial passages in pigs

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Highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV), the causative agent of porcine reproductive and respiratory syndrome, is responsible for a serious disease in pigs that ultimately results in substantial economic losses in the porcine industry. PRRS viruses are recognized to possess a high degree of genetic and antigenic variability. The purpose of this study was to evaluate the virulence of a modified live HP-PRRS TJM-F92 vaccine by serial passages in pigs and to analyze the genetic changes ofTJM-F92 virus during replication in pigs. Animals were inoculated withTJM-F92 vaccine virus derived from HP-PRRS TJ strain. Five passages of in vivo replication were maintained for 112 days by pig-to-pig passage. All animals were observed for clinical sighs and rectal temperature. The complete genomes of isolates from five animal passages (P1 to P5) were sequenced and compared to the TJM-F92 vaccine virus and its parent strain TJ. All pigs (from P1 to P5) showed no clinical symptoms related to PRRS although the viremic level in pigs from P1 to P5 increased. Sequence analysis showed the continuous 120 amino acid deletion in nsp2 in TJM-F92 vaccine virus existed persistently in vivo, however,29 nucleotide changes resulting in 21 amino acid changes distributed in nsp1β, nsp2, nsp3, nsp9, GP3, GP4, GP5, and N during the experiment. Results showed that TJM-F92 vaccine virus did not show any reversion to virulence.

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Changes of different lymphocyte contents in peripheral blood from pigs co-infected with porcine circovirus type 2 and porcine parvovirus

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Objectives
Porcine circovirus type 2 associated diseases (PCVAD) is one of the most important immunosuppressive diseases seriously endangering the world's pig production. Co-infection with porcine circovirus type 2 (PCV2) and other pathogens is usually necessary for expression of PCVAD, and porcine parvovirus (PPV) is an important synergic pathogenic factor of PCV2. Immunopathologic mechanism of PCV2 and PPV co-infection is, however, unclear up to now. In order to provide the basis for illuminating pathogenisis of PCVAD, this study analyzed the dynamic changes of contents of B lymphocytes, T lymphocyte subpopulations and natural killer (NK) in peripheral blood from pigs co-infected with PCV2 and PPV by flow cytometry.
Methods
Forty-eight healthy, 5-week-old, piglets were randomly divided into four groups (PCV2, PPV, PCV2/PPV and control), twelve per group. Group PCV2 were inoculated oronasally with PCV2 (105.61 TCID50), Group PPV with PPV (106.69TCID50), Group PCV2/PPV with PCV2 (105.61 TCID50) and PPV (106.69TCID50) and pigs in control group with cell culture supernatant from uninfected PK-15 cells. The contents of CD21+MHC-I+ and CD21+MHC-II+ B cells, T cell subsets and NK in peripheral blood from three random pigs in each group were measured on 3, 7, 14 and 35 days post infection (dpi) by FCM.
Results
In pigs co-infected with PCV2 and PPV, contents of CD21+MHC I+ B cells on 3, 7 and 14 dpi and CD21+MHC II+ B cells on 3 and 7 dpi increased significantly and then decreased; contents of both CD3+CD4-CD8+ and CD3+CD8+CD45RA+ T cells on 7 and 14 dpi decreased markedly, and the latter maintained a lower level compared with other groups within the experimental period; contents of CD3+CD4+CD8- T cells on 3, 14 and 35 dpi and CD3+CD4+CD8+ T cells on 14 and 35 dpi rose obviously, and CD3+CD4+CD25+ regulatory T cells sustained a high level; contents of CD3+CD4-CD8- γδT cells on 14 and 35 dpi and CD3-CD4-CD8+ NK on 3, 14 and 35 dpi dropped distinctly.
Discussion
Variation trends of various lymphocytes contents in peripheral blood from pigs co-infected with PCV2 and PPV and those solely with PCV2 were similar, in which the contents of CD3+CD8+CD45RA+ T, CD3+CD4-CD8- γδT, CD3+CD4+CD25+ regulatory T cells and CD3-CD4-CD8+ NK were under serious changes, but the contents' rising or falling in pigs co-infected with PCV2 and PPV was more severe than those in pigs infected alone with PCV2. The results suggested that participation of PPV aggravated immunosuppression induced by PCV2.

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Peptide nanofiber hydrogel: A novel adjuvant for Porcine Reproductive and Respiratory Syndrome virus modified live vaccine

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Porcine reproductive and respiratory syndrome virus (PRRSV) is well known for its genetic variation and ability to change constantly, avoid host defense and establish a long-term infection. Although current vaccines can confer protection against homologous reinfection, their efficacy against heterologous infection is questionable. The objective of this study was to determine whether peptide hydrogel H9e can be used as an adjuvant for PRRS modified live virus (MLV) vaccine and its immunological mechanisms of adjuvanticity. Thirty-five pigs (3-weeks old) were divided into 7 groups (5 pigs/group) including PBS control groups, pigs vaccinated with Ingelvac PRRS MLV vaccine only, and pigs vaccinated with Ingelvac PRRS MLV vaccine adjuvanted with H9e hydrogel. Pigs were challenged with PRRSV VR-2332 or MN184A on day 28 post vaccination. Blood samples were collected weekly after vaccination and blood, lung, and lymph node samples were collected at necropsy (14 days post challenge). It was found that pigs vaccinated with H9e adjuvanted MLV had a higher and prolonger vaccine virus circulating in the blood than pigs that received the unadjuvanted MLV vaccine. Pigs vaccinated with H9e-MLV also developed PRRSV-specific antibodies earlier and had a higher titer of neutralizing antibodies than that from pigs in other groups. More importantly, pigs vaccinated with H9e-MLV had improved protection against both strains of PRRSV demonstrated by reduced viremia, less lung pathology and a robust Th1 type of immune response over pigs vaccinated MLV alone. Although pigs vaccinated with H9e-adjuvanted vaccines had lower frequency of T-regulatory cells at the end of this study, only pigs vaccinated with H9e- MLV had higher frequency of Th/memory cells in tracheobronchial lymph nodes, lung mononuclear cells, and peripheral blood, increased blood concentration of IFN-gamma, and reduced level of IL-10 in the blood. Taken together, our studies suggest that peptide hydrogel H9e is a novel adjuvant that can enhance vaccine efficacy by modulating host humoral and cellular immune responses when it is formulated with PRRS MLV vaccine.

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Attenuation of interferon antagonizing function of PRRSV by targeted mutations in a highly conserved motif of nsp1beta protein

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Nonstructural protein 1 beta (nsp1beta) of porcine reproductive and respiratory syndrome virus (PRRSV) contains a papain-like cysteine protease (PCPbeta) domain, and was determined to be the main viral protein antagonizing host innate immune response. In this study, nsp1beta was determined to suppress the expression of reporter genes as well as suppress "self-expression" in transfected cells, and this activity appeared to associate with its interferon (IFN) antagonist function. To knock down the effect of nsp1beta on IFN activity, a panel of site-specific mutations in nsp1β was analyzed. Double mutations K130A/R134A (type 1 PRRSV) or K124A/R128A (type 2 PRRSV), targeting on a highly conserved motif of nsp1β, GKYLQRRLQ, impaired the nsp1beta's ability to suppress IFN-beta and reporter gene expression, as well as to suppress "self-expression" in vitro. Subsequently, viable recombinant viruses, vSD01-08-K130A/R134A and vSD95-21-K124A/R128A, containing double mutations in GKYLQRRLQ motif were generated using reverse genetics. In comparison to wild-type viruses, these nsp1beta mutants showed impaired growth ability in infected cells, but the PCPbeta cleavage function was not directly affected. The expression of selected innate immune genes was determined in vSD95-21-K124A/R128A mutant-infected cells. The results consistently showed that gene expression levels of IFN-alpha, IFN-beta and ISG15 were up-regulated in cells that were infected with the vSD95-21-K124A/R128A compared with that of wild-type virus. These data suggest that PRRSV nsp1beta may selectively suppress cellular gene expression, including those involved in host innate immune function. Modifying the key residues on the conserved GKYLQRRLQ motif could attenuate the virus growth and improve cellular innate immune responses.

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Comparative respiratory pathogenicity and dynamic distribution of highly pathogenic porcine reproductive and respiratory syndrome virus and its attenuated vaccine strain in piglets

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Objective
The objective of the present study was to compare the pathogenic properties of highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) GD strain and its attenuated vaccine strain GDr180 in piglets.
Methods
For this purpose, a total of thirty 35-day-old piglets were divided in 3 groups, each twelve piglets in group 1 and group 2 were separately intramuscularly inoculated with the GD strain of HP-PRRSV and its attenuated vaccine strain GDr180, six piglets in group 3 served as uninoculated controls. Clinical signs and rectal temperatures were recorded daily. Each two piglets from group 1 and group 2, one piglets from group 3 were necropsied at 3, 7, 14, 21, 28 and 35 days postinoculation(DPI), respectively. The trachea, lung and pulmonary alveolar lavage fluid were collected for H.E. staining, immunohistochemical staining and scanning electron microscopic observation, besides, fluorescent quantitative RT-PCR (FQ-RT-PCR) were applied to determine the viral organic distribution.
Results
The results obtained indicate that piglets of group 1 which inoculated with PRRSV GD strain showed typical clinical symptoms of HP-PRRS, such as persistently high fever, anorexia and death, the macroscopic and microscopic lesions of lung and trachea are prominent; while piglets of group 2 which inoculated with attenuated vaccine strain GDr180 appeared neither clinical symptoms nor macroscopic and microscopic lesions, no difference with negative control piglets of group 3. Nucleic acid or positive cells of PRRSV GD strain were significantly higher than GDr180 strain in lungs and tracheas of the different periods.
Discussion
Taken together, there are significant differences between PRRSV GD strain and GDr180 strain on pathogenicity and virus distribution in the respiratory system of piglets. This difference in pathogenicity may help explain the variation in severity of clinical disease observed in pigs inoculated with attenuated vaccine and should provide for meaningful comparison of PRRSV genotypes.
Reference
[1] Martinez F, Diez F, Segales J, et al. Comparative pathogenicity of type1 and type 2 isolates of porcine reproductive and respiratory syndrome virus (PRRSV) in a young pig infection model[J]. Veterinary Microbiology, 2011, 154: 58-68.
[2] D.-S. Cheon, C. Chae. Distribution of a Korean Strain of Porcine Reproductive and Respiratory Syndrome virus in Experimentally Infected Pigs, as Demonstrated Immunohistochemically and by In-situ Hybridazition[J].J. Comp. Path. 1999 120:79-88.

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Construction and biological characteristics of a recombinant porcine circovirus type 2 expressing the VP1 neutralizing epitope of foot-and-mouth disease virus

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Background: Porcine circovirus type 2 (PCV2) is associated with post-weaning multisystemic wasting syndrome (PMWS) in pigs. The C-terminus of PCV2 capsid protein tolerates insertions of exogenous epitope, and elicits both anti-epitope tag antibodies and anti-PCV2 neutralizing antibodies. The recombinant PCV2 expressing the neutralizing VP1 epitope (aa 141–160) of foot-and-mouth disease virus (FMDV) type O was generated, and its immunogenicity was evaluate in the mice model.

Results: The antigenicity of the rescued virus as recPCV2-CL-VP1 was identified using PCV2- or FMDV-positive serum by an immunoperoxidase monolayer assay (IPMA) and a capture enzyme-linked immunosorbent assay (ELISA). The cloned virus strain was stably multiplied through ten passages in PK-15 cells. The morphological features of the virus were not discernibly different from those of its parental virus (PCV2-CL strain). However, the recombinant virus could be differentiated from its parental virus by PCR and capture ELISA. An in vivo infection experiment using BALB/c mice showed that both recPCV2-CL-VP1 and PCV2-CL could replicate in the mice, cause various pathological changes, and induce a high level of anti-Cap antibodies. The recombinant viral antigen emulsified with Freund's adjuvant to repeatedly immunize the mice induced the generation of antibodies to FMDV-VP1 epitope.

Conclusions: The recombinant virus of PCV2 expressed the neutralizing VP1 epitope of FMDV type O was successfully constructed, and the rescued virus had immunogenicity. It provides a valuable platform to develop novel genetic vaccines.

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Enhanced protective immune response to PCV2 subunit vaccine by co-administration of recombinant porcine IFN-gamma in mice

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Background: The capsid (Cap) protein of PCV2 is the major immunogenic protein that is crucial to induce PCV2-specific neutralizing antibodies and protective immunity; thus, it is a suitable target antigen for the research and development of genetically engineered vaccines against PCV2 infection. IFN-gamma has exhibited potential efficacy as an immune adjuvant that enhances the immunogenicity of certain vaccines in experimental animal models, consideration of IFN-gamma as a component of vaccine formulations is therefore warranted.

Methods: Three recombinant proteins: the PCV2-Cap protein, porcine IFN-gamma (PoIFN-gamma), and the fusion protein (Cap-PoIFN-gamma) of the two proteins were expressed and identified by Western blot and indirect ELISA. Three PCV2 subunit vaccines (the Cap-PoIFN-gamma, PCV2-Cap, and PCV2-Cap+PoIFN-gamma vaccines) were generated and their immunogenicity was investigated in this study. IPMA assay and blocking ELISA were performed to detect, respectively, PCV2-specific IPMA antibodies and neutralizing antibodies in serum samples. Lymphocyte proliferation assay was conducted to evaluate the ability of T lymphocyte proliferation in immunized mice. In addition, routine PCR was used to detect PCV2 viraemia, and qPCR was employed to quantify PCV2 viral loads.

Results: In this study, the results demonstrated that PoIFN-gamma did not only enhance PCV2-specific T lymphocyte proliferative response, but also the humoral immune response to the PCV2-Cap protein when co-administrated with the protein in mice. Following challenge, the mock group developed viraemia, whereas all immunized groups did not develop viraemia; the PCV2-Cap+PoIFN-gamma vaccine significantly reduced the viral loads in lungs compared to the Cap-PoIFN-gamma vaccine and the PCV2-Cap vaccine.

Discussion and conclusion: One potential reason for the enhanced immunogenicity of the PCV2-Cap protein via co-administration with PoIFN-gamma is that it may facilitate the efficiency of the PCV2-Cap protein antigen presentation. However, there was no potent adjuvant effect of PoIFN-gamma on enhancement of both lymphocyte proliferation and antibody responses when delivered as a Cap-PoIFN-gamma; two hypotheses could explain this phenomenon: (1) the conformational changes of fused PCV2-Cap protein and PoIFN-gamma may occur in the Cap-PoIFN-gamma, and thus the two proteins failed to sufficiently exhibit their biological activities; and (2) Cap-PoIFN-gamma antigen presentation was influenced by the decreased function of fused PoIFN-gamma. In summary, our findings demonstrated the effectiveness of co-administration of PoIFN-gamma in enhancing the immunogenicity of the PCV2-Cap protein in a mouse model. The generation of the PCV2-Cap+PoIFN-gamma vaccine against PCV2 infection that we reported here would be widely applicable to other antigens and cytokines.

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Immunity induced by an experimental vaccine based on the recombinant PRRSV GP5 protein without ADE epitope expressed in adenovirus in pigs

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Background: Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of porcine reproductive and respiratory syndromes (PRRS). This disease caused the significant economic loss to the swine industry worldwide, due to reproductive disorders, respiratory illness and systemic diseases in adults and younger pigs. Open reading frames (ORF5) of PRRSV encode the envelope glycoprotein (GP5), which induces neutralizing antibodies in pigs. During PRRSV infection, two epitopes were identified located in ectodomain of GP5. One of these epitopes, the named epitope A is immunodomonant and the epitope B is recognized neutralizing antibodies (NA). In this study, a recombinant PRRSV GP5 protein without ADE epitope expressed in adenovirus in intend to develop new vaccines candidate against PRRSV.
Materials and methods: A PRRSV JL07/SW strain obtained from the Marc-145 cells supernatant, virus RNA was transcribed to cDNA. A desired mutation was designed and GP5 gene was amplified by PCR, using the primers containing the appropriate restriction sites. The amplified GP5 fragment was introduced into the pMD-18T vector and the correct fragment was verified by sequencing. The GP5 gene was digest using Xho I and Hind III, and introduce into pacAd5 CMVK-N pA, (pAD-aGP5). After being treated with Pac Ⅰ and ethanol precipitation, pAd-aGP5 and pacAd5 9.2-100 bone were transfected into 293T cells, when the cells obtain 70–80% confluence. After three freeze-thaw cycles, the natural recombinant adenovirus were harvested.
Results: The sequencing results showed that the obtained aGP5 gene was 588bp, which is theoretical match. The obtained recombinant plasmid pAd-aGP5 was confirmed containing the fragment by endonuclease digestion. PCR product was confirmed by sequencing and the constructed plasmid was correct. The obtained recombinant adenovirus was detected by introduction of green fluorescent gene in the plasmid and by indirect immunofluorescence assay. The negative control cells have no fluorescent; the result showed that the recombinant adenovirus may effectively express the target protein.
Discussion: Currently, the research of developing efficient PRRS vaccine has been on the forefront of clinical research. Although modified live vaccines and inactivated vaccines are now commercially available and widely used, no ideal vaccines were achieved so far. There is an urgent requirement to develop vaccines more effective and to alter the strategies against this viral disease. Recombinant virus design is one of the common and useful methods. An epitope in GP5 ectodomain rapidly induced a strong non-neutralizing antibodied in the immune response, and covered B epitope to lead to neutralizing antibody delayed to three weeks to produce. In this study, through transformated GP5 gene without ADE epitope, the recombinant adenovirus was constructed, to lay the foundation for future research ADE generating mechanism and novel PRRS vaccine.
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Modulation of swine interleukin-4 to induce virus-specific immune responses elicited by modified live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine

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Porcine reproductive and respiratory syndrome (PRRS) has become one of the most economically important diseases to the global pork industry. Currently available commercial vaccines provide limited protection due to delayed and weak neutralizing antibody production and cell-mediated immunity. We investigated the effect of swine interleukin 4 (IL-4) on the development of virus-specific immune responses elicited by modified live PRRSV (MLV) vaccine. The level of antibodies against PRRSV membrane proteins in pigs elicited by MLV vaccine plus recombinant plasmid encoding IL-4 (group 3) were significantly higher than those by MLV vaccine alone (group 1) and MLV vaccine plus empty plasmid (group 2), with the exception of primary 14 days post-inoculation. Similarly, the neutralizing efficacy of sera from group 3 was markedly enhanced compared with group 1 and group 2. In cellular immunity, the ratio of CD3+CD4+/CD3+CD8+ T lymphocytes subpopulations from group 3 monitored by flow cytometry (FCM) was significantly higher than those from group1 and group 2 from 42 days post-inoculation to 21 days post-challenge. After challenge, pigs in group 3 showed significantly lower virus loads in peripheral blood measured by a quantitative real-time PCR (RT-qPCR), as compared to those in group 1 and group 2. Pigs in group 1 and group 2 had a low fever and displayed slight inappetence, lethargy, rough hair coats and no lung lesions, meanwhile those in group 3 showed almost no clinical signs, no lung lesions. The scores of clinical signs of pigs in group 3 were significantly lower than those in both group 1 and group 2. The scores of lung lesions in group 3 were only numerically lower than those in the latter two groups, but it had no significant difference among three groups. This study indicated swine IL-4 markedly enhanced the protective immune response of hosts and improved the efficacy of the MLV vaccine in preventing PRRS disease.

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Construction and immunogenicity of DNA vaccine coexpressing GP3 and GP5 proteins of European-type Porcine Reproductive and Respiratory Syndrome virus

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Introduction and Purpose: Porcine reproductive and respiratory syndrome virus (PRRSV) causes the most important infectious disease which was a worldwide and serious threat to pig production. Based on serological characteristics, there are two main serotypes of PRRSV, named the European isolate (EU, type 1) and the Northern American isolate (NA, type 2),which share nucleotide homology of 54%～67%. The European type of PRRSV was restricted to Europe, while the American type of PRRSV was restricted to North America and Asia. However, the epidemic of PRRSV has break the regional restrictions, the European type of PRRSV have been reported in Asia and North America, and American wild-type PRRSV has been isolated in Europe recently. A PRRSV strain coexisting with NA and EU genotype could cause potential problems to PRRSV diagnostics and managements. In recent years, several the European type PRRSV field isolates were reported in several Asia countries such as South Korea, Thailand etc, and the European type PRRSV was also reported in China. The emergence of European type PRRSV in China brought great challenge to the prevention and control of PRRSV. In this study, DNA vaccine pVAX-EU-ORF3-ORF5, pVAX-EU-ORF3 and pVAX-EU-ORF5 were constructed basis on the European LV strain (M96262), analysis of its expression at the cellular level and comparison of its immunogenicity by animal experiment.

Materials and methods: Three expression plasmids of pVAX-EU-ORF3-ORF5, pVAX-EU-ORF3 and pVAX-EU-ORF5 were constructed in use of pVAX expression vector, and then were transfected into BHK-21 cells to verify the expression. Sixty 6-week-old female BALB/c mice were randomly separated into five groups. Three groups of the mice were immunized with plasmid pVAX-EU-ORF3-ORF5, pVAX-EU-ORF3 or pVAX-EU-ORF5 respectively, while other two groups were immunized with plasmid pVAX and PBS respectively as negative control. The secondary immunization was strengthened after three weeks. Sera were collected at 0, 7, 14, 21, 28th day to detect specific ELISA antibodies and cytokine analysis in PBMC. Two weeks after secondary immunization, lymphocytes were separated for analysis of T-cell subtypes.

Results and discussion: The results of EU PRRSV GP3, GP5 antibody showed that one week after primary immunization, GP3 and GP5 specificity antibody could be detected in all experimental groups, but no significant differences existed comparing to control groups. Two weeks later, the antibody levels showed a rising trend and significant difference compared with the control groups ($P < 0.05$), but decreased slightly in the third week. The antibody levels were rising after the boost immunization, but the trend is slightly, and the antibody level of pVAX-EU-ORF3-ORF5 group was slightly higher than other two experimental groups after the secondary immunization, but the difference showed no significance. The detection result of peripheral blood cytokines (IL-2, IL-4, IL-10） showed that secretion of cytokines IL-2, IL-4 and IL-10 was emerged in mice in all the experimental groups, and showed significant difference comparing to control groups ($P < 0.01$) , but there was no significant difference between experimental groups. Peripheral blood test showed that IFN-γ in experimental groups was all higher than control groups, the difference between experimental groups and control groups was significant ($P<0.05$), and no significant difference was observed between the experimental groups. Lymphocyte proliferation experimental results showed that the lymphocyte stimulation index of experimental groups was significantly higher than that of control groups ($P < 0.05$), no significant difference was observed between the experimental group. The results of lymphocyte subgroup analysis showed that the T lymphocytes sub-types CD3+CD4+ and CD3+CD8+ of pVAX-EU-ORF3-ORF5 group was higher than pVAX - EU - ORF3 and pVAX - EU - ORF5 group, but no significant difference; the three experimental groups has significant difference comparing to the control groups ($P<0.05$). The results showed that both EU PRRSV GP3 and GP5 protein could induce specific antibodies production in body, whether GP3 have ability to induce neutralizing antibodies against PRRSV or not will need further experiments. Scholars have confirmed that the LV strain GP3 monoclonal antibody having a neutralizing activity, however, some scholars believe the specific antibody has nothing to do with the neutralization even the antigenic determinant is contained in GP3 protein. There has been reported that the expression products of Spanish isolates Olot/91 ORF3 baculovirus may provide partial protection of reproductive failure in pregnant sows. Some research confirmed that the neutralizing antibody could not be detected after swine vaccinated with expressed GP3 protein, therefore we presumed GP3 might induce cellular immunity. Now it is generally accepted that GP5 has the ability of inducing body to produce neutralizing antibody, and the six epitopes of GP5 can induce specific neutralizing antibody, one of the serotype-specific linear epitopes can neutralize virus in vitro, the neutralizing ability is stronger than the neutralizing antibodies induced by GP4, the neutralization induced by GP5 protein has significant correlation with GP5 antibody titer. This experiment concludes that Europe type PRRSV GP3 and GP5 protein has better immunogenicity. The immunological function of GP3 and GP5 protein induces in the virus proliferation, replication and the process of viral infection will need further studies.

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Inhibition of porcine circovirus type 2 replication by cap protein gene DNA vaccine in mice

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Short statement: The post-weaning multisystemic wasting syndrome (PMWS) emerged in the 1990s. It was a shock for the swine industry worldwide. PCV2 was implicated in PMWS and in high economic losses. PCV2 genome encodes two expressions open reading frames (ORFs), ORF1 encode the Rep protein gene and ORF2 encode Capsid (Cap) protein gene. The only major structural protein, Cap is the immunogenic protein of PCV2; it is also target for development of vaccines and serodiagnostic way for PCV2 specific immune response detection. Evaluate the inhibition effect of PCV2 high pathogenic strain on lymphoid tissues, and immunogenicity of the recombinant plasmid pEGFP-Cap protein gene (ORF2-pEGFP) of PCV2 and compared it to that induced by an available vaccine were the aims of this study.
Methods: The ORF2 gene was amplified then inserted into the pEGFP-N1 vector. The PK-15 cells were transformed with constructed plasmid, and the cap protein expressed in the cells was identified by immunofluorescence assay. The same cap protein gene was introduced into the pET32a vector for expressed and purified the Cap protein. The protein obtained was identified by western blot. For the immunogenicity evaluation, twenty-five six-week old mice were immunized two times at two week interval with OFR2-pEGFP plasmid and available vaccine (LG-strain). The control groups were inoculated with pEGFP-N1 vector and 0,9mol/L of sodium chloride (NaCl). At day 7 after the second immunization all immunized groups were challenged with 10^5 $TCID_{50}$ of PCV2 viruses JL01 strain. The antibody levels in the sera from the vaccinated mice were determinate by indirect Enzyme-Linked Immunosorbent assay (iELISA). The levels of interferon-gamma and interleukin-10 secreting cells in the sera from the mice were determined and the neutralizing antibody was determine in the same sera. At the days 7 and 14 after challenge, five mice were randomly selected from each group and sacrificed; the inguinal lymph from each sacrificed mouse was collected for histological examination and immunohistochemical (IHC) staining. SPSS system for windows version 13.0 was used for statistical analysis and *P*-values < 0.05 were considered as statistically significant.
Results: The sera from the groups inoculated with constructed plasmid (ORF2-pEGFP /adjuvant and ORF2-pEGFP) presented a high titer of the antibody against PCV2. However the induced antibody titer of LG-strain vaccine was widely elevated and the difference was statistically significant ($p<0.001$). The induced levels of the interferon-gamma and interleukin-10 were also high in the sera from the mice inoculated with constructed plasmid. But the interferon-gamma and interleukin-10 levels obtained in the sera from LG-strain group were statistically superior ($p<0.02$). The levels of induced antibody or interferon-gamma and interleukin-10 in the sera from ORF2-pEGFP /adjuvant were highest that induced by ORF2-pEGFP and the different was statistically significant ($p<0.05$). The high titers of neutralizing antibody were obtained in the sera from the mice inoculated with constructed plasmid. However the neutralizing titers obtained in the sera from the groups inoculated with LG-strain were highest ($p<0.05$). The results suggest that ORF2-pEGFP adding an adjuvant can induce a high immune response in the mice. Compared to the control groups, the histological lesion in lymph node was decreased in the immunize group with ORF2-pEGFP plasmid. The positive stain in the immunized mice was reduced after the immunohistochemical staining, compared to the positive control mice.
Conclusion: Although the Cap protein of the PCV2 was considered as a major protein and immunogenic of the PCV2, the cap protein cannot induce a sufficient immunity in any conditions. This is the case in this present study, the constructed plasmid ORF2-pEGFP induced immune response in mice after the second inoculation of 15µg. But the obtained immune response was far from giving sufficient protection against a possible PCV2 infection.
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Preliminary study of recombinant Bordetella Bronchiseptica expressing cap protein of Porcine Circovirus Type 2 as a candidate vaccine against Porcine Circovirus disease

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Objective: To use a Bordetella bronchiseptica (Bb) aroA mutant to expressing Cap protein of Porcine circovirus type 2(PCV2), develeping a new vaccine strain, which can deliver the immunogenic protein of PCV2 to respiratory mucosa, for prevention of swine multi-system dysfunction caused by PCV2. To evaluate the effective and time-limitation of colonization of this vaccine strain in the porcine respiratory mucosa, and the ability of stimulation the mucosal immune, providing a scientific basis for it as a candidate vaccine.

Result: we used an attenuated Bb strain（QH0814ΔaroA） as a vector to express PCV2 antigens gene ORF2,which can effectively produce the Cap protein. The LD50 of this vaccine strain is 2.0×107CFU, that decreased by 8-fold approximately compared with the parental Bb strain (QH0814) Following intranasal immunization in Balb/C mice with the vaccine strain, Serum antibody against PCV2 Cap was measured by ELISE, and the average antibody level（OD630）of immunity group arrives 1.3 at 14d after second immunization. The respiratory bacterial colonization assay in mice, the vaccine strain （QH0814 ΔaroA/ORF2）and the attenuated Bb strain (QH0814ΔaroA) had been cleared from lung, endotracheal and nasal until 14d, 21d and 28d, respectively after inoculated, while the duration of the parental Bb strain in lung was 28d, and in endotracheal and nasal was both over 35d. Optimal intranasal immunization dose was determined in 6×109 CFU/mL for 20-day-old piglets (PCV2 negative) , the nasal swabs of inoculated piglets had been undetected the vaccine strain until 5d after challenge, Following intranasal immunization in 20-day-old piglets with the vaccine strain, and the average level（OD630）of antibody against PCV2 Cap of immunity group arrives 0.707, the values are similar to those of groups inoculated commercial PCV2 vaccines.

Conclusion: The recombinant B. Bronchiseptica expressing Cap protein of PCV2 can colonize in the porcine respiratory mucosa effectively and time-imitated, which stimulate the mucosal immune increasing the level of antibody against PCV2, protecting the herd from the virus. Therefore, this recombinant strain can be development of a candidate vaccine against PCV2.

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Isolation of the European genotype porcine reproductive and respiratory syndrome virus (PRRSV) in Kazakhstan

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Objective: To identify the cause of disease emergence in pigs (bluish-red coloration of the skin of the ears, high temperature [41ºC], signs of depression and feed refusal) eventually leads to reproductive failure and respiratory distress in Kostanay region, Kostanayskaya oblast, Republic of Kazakhstan in 2008.
Materials and Methods: In October 2008, RIBSP researchers collected pathological material from 18-month-old sows with clinical symptoms of the disease (bluish-red coloration of the skin of the ears, high temperature [41ºC], signs of depression and feed refusal) from private farmsteads in Zatobol village (Kostanayskiy rayon). RNA was extracted from the blood and internal organ samples (mediastinal lymph nodes, spleen, lungs, heart and liver) using the QIAamp Viral RNA mini kit (Qiagen, Germany). PRRSV RNA was detected using NextGen Real-Time RT-PCR Target Specific Reagents for the Detection and Differentiation of North American and European PRRSV viral RNA (Tetracore, USA). The presence of PRRSV virions were confirmed by negative staining (2% aqueous solution of phosphotungstic acid) of MARC-145 cell cultures and transmission electron microscopy using a Jem-100CX II (Jeol Ltd., Tokyo, Japan; accelerating voltage, 80 kW; magnification, x100000). Healthy 3-month-old Large White breed pigs were intranasally infected with the isolated virus ($10^{5.25}$ $TCID_{50}$/pig); PRRSV antibodies were quantified in the sera using the IDEXX Herd Check Virus PRRS ELISA (IDEXX Laboratories, USA).
Results: RNA corresponding to the genome of the European genotype of PRRSV was detected by real-time-RT-PCR in the blood samples taken from the sows. *In vitro* culture of blood samples taken from the sows with MARC-145 cells enabled isolation of the cytopathogenic agent. Based on its morphological properties (spherical virion with an average diameter of 57 nm), the virus was attributed to the family *Arteriviridae* and genus *Arterivirus*. Specific cytopathogenic effects of the virus appeared in MARC-145 cells on day 3 of *in vitro* culture, followed by the accumulation of spherical cells rising above the monolayer. Healthy Large White breed pigs infected with the isolated virus displayed signs of disease without overt clinical symptoms, except for minor blue coloration of the ears and increased body temperature (40.6ºC). Antibodies to PRRSV were detected in the sera of infected pigs, ranging in titer from 1:40 (at 14 day) to 1:320 (at 35 day). The isolated PRRS strain (called Kostanay-CM/08) was successfully adapted to continuous culture in MARC-145 cells by serial passaging, and has been deposited in the Pathogen Depository of Especially Dangerous Infections Agents of the RIBSP.
Conclusion: Comprehensive analysis of pathological samples from sick sows from the Kostanay region led to the isolation and identification the European genotype of PRRSV for the first time in Kazakhstan. We are currently using strain Kostanay-CM/08 for the production of an inactivated vaccine against PRRS; this work is being funded by Grant Project 055/101 Development of Technology for the Production of Inactivated Vaccine Against PRRSV (2012-2014), which is financed by the Ministry of Education and Science of the Republic of Kazakhstan.

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Effect of vaccination with Ingelvac Circoflex® on fattening performance in a farm in north China

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Introduction
PCV2 causes significant losses in the pig industry worldwide. PCV2 piglet vaccination is a highly useful tool in controlling PCVD [1]. The objective of this study was to evaluate the efficacy of Ingelvac CircoFLEX® by measuring fattening performance on a farm in the north of China.

Materials and methods
The study was conducted on a 2-site, 1,000 sow level farm located in the north of China. The farm operates with an all-in-all-out system. At 10 weeks of age, the pigs are moved to the fattening barns. The breeding herd in this farm is vaccinated against CFSV, PRV, PPV, JEV and atrophic rhinitis while piglets are vaccinated against CSFV, PRV, PRRS and Mycoplasma hyopneumoniae.
The main clinical signs in the fattening period in this farm were growth retardation and PDNS, indicating PCV2 infection. Laboratory diagnosis (PCR and ELISA test) confirmed that PCV2 was the main viral pathogen. Based on these findings a field trial was conducted between May and November 2011 to evaluate the efficacy of PCV2 vaccination with Ingelvac CircoFLEX®.
816 piglets at 14 days of age were randomly divided into 2 groups. The piglets in the vaccinated group were injected with Ingelvac CircoFLEX®, 1ml per pig. The piglets in the control group were treated with physiological saline, 1ml per pig. Pigs in these two groups were raised in different barns on the same site under the same management and housing conditions. Mortality and culling rate, feed conversion rate (FCR) and slaughter weight were recorded. The livability, calculated as (100-mortality-culling rate), in the two treatment groups was compared with Chi-square test.

Results
Mortality and FCR of the vaccinated pigs were reduced, while ADG was increased in this trial (Table 1). The ADG in the vaccinated group was on average 97g higher than in the control group. The livability rate in the vaccinated group was significantly higher than in the control group ($p<0.01$).

Table1 *Relevant parameters in the control and the vaccinated group*

	Control	Vaccinated
Number of pigs	398	418
Mortality	10.05%	7.42%
Culling rate	7.54%	1.20%
Livability	82.41%	91.38%
FCR	3.27	3.20
ADG (g)	506	603

Conclusion and discussion
In this farm with PCV2 infection, Ingelvac CircoFLEX® successfully reduced the mortality and the number of unthrifty pigs (thus contributed to a much lower culling rate) in fattening. It also improved FCR and ADG. Based on the positive findings in this study, the farm has been using Ingelvac CircoFLEX® since October 2011. The 5-year record of mortality and culling rate of fattening pigs in this farm also showed the big improvement in performance after implementing PCV2 piglet vaccination (table 2). The results demonstrate that PCV2 vaccination can reduce the clinical symptoms of PCVD and improve the productivity.

Table2 *5-year mortality and culling rate for fattening pigs in this farm.*

Year	Number of fattening	Mortality %	Culling rate %	Mortality and culling%
2008	16547	6.11	3.17	9.28
2009	16205	3.54	3.44	6.98
2010	14411	5.32	4.63	9.95
2011	15737	5.44	3.83	9.27
2012	13852	3.89	0.40	4.29

Reference
[1] Harding JCS, 2009. Porcine circoviral disease-from inception to successful control. Boehringer Ingelheim Asian PRRSpective Symposium: 28-37.

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Construction of recombinant PRRSV expressing granulocyte-macrophage colony-stimulating factor

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Introduction

Porcine Reproductive and Respiratory Syndrome virus (PRRSV) suppresses and delays the development of neutralising antibodies and induction of cell-mediated immune responses after the host infected. DCs, as the most effective antigen presenting cells, which are recruited and activated by GM-CSF, could initiate humoral and cellular immunity. Here, we are trying to rescue a recombinant PRRSV expressing GM-CSF to be a more efficacious PRRSV candidate vaccine strain.

Materials and Methods

Porcine GM-CSF and a copy of the transcription regulatory sequence for ORF6 (TRS6) were inserted between ORF1b and ORF2a of the infectious molecular clone of HuN4-F112 vaccine strain by the method of overlap PCR. The recombinant full-length cDNA clones were assembled by cloning and splicing of the gene fragments, and confirmed by sequencing and enzyme digestion of Swa I. To rescue the virus, capped RNAs of the viral genome were transcribed in vitro from recombinant plasmid and transfected into BHK-21 cells. Then, the rescued recombinant virus was passaged in MARC-145 cells for further analysis.

Results

1. Cytopathic effects (CPE) were obviously observed on MARC-145 cells 72 hours after infection. The specific fluorescence of the PRRSV nucleocapsid protein and GM-CSF protein inserted could be detected through immunofluorescence. These results indicate that the recombinant virus HuN4-F112-GM was successfully generated.
2. We next investigated the genetic stability of inserted gene in the virus of HuN4-F112-GM. As expected, the sequence of GM-CSF was absent of mutation or deletion after 20 passages.
3. The virus HuN4-F112-GM passaged 5, 10, 15, and 20 times were respectively detected for viral multi-step growth curve. The HuN4-F112-GM virus replicated to levels similar to those of the parent PRRSV virus, indicating that the insertion of GM-CSF gene did not affect the replication of the vaccine strain.

Conclusions

We successfully rescued a recombinant PRRSV expressing GM-CSF protein. The inserted gene could be stably maintained in HuN4-F112-GM and did not affect the growth characters of the parental strain. Our future work will focus on investigating whether the inserted gene could enhance humoral and cellular immunity.

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Evidence for a broadly neutralizing antibody response to PRRSV

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Introduction: The neutralizing antibody (nAb) response following PRRSV infection is generally described as weak and delayed. Furthermore, when present, nAb is primarily directed against only the homologous isolate (or genetically related isolates). The absence of heterologous protection is a major impediment to the development of vaccines. Based on observations for other viruses, we hypothesize the existence of a subpopulation of pigs that produce a strong nAb response to homologous and multiple heterologous viruses. **Methods:** Serum samples were obtained from 1,211 pigs at 42 days after infection, challenged with either the Type II isolate NVSL (854 pigs) or KS06 (357 pigs). All pigs were genotyped using a 60K SNP chip (Illumina). Three types of phenotypic antibody data were collected: total antibody (tAb), homologous nAb, and heterologous nAb. Total Ab was measured using a fluorescent microsphere immunoassay (FMIA) incorporating the PRRSV nucleocapsid (N) protein. Virus neutralizing activity was measured using a standard PRRSV neutralization assay against four genetically diverse PRRSV isolates (5-11% homology at the nucleotide level of ORF5), with titer as the assay endpoint. Estimates of heritability and genome wide association studies (GWAS) were performed as previously described (Boodicker et al, 2012, J Animal Science, 90(6), 1733-1746). For the purpose of classification, heterologous nAb responses were placed into one of four groups: Grp1, no nAb; Grp2, nAb to only the homologous isolate; Grp3, nAb to the homologous and 1 or 2 additional isolates; and Grp4, nAb to all isolates. For the purpose of definition, the Grp4 pigs are described as possessing broadly neutralizing antibody (bnAb). **Results:** The log2 homologous nAb titers for pigs infected with NVSL and KS06 ranged from <1 to 8 (mean=2.7+/-1.8) and <1 to 7 (mean=1.7+/-1.5) respectively. Mean log2 homologous nAb titers for pigs challenged with NVSL or KS06 were significantly different ($p<0.0001$), suggesting the challenge isolate plays a role in the magnitude of the homologous nAb response. Analysis of 457 samples from pigs challenged with NVSL showed an inverse relationship between virus load and homologous nAb titer, confirming the importance of nAb in controlling virus replication. Heritability estimates for homologous nAb showed a weak association (5.7+/-9%). Heritability estimates from 457 NVSL challenged pigs showed the tAb response was moderately heritable (13.5±13.2%). GWAS identified a quantitative trait loci (QTL) in a 1Mb region of *Sus scrofa* chromosome 7 (SSC7) that explained most of the genetic variation. Heterologous nAb response distributions for pigs challenged with NVSL or KS06 were as follows: Grp1, NVSL-8.9%, KS06-24.9%; Grp2, NVSL-57.3%, KS06-46.5%; Grp3, NVSL-29.6%, KS06-24.1%; and Grp4, NVSL-4.3%, KS06-4.5%, showing the production of bnAb was independent of the challenge isolate. **Discussion and Implications:** The antibody response possesses a heritable component. Establishment of a genetic basis for a nAb response will create the opportunity to develop pig lines that are tailored to respond optimally to PRRS vaccines, bringing the pig closer to the vaccine. Pigs possessing bnAb suggests the presence of conserved bnAb epitopes. Identifying conserved bnAb epitopes creates the opportunity to design PRRS vaccines that are tailored to elicit a bnAb response.

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Host recognition of the structural form of the PCV2 capsid protein results in protective immunity or immunopathogenesis

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Porcine circovirus associated disease (PCVAD) describes a group of complex syndromes such as porcine multi-systemic wasting syndrome (PMWS) and porcine dermatitis and nephropathy syndrome (PDNS). The central feature of PCVAD involves infection with porcine circovirus type 2 (PCV2). The capsid protein (CP) of PCV2 possesses an immunodominant epitope, CP(169-180), which is linked to infection with PCV2 and pathogenesis of porcine circovirus-associated disease (PCVAD). For example, sera from pigs diagnosed with PCVAD, experimentally infected with PCV2, or vaccinated with baculovirus expressed CP recognize a polypeptide comprised of the entire CP minus the nuclear localization signal, CP(43-233) in an ELISA. In contrast, only sera from pigs diagnosed with PCVAD or experimentally infected with PCV2 recognize the oligopeptide CP(169-180). Recently, the structure of the CP monomer and model of a baculovirus-expressed PCV2 virus like particle (VLP) were reported. The homopolymer virion capsid of PCV2 is comprised of 60 CP molecules arranged in a T=1 icosahedral form. Analysis shows CP(169-180) locates to an outer loop of the monomeric form of CP, and therefore, is available for recognition by host immunity. In contrast, in the context of the VLP, CP(169-180) is buried between CP subunits. We propose that CP(169-180) in monomeric CP serves as a decoy to divert the host humoral response away from protective epitopes formed by the VLP. The overall purpose of this study was to investigate the role of the immune response to monomer CP in the development of PCVAD. In this study, Group 1 pigs were immunized with recombinant bacterial expressed CP(43-233) in monomeric form, then challenged with PCV2 and porcine reproductive and respiratory syndrome virus (PRRSV), a pathogen known to increase replication and the pathogenesis of PCV2. The CP immunogen was maintained as a stable monomer by fusion of CP(43-233) to ubiquitin (Ub-CP). Size exclusion chromatography confirmed Ub-CP was in monomeric form. Control groups consisted of pigs vaccinated with a commercial baculovirus expressed CP product then challenged with PCV2 and PRRSV (Group 2), pigs challenged with PCV2 and PRRSV alone (Group 3), and non-treated pigs. Group 1 pigs (Ub-CP) produced a strong total antibody response to PCV2 and CP(169-180), however, produced low levels of neutralizing antibodies, and failed to protect against PCV2-PRRSV challenge. Viremia and humoral immunity was similar to the outcome of infection with PCV2-PRRSV alone (Group 3 pigs). In contrast, Group 2 pigs were protected from virus challenge, produced high levels of neutralizing antibody, and low levels of anti-CP(169-180) antibody. Overall, the data support the role of CP(169-180) as an immunological decoy and illustrate the importance of the structural form of the CP immunogen in determining the outcome following infection.

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The comparison of immune efficacy between combined PCV2, MH vaccine inactivated and the corresponding single vaccines

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Introduction

The immune efficacy of bivalent inactivated PCV2-MH vaccine (DBN-SX07 Strain + DJ-166 Strain) and corresponding monovalent vaccines, developed by Animal Medicine Research Center of Da Bei Nong Group (DBN), were compared in this study. The results indicated that there are no significant differences of immue efficacy between these vaccines.

Materials and Methods

The pigs used in this study were from a closed commercial pigfarm, which is known to be free of PCV2 and MH antibodies. Seventy pigs aged from 14-21 days were allocated into seven groups of ten pigs. Group 1 and 2 were vaccinated bivalent inactivated PCV2-MH vaccine (PMV), group 3 was vaccinated with inactivated PCV2 vaccine (PV), group 4 was vaccinated with inactivated MH vaccine (MV), group 5 and 6 were control groups, group 7 was blank group. Vaccination dosage was 2 ml for all vaccinated pigs, and pigs in group 1-4 were boosted 14 days post the first vaccination. Pigs in group 1, 3 and 5 were challenged with PCV2 (DBN-SX07) 35 days post the first vaccination, and pigs in group 2, 4 and 6 were challenged with MH (HB) 35 days post the first vaccination. Necropsies were performed at 25th day post the challenging. Lung lesion scoring and immunohistochemical method was used to determine the protection rate.

Results

No adverse reaction was observed in any of the vaccinated pigs during the 1-35 days post the first vaccination. The average PCV2 serum antibody titer of PMV group and PV group were both 1:800 at 35th day post the first immunization. All pigs in vaccinated groups stayed healthy after the challenging, while 90% of the pigs in control groups got infected (Figure 1).

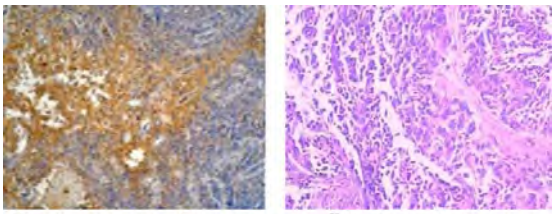

Figure 1. Immunohistochemical figure of lymph nodes. Yellow color indicates PCV2 infected cells in the lung.

The average MH serum antibody S/P value of PMV group was 2.31±0.23 and the value of MV group was 2.44±0.21 at 35th day post the first immunization, and average lung lesions of pigs in immune group depletion rate was 80.0% and 81.8% after challenged(Figure 2).

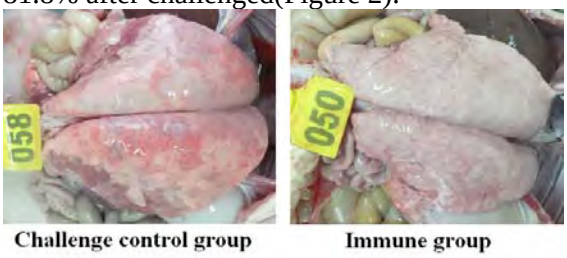

Figure 2. The pictures of lungs: Lung lesion scores of pigs in control groups was 11; lung lesion scores of pigs in immune group was 2.2.

Discussion and Conclusion

It was shown that the vaccines of MH and PCV2 could be given concurrently without any negative consequences for the immune efficacy in a previous study1. Simultaneous use of both vaccines has been practiced in the pig production without evidence of mutual interference or apparent safety problems2. The results of this study indicated that there was no significant differences of immune efficacy between the bivalent PCV2-MH vaccine and corresponding monovalent vaccines.

Literature cited

1. Segales, Q. 2008. IPVS I/S-P satellite symposium.
2. Farreres, J. et al. 2010. IPVS.

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Identification of a B-cell epitope in the glycosylated protein 3 of highly pathogenic porcine reproductive and respiratory syndrome virus HuN4 strain

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Highly pathogenic PRRS (HP-PRRS) is one of the most economically significant viral diseases, causing great losses in China and continuing to be a threat for the swine industry. The glycosylated protein 3 (GP3) is considered to be associated with protective immunity, but its function is poorly known.

In this study, BALB/c mice were immunized with HP-PRRSV HuN4 to generate monoclonal antibodies (MAb) against GP3 protein. Indirect immunofluorescence assay (IFA) based on HuN4 and eukaryotic expressed HuN4 GP3 protein was used to screen the positive hybridoma cell clones. A hybridoma cell named as 4G5 that secreted MAb against HP-PRRSV GP3 protein was obtained. Western blot showed that 4G5 could react with GP3 expressed in E.coli BL21 (DE3). In order to identify the minimal epitope recognized by 4G5, the ORF3 gene was truncated into several overlapping fragments, and fusion expressed with GST-tag in E.coli, then the proteins were probed with 4G5 by western blot. Based on the analysis above, we determined the minimal epitope was localized to GP3P3 W74CRIGHDRCS83. Sequence alignments of 23 PRRSV isolates revealed that the epitope sequence was relatively conserved except for H79→979and S83→383/E83. In addition, the IFA showed that 4G5 had reactivity to PRRSV attenuated live vaccine strains HuN4-F112 (H79), CH-1R (N79) and JXA1-R (H79), but not to RespPRRS MLV (Y79), which indicated that the mutation (H79/N79→979) may change the antigenicity of the epitope. In order to determine the effect of the mutated amino acid (S83→G83) on epitope antigenicity, the mutated epitope (W74CRIGHDRCG83) was expressed and examined. This suggested that the mutated amino acid couldn't cause the antigenic differences of the epitope. So the antigenicity of the epitope is coincident among domestic classic and high pathogenic PRRSV strains. The epitope GP3P3 was fusion expressed, purified, and employed as coating antigen to detect the pig sera by ELISA. The results showed that antibodies against the epitope experienced an increasing process in 30% of the pigs from 3-4 weeks post HuN4-F112 vaccine immunization, but others had little changes during the process.

Compare to the epitope GP3EP3 (W74CRIGHDRCGED85) identified by Zhou, GP3P3 (W74CRIGHDRCS83) was two amino acids shorter. In addition, the MAb against GP3EP3 developed by Zhou couldn't recognized the epitope GP3P3, indicated that there are multiple epitopes in this region. 4G5 could be useful for further study of the structure and function of HP-PRRSV GP3.

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Anti-idiotypic antibody vaccine against highly pathogenic PRRSV infection

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Objectives: PRRS is one of the most economically significant diseases of swine. Vaccination is one strategy used to control PRRS. However, the current vaccines do not provide adequate long term protection at the herd level and thus an alternative approach is needed. We have produced a monoclonal anti-idiotypic antibody (designated Mab2-5G2) which mimics PRRSV GP5 antigen, binds non-muscle myosin heavy chain II-A, blocks PRRSV infection of the cells and protects pigs against type 1 PRRSV infection. The objective of this study was to evaluate the efficacy of Mab2-5G2 as a vaccine to protect pigs against highly pathogenic PRRSV (HP-PRRSV) infection.
Material and Methods: Twenty PRRSV negative piglets were randomly divided into 4 groups (5 piglets/group). Group 1 piglets were immunized intramuscularly with Mab2-5G2 emulsified in MONTANIDE ISA 206 VG adjuvant at dosage of 1.0 mg/piglet. Group 2 piglets received an isotype matched mouse IgG emulsified in the same adjuvant. Group 3 and Group 4 piglets received the adjuvant or PBS, respectively. All piglets were boosted on 14 days post immunization (DPI) with the same immunogens. Two weeks later, Group 1 to Group 3 pigs were challenged intranasally with HP-PRRSV NVDC-JXA1 strain and Group 4 piglets was inoculated with PBS. Pigs were monitored daily for body temperature, clinical signs and death. Serum samples were collected from all pigs at 0, 7, 14, 21 DPI and 0, 3, 5, 7, 14, 21, 28 days post challenge (DPC) for detection of antibodies, cytokines and viremia. All pigs were euthanized on 28 DPC and tissue samples were collected post mortem for virus detection and isolation.
Results and Discussion: Group 1 piglets showed relatively lower body temperature, mild clinical signs and survived throughout the experiment. In contrast, Group 2 and Group 3 piglets developed higher body temperature and severe clinical signs, piglets were dead from 7 DPC to 21 DPC. Serum antibodies to PRRSV N protein were positive starting at 7 DPC and peaked at 28 DPC without significant differences among the challenged groups. However, serum antibodies to GP2/GP5 were significantly higher in Group 1 pigs than other groups from 10 DPC to 14 DPC. The levels of IL-4 and IL-10 in serum were significantly up-regulated in Group 1 piglets from 3 DPC to 10 DPC compared with other groups. Viremia was detected starting at 3 DPC and peaked on 10 DPC in all challenged pigs, however, the amount of virus in Group 1 piglets was significantly lower than that in other challenged groups. Piglets in group 4 were healthy throughout the experiment, the cytokines unchanged and no antibodies or virus were detected .
Conclusion: Anti-idiotypic antibody Mab2-5G2 can be used as an alternative safe vaccine against the HP-PRRSV infection.
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The nonstructural protein 1 papain-like cysteine protease was not necessary for porcine reproductive and respiratory syndrome virus nonstructural protein 1 to inhibit interferon-beta function

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Type I IFN(IFN-alpha and IFN-beta) is the first responder against animal virus infections. When virus infects, the virus could be recognized by the pathogen-associated molecular pattern receptors and leads to the transcription of IFN-beta. Then, IFN-beta binds to the IFN-alpha/beta receptor, initiating the phosphorylation of STAT1 which induced the activation of the genes containing interferon-stimulated response element (ISRE) in their promoters and resulted in transcriptional up-regulation of these genes and establishment of an antiviral state.

Porcine reproductive and respiratory syndrome virus (PRRSV) has caused one of the most economically devastating and pandemic diseases of swine. Its nonstructural protein 1(nsp1) could be auto-cleaved into nsp1alpha and nsp1 beta, both of which had the papain-like cysteine protease activities. The papain-like cysteine protease of nsp1alpha directed the release of nsp1alpha by cleavage of the nsp1alpha/nsp1beta site, while the papain-like cysteine protease of nsp1beta was responsible for the cleavage of the nsp1/nsp2 site to release the nsp1beta. The papain-like proteinases were characterized by a catalytic dyad that consists of a nucleophilic cysteine residue and a downstream histidine. Site-directed mutagenesis studies found that PRRSV Cys-76 and His 146 were the catalytic Cys and His residues of the papain-like cysteine protease of nsp1alpha, and Cys-270 and His339 was that of the papain-like cysteine protease of nsp1beta. Substitution of Cys with Ser or substitution of His with Ala completely inactivated the proteolytic activity. Previous studies have shown that PRRSV nsp1 was an inhibitor to interferon signaling. Whether the papain-like cysteine protease activities were required for nsp1 to interferon signaling attracted our attention.

Here we constructed the report plasmid pISRE-Luc, which contained the interferon-stimulated response element promoter in the upstream of luciferase gene, and used site-directed mutagenesis that inactivated papain-like cysteine protease activities of nsp1 to explore whether the papain-like cysteine protease activities were required for nsp1 to disrupt the IFN-beta-induced activation of pISRE-Luc. The results showed that mutations that inactivated papain-like cysteine protease activities of nsp1alpha (PCPalpha) and PCPbeta didn't influence nsp1 to inhibit IFN-beta-induced activation of pISRE-Luc. In conclusion, our present work indicated that the papain-like cysteine protease activities of nsp1alpha (PCPalpha) and PCP beta were not necessary for nsp1 to inhibit the IFN-beta function.

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Toll-like receptor ligands enhance the protective effects of vaccination against porcine reproductive and respiratory syndrome virus in swine

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Porcine reproductive and respiratory syndrome virus (PRRSV) is mainly responsible for the heavy economic losses in pig industry in the world. Current vaccination strategies provide only a limited protection. Previous studies have demonstrated the immunostimulatory adjuvant effects of Toll-like receptor (TLR) ligands, synthetic double-stranded RNA polyriboinosinic polyribocytidylic [poly(I:C)], lipoteichoic acid (LTA) and CL097 in humans and animals.

To study the effects of these compounds on the induction of PRRSV-specific immune responses, mice were immunized subcutaneously with killed virus (KV) antigens incorporating pairs of TLR ligands. It was found that poly(I:C) and CL097 induced the higher IFN-gamma levels and PRRSV-specific antibodies, comparing with that KV with or without LTA in mice. Piglets were vaccinated with the KV mixed with poly(I:C) or CL097 and the protective effects of the vaccination were evaluated. The results showed that PRRSV-specific antibodies and T lymphocyte proliferation levels in KV mixed with poly(I:C) or CL097 groups were higher than those in KV group. Following challenge with PRRSV, pigs inoculated with KV mixed with poly(I:C) or CL097 showed lighter clinical signs, lower viremia and less pathological lesion of lungs, as compared to those of KV and challenge control groups. It indicated that co-administration of poly(I:C) and CL097 with killed PRRSV vaccine conferred higher protection against PRRSV challenge.

In summary, our findings indicated that immunization of poly(I:C) and CL097 could augment the humoral and cell-mediated immune responses against inactivated PRRSV vaccine and enhance the protection against homologous virulent PRRSV challenge in pigs. TLR3 and TLR7/8 ligands might be a useful adjuvant in improving PRRSV immune response and maybe it will be used in PRRSV further vaccine.

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The changes of the lymphocyte subpopulations in peripheral blood of the pigs inoculated with highly pathogenic PRRSV GD wild and vaccine type

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Objective: Porcine reproductive and respiratory syndrome (PRRS) viruses are recognized as possessing a high degree of variability, and different strains have various Pathogenicity of the host. To investigate changes in lymphocyte subpopulations in Peripheral blood of the SPF pigs inoculated with highly pathogenic PRRSV GD wild and vaccine type, which was in a cellular way to discuss the effect between the lymphocyte subpopulations in peripheral blood of the SPF pigs and highly pathogenic PRRSV GD wild and vaccine type, and provide a theoretical basis for the vaccine development.

Methods: We used an immunofluorescence assay and routine blood tests to investigate changes in lymphocyte subpopulations in Peripheral blood of the SPF pigs inoculated with highly pathogenic PRRSV GD wild and vaccine type. In the experiment, twenty SPF pigs of thirty-days were randomly divided into three groups , of which, one group was inoculated with highly pathogenic PRRSV GD fifth, another group highly pathogenic PRRSV GDr 180 vaccine type , the left was negative control . Blood samples were taken at 0, 3, 7, 10, 14, 17, 21, 28 and 35 DPI, and evaluate by Automatic blood analyzer and flow cytometry, then the changes of lymphocyte subpopulations were analyzed.

Results: It showed that specific-pathogen-free pigs inoculated with PRRS wild virus showed remarkable decreases in total lymphocytes, white blood cells, monocytes, granulocytes, B cells, Tc cells, Th cells, Tm cells and gamma-delta T cells, and absolute values were smaller than that of the negative control group, while specific-pathogen-free pigs inoculated with PRRS vaccine virus had no difference with that of negative control group,and increases in total lymphocytes, white blood cells, monocytes, granulocytes, B cells, Tc cells, Th cells, Tm cells after DPI 14, and has little effect in subpopulations of gamma-delta T cells, and the numbers of all cells remain substantially stable in the negative control group.

Discussion: That the specific-pathogen-free pigs inoculated with PRRS wild virus showed remarkable decreases in total lymphocytes, white blood cells, monocytes, granulocytes, B cells and T cells, and absolute values were smaller than that of the negative control group indicated that PRRSV GD wild virus can do harm to swine's immune cells , have immunosuppressive effects on immune organs and the immune system; lymphocytes and white blood cells of specific-pathogen-free pigs inoculated with PRRS vaccine virus had no difference with that of negative control group in the beginning and rose about two weeks after immunization, which indicated that PRRSV GDr 180 vaccine strain will not cause immunosuppression, and can effectively stimulate the body to produce an immune response, including cellular and humoral immunity. Thus, we conclude this vaccine is safe for the thirty-day-old piglets, and provides a viable reference for attenuated PRRSV virus vaccine research. From these results we can conclude that HP-PRRSV GD wild type can destroy immune cells, while HP-PRRSV GD wild type can stimulate immune cell proliferation, provides a good immune response to the pigs.

Reference

[1] Tian K, Yu X, Zhao T, et al. Emergence of fatal PRRSV variants: unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark.[J]. PLoS One. 2007, 2(6): e526.

[2] Duan X, Nauwynck H J, Pensaert M B. Virus quantification and identification of cellular targets in the lungs and lymphoid tissues of pigs at different time intervals after inoculation with porcine reproductive and respiratory syndrome virus (PRRSV).[J]. Vet Microbiol. 1997, 56(1-2): 9-19.

[3] E. Mateu , I Diaz. Changes of lymphocyte subpopulations in pigs infected with porcine reproductive and respiratory syndrome (PRRS) virus. [J]. The Veterinary Journal.2007,5(1-7):2-3.

[4] Pauly T, Weiland E, Hirt W, et al. Differentiation between MHC-restricted and non-MHC-restricted porcine cytolytic T lymphocytes.[J]. Immunology. 1996, 88(2): 238-246.

[5] Yoon K J, Zimmerman J J, Swenson S L, et al. Characterization of the humoral immune response to porcine reproductive and respiratory syndrome (PRRS) virus infection.[J]. J Vet Diagn Invest. 1995, 7(3): 305-312.

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Investigation of recombination in Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

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Sources of genetic diversity in PRRS virus include substitutions, insertions-deletions and recombination. The goal of this research is to develop an in-vitro model to study recombination. The first step is to determine if cells can be co-infected by different PRRS viruses. MARC-145 cells were co-infected with two identical viruses carrying GFP or RFP and confocal microscopy identified dual-fluorescence. Flow cytometry estimated a 17% co-infection rate. The model system developed to study recombination is a two-step process, co-transfection of HEK cells followed by infection of MARC-145 cells. Two infectious clones were constructed: a non-fluorescent virus, PRRS-EGFP-97C, containing mutated EGFP gene and a defective virus, PRRS-EGFP-d(2-6) containing wild-type EGFP gene but lacking ORFs 2-6. Recombination in the mutated EGFP region resulted in restoration of fluorescence which was detected as green fluorescent plaques in MARC-145 cells. The recombination frequency was approximately 0.1%. Recombination between sequences that lack significant homology was investigated by co-transfection of a defective construct, PRRS-GFP-d(2-6), with PRRS-EGFP-97C. Recombination was not detected in this experiment implying the need for greater than 83% homology for template-switching. Wild-type GFP gene was mutated to GFPm which carried a 429 nucleotide stretch of identity flanking the marker site. Recombination was detected when PRRS-GFPm-d(2-6) and PRRS-EGFP-97C were co-transfected, demonstrating that long stretches of identity favor recombination. Further, this system was applied to determine if PRRS-EGFP-97C could recombine with mRNA derived from a plasmid. A stable HEK cell line that expressed wild-type EGFP was transfected with pPRRS-EGFP-97C. MARC cells were productively infected but failed to yield a green virus. This suggested that co-localization of viruses within replication complexes is important for recombination. This in-vitro system is being applied to determine the percentage and pattern of homology required for template-switching during RNA recombination.

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A novel isolate with deletion in GP3 gene of Porcine Reproductive and Respiratory Syndrome virus from the mid-east of China

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Porcine reproductive and respiratory syndrome virus (PRRSV) is the etiologic agent of porcine reproductive and respiratory syndrome, which continues to affect the Chinese swine industry and emergency of new strains by genomic recombination or mutation. Inspection of genomic variant strains is very important to control this disease.

PRRSV strain SH1211 was obtained from the lung tissues of the piglets with clinical signs from a large scale pig farm with mortality of 50% and mobility of 30% approximately in the Mid-east of China in 2012. The full-length genome of the isolate was amplified with RT-PCR as seven overlapping fragments. The non-coding regions were amplified by using RACE Core Set. The complete genome sequence was subsequently assembled and analyzed using DNAStar version, MEGA 5.05 and the recombination detection program version 4.1.3 (RDP4).

The genomic full-length of the isolate was 15,313 bp, excluding the poly(A) tails. It shared a 95% similarity with HP-PRRSV strain JXA1, but only 89.2% nucleotide identity with prototype North American strain VR-2332. Phylogenetic analyses indicated that SH1211 strain form a separate branch which belongs to large phylogenetic branch of highly pathogenic strains. The Nsp2 also had the same discontinuous deletion of 30 amino acid (aa) as HP-PRRSV JXA1, comparing with that of VR2332. GP2 and GP5 shared 91.5% and 85.1% homology with JXA1, respectively. The recombination events analyze result indicated that the nucleotide positions 11697-12568 located in GP2 and 13613-14266 in GP5 encoding regions of SH1211 came from the regions of PRRSV strain QYYZ, a recently emerging prototype Chinese HP-PRRSV field strain. And the remaining areas of the isolate genome maintained the greatest similarity with the prevalent HP-PRRSV in China, such as GDBY1 and HUN4. Most interesting, a deletion of two continuous amino acids position 67-70 was found in GP3 of the isolate, comparing with that of other PRRSV isolates, which being located in an important immuno-dominant domain of the GP3 of North American strains of PRRSV. And the deletion improved the hydrophobicity of GP3 protein of the isolate, comparing to that of JXA1 and VR2332.

In summary, it was first found a novel virulent PRRSV isolate with the deletion within GP3 gene emergency from piglets with clinical signs. It should be helpful for elucidating the molecular characteristics and evolution trend of PRRSV.

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swIFN-beta promotes genetic mutation of porcine reproductive and respiratory syndrome virus in Marc-145

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) can cause respiratory tract disease, death, sow abortion and serious immunosuppression. It is one of the most economically important diseases to the pork industry world-wide. The existence of a virus in a host stimulates immune surveillance mechanisms to eliminate invading viruses. PRRSVs were isolated in the antibody positive pigs, some experiments demonstrate viruses can evolve to escape immune surveillance system for efficient proliferation in the host under continuous antibody pressure. SwIFN- beta is broad-spectrum and used as a new antiviral drug. The purpose of this study is to test whether PRRSV will mutate under the swIFN- beta pressure. This result, for the first time, demonstrates that under swine interferon-β (swIFN- beta) immune pressure molecular variation of porcine reproductive and respiratory syndrome virus (PRRSV) accelerates.

Materials and methods

PRRSV-A1 strain was continuously propagated in Marc-145 cells primed with or without swIFN-beta for 20 passages to develop RRRSV-A1Nf20 strains and PRRSV-A1f20. NSP2, ORF3, ORF5 and ORF6 genes of these strains were amplified, cloned and sequenced.

Results

Sequencing analysis indicated that PRRSV-A1Nf20 had 43 nucleotide mutations while PRRSV-A1f20 had only 14 in NSP2, ORF3, ORF5 and ORF6. The ratio of mutative nucleotides between them was 307%. Amino acid variations were 24 in RRRSV-A1Nf20 and 5 in PRRSV-A1f20, so that mutation in RRRSV-A1Nf20 appeared to be 4.8 times faster than that in PRRSV-A1f20. The ratio of non-synonymous(NS) mutations to synonymous(S) mutations, named NS/S, was 3 in RRRSV-A1Nf20 and 0.625 in PRRSV-A1f20. The nucleotide mutation rate of NSP2、ORF3、ORF5 and ORF6 of PRRSV-A1Nf20 were 3.8%, 2.3%, 4.5%, 0.57% and their NS/S were 5, 2, 6 and 1 respectively, which is much higher than those in PRRSV-A1f20.

Discussion

Our results indicated that the mutative frequency of PRRSV passaged under swIFN-beta immune pressure was significantly faster than that without swIFN-beta. The immuno-pressure of swIFN-beta accelerated genetic variation on PRRSV ORF5, NSP2 and ORF3, but not on the ORF6 gene. PRRSV NSP2 and ORF5 genes may play a key role in escaping from inhibition of swIFN- beta.

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Experimental infection of pigs with two East European variants of Type 1 PRRSV

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Porcine reproductive and respiratory syndrome viruses (PRRSV) have been divided into Type 1 (European) and Type 2 (North American) viruses. PRRSV are very diverse and Type 1 viruses have even been further divided into subtypes. While Type 1 viruses from Western Europe belong to subtype 1, viruses from Eastern Europe have been divided into at least 3 different subtypes based on the length of ORF7 and in addition, atypical Type 1 viruses do not readily group into the subtype groups. In experimental trials it has been shown that some of these viruses, e.g. strain Lena, are more virulent than the subtype 1 strains.

The aim of this project was to study the infection dynamics and clinical and pathological impact of two east European Type 1 strains. In an experimental trial, infection of pigs with the Russian subtype 2 strain "Ili6" and the Belarusian atypical isolate "Bor59" were compared to an early "Lelystad-like" Danish subtype 1 isolate "18794". Groups of seven pigs of unique high sanitary status were infected with one of the three PRRSV isolates, and a fourth group served as sham-inoculated controls. The pigs were monitored for 24 days, and nasal swabs and blood samples were taken at 0, 3, 7, 10, 14, 17, 21 and 24 days post infection (dpi).

The pigs infected with the "Bor59" virus developed higher body temperature and more severe clinical symptoms compared to the other three groups, although the clinical signs in general were mild. The acute phase response was measured in serum samples as an objective indicator of infection. Acute phase protein C-reactive protein (CRP) showed an increase in levels in pigs infected with the Eastern European viruses with an earlier rise for Bor59 than for Ili6, both peaking at 10 dpi. In contrast, the CRP level did not increase significantly in neither the subtype 1 virus inoculated pigs nor the sham-inoculated controls. Acute phase protein haptoglobin showed a very early increase in Bor59 infected pigs, peaking at 3 dpi, while no increase was observed in Ili6 infected pigs.

All of the virus inoculated pigs seroconverted, as measured by IPMA and ELISA, around 7 dpi, and virus was detected by real-time RT-PCR in serum at various quantities and times after infection; detailed PCR analyses are ongoing.

Taken together, these preliminary data suggested that the east European subtype 2 isolate Ili6 and the atypical Bor59 strain induced more severe infection compared to the type 1 "Lelystad-like" virus isolate. This correlates with results obtained from studies of other east European PRRSV strains.

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Fast and robust methods for full genome sequencing of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Type 1 and Type 2

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The high level of diversity among PRRS viruses makes it very important to monitor the overall genetic variations in relation to the sensitivity of diagnostic tests and vaccination efficacy, but only few full genome sequences of PRRSV strains isolated in Europe have been made public available. In the present study, fast and robust methods for long range RT-PCR amplification and subsequent next generation sequencing (NGS) of PRRSV Type 1 and Type 2 viruses were developed and validated on nine Type 1 and nine Type 2 PRRSV viruses. The methods were shown to generate robust and reliable sequences both on primary material and cell culture adapted viruses and the protocols were shown to perform well on all three NGS platforms tested (Roche 454 FLX, Illumina HiSeq 2000, and Ion Torrent PGM™ Sequencer). To complete the sequences at the 5' end, 5' Rapid Amplification of cDNA Ends (5' RACE) was conducted followed by cycle sequencing of clones. The genome lengths were determined to be 14,876-15,098 and 15,342-15,408 nucleotides long for the Type 1 and Type 2 strains, respectively. These methods will greatly facilitate the generation of more complete genome PRRSV sequences globally which in turn may lead to identification of markers of virulence and improve our understanding of PRRSV evolution and pathogenesis.

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The diversity of Porcine Reproductive and Respiratory Syndrome Virus Type 1 and 2 in Denmark

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Both Type 1 and Type 2 PRRS viruses are circulating among Danish pigs. The first appearance of Type 1 PRRSV in Denmark was in 1992 whereas the Type 2 PRRSV was introduced in 1996 after the use of a live attenuated vaccine that reverted to virulence. Since then, vaccination to control the disease for both PRRSV genotypes has been widely used in Denmark and it is therefore highly relevant to monitor the diversity of currently circulating PRRSV strains. Only subtype 1 of the Type 1 PRRSV strains and vaccine-like Type 2 PRRSV strains were previously detected in Denmark, however, only few Danish PRRSV strains were sequenced. Denmark exports more than 50.000 living pigs each month. A portion of these pigs inevitably harbor PRRSV. Thus, the diversity of PRRSV in Denmark is of interest to other countries besides Denmark. The main objective of the present study was to close the gap in knowledge on the genetic diversity of currently circulating PRRSV stains in Danish pigs by sequencing ORF5 and ORF7 of approximately 41 Type 1 and 50 Type 2 strains isolated between 2003 and 2013. Furthermore, full genome analysis was performed on nine Type 1 and nine Type 2 selected strains. The preliminary assessment of the results showed that the Type 1 strains all belonged to subtype 1. Based on the ORF5 sequences, the Danish Type 1 viruses clustered into two groups. These two groups shared 84 % to 92 % and 94 % to 99 % nucleotide identity to the Lelystad virus, respectively. The sequenced Type 2 viruses showed a significant higher level of identity in that the ORF5 sequences were 94 - >99 % identical at the nucleotide level. Most of the Type 2 viruses, shared high level of identity to the VR2332 vaccine strain (Ingelvac MLV), but a few more diverse isolates were also identified, including strains with interesting deletions in NSP2 and other genes. The full genome sequences of Danish strains showed an overall nucleotide identity of 88-98 % (Type 1) and 94 % to >99 % (Type 2). The impact of these results will be discussed.

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The evidence of porcine respiratory and reproductive syndrome virus evolved under pressure from deaminases

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Porcine reproductive and respiratory syndrome (PRRS) is an economically important infectious disease infected with PRRS virus (PRRSV) for the pig industry worldwide. Especially, the highly pathogenic PRRSV (HP-PRRSV) was emerged in China in 2006, which caused atypical PRRS or "High Fever" disease. Since then, PRRSV evolutionary mechanism, especially the question that PRRSV evolved from low pathogenesis to high pathogenesis was never failed to fascinate us.

In this study, we compared the genomic sequences of six PRRS attenuated live vaccine strains with the sequences of their parental strains (CH-1a/CH-1R, HuN4/HuN4-F112, JXA1/JXA1-R, VR-2332/respPRRS MLV and JA142/Ingelvac ATP), respectively by DNAStar program 7.0. Interestingly, the results indicated that the whole genome of the six PRRSV parental strains experienced some types of prone hypermutation in the process of successive passages on Marc-145 cells. Among mutations of all six PRRSV attenuated strains, substitutes of A→G/G→A and C→U/U→C were significantly higher than the other types of substitution, especially the pair of VR-2332/respPRRS MLV. In addition, we compared PRRSV classic strain CH-1a with 10 isolated epidemic HP-PRRSV strains (HuN4, JXA1, NM1, SY0909, JXwn06, CWZ-1-F3, GS09-16, GS09-29, GS09-32 and HEB), respectively. What amazed us was that significant hypermutation was similar to observed in vitro attenuation. Furthermore, we monitored the evolution of PRRSV in vivo. Four piglets (#51, #53, #55 and #56) were infected with HP-PRRSV HuN4 strain. Circulating viruses were collected at 3, 7 and 14 days post infection (DPI). Sequence analysis of the viral structure gene GP5 and non-structure gene NSP2 demonstrated that a prevalent A→G/G→A and C→U/U→C substitutions were observed in viruses isolated from all the four animals and at all the time points examined. In addition, NSP2 were more prone to be hypermutated. The aforementioned results implicated that the PRRSV evolved in vitro and vivo under certain strong pressure(s) of host factors.

Some previous studies clearly indicated that C→U and G→A hypermutations were caused by the action of APOBEC3G and that A→I(G) and U→C hypermutation for several different RNA viruses attributed to the action of ADARs. Based on the specificity of substitution types of the sequences examined, we speculated that deaminases might play a major role in this process, especially adenosine deaminases and cytidine deaminases, such as the APOBEC family and ADAR family. These data implicate that the APOBEC family and ADAR family largely involve in the evolution of PRRSV. However, it remains to be explored that which member of deaminases families predominates the hypermutation. This study shows for the first time that in the evolution of PRRSV, the hypermutation induced by deaminases largely contributes to the viral genomic variation.

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Genomic sequencing and genetic variation analysis of highly pathogenic porcine reproductive and respiratory syndrome virus

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Objective
To investigate the variation of highly pathogenic porcine reproductive and respiratory virus (HP-PRRSV) prevailing in mainland of China for the past few years.
Methods
The whole genome of a strain of PRRSV GD-2011 isolated from one pig farm of Guangdong province with clinical outbreak of PRRS was sequenced and analyzed by RT-PCR amplification.
Results
The results indicates, excluding the poly(A) tail, the genomic sequence of GD-2011 was 15323 nucleotides (nt) in length. Genetic analysis revealed that the virus shared 97.01-98.78% identity with the representative strains of highly pathogenic PRRSV (JXA1, HuN4 and GD), 87.68%-93.29% identity with the representative strains of classic PRRSV (VR-2332 and CH1-a), and 51.20% identity with the representative strain of European PRRSV (Lelystad), and exhibited two unique discontinuous deletions of 30 amino acid (aa) deletion within Nsp2-coding region just as JXA1. In addition, variation of 1, 9, 10, 10, 1 and 2 aa was found in GP2, GP3, GP4, GP5, M and N-coding regions of its genome, respectively; Compared with 55 HP-PRRSV strains, it's worth noting that one aa insertion in the GP5 region of GD-2011 was found. Phylogenetic analysis illustrated that GD-2011 belonged to the same subgroup with other highly pathogenic PRRSV, and its homology with HP-PRRSV of 2010 is higher than 2006-2009.
Discussion
After multiple alignment of GD-2011 and 86 Northern America PRRSV strains, we found many mutations in the nucleocapsid-coding region that is the most conservative region of whole genome, which prompt that a combination of detection methods should be considered to boost the exactitude of PRRSV infection, while using RT-PCR, IFA or IPMA that rely on nucleotide sequence recognition or epitope recognition by monoclonal antibody. Our study provides beneficial reference for exploring the genetic features, variation pattern and molecular detection of PRRSV.
Reference
[1] Wang Chengbao, Zhao Qin, Liang Chao, et al. complete genome sequence of a highly pathogenic porcine reproductive and respiratory syndrome virus variant[J]. J. Virol, 2012, 86(16): 8906.
[2] Jean-Pierre Frossard, Catherine Fearnley, Brindha Naidu, et al. Porcine reproductive and respiratory syndrome virus: antigenic and molecular diversity of British isolates and implications for diagnosis[J]. Veterinary Microbiology, 2012, xxx-xxx.

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Recombination events are common in genotype 1 and 2 PRRS virus worldwide and may generate mosaic viruses

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In the last years a number of papers provided increasingly evidence that recombination in PRRS may contribute to viral diversity. However, the frequency of such recombination events and how the occur are not well known. In the present study we initially focused in the detection of recombinant isolates by examining previously published ORF5 datasets of genotype 1 and 2 sequences obtained worldwide. Then, we examined full length genome sequences in order to determine potential recombination breakpoints. For genotype 1, 11 sets were examined containing 558 sequences; 10 datasets corresponded to previously published papers containing isolates from Germany (n=48), Hungary (two sets, n=39+11), Italy (n=63), Republic of Korea (n=25), Romania (n=15), Thailand (n=69); United Kingdom (n=140), United States (n=24) and Eastern Europe (n=74) and one dataset comprised newly sequenced isolates from Spain (n=50). Sets of ORF5 sequences for genotype 2 PRRSV strains (n=237) corresponded to previously published sequences from China (n=38) and United States (n=51) plus a set of 148 sequences from isolates of Sonora (Mexico) (2003 to 2011). Sequences were initially analyzed using the single breakpoint detection utility (SPB) and the general A recombination detection (GARD) of the Hyphy package. Then, each set was subjected to a phylogenetic analysis in MEGA 5.1 by the Maximum Likelihood (ML) method (general time reversible model) and with MrBayes 3.2. For sets of sequences where potential recombination breakpoints were detected, phylogenies were re-examined after splitting sequences in two segments, up to and after the breakpoint. The resulting trees were compared in order to search for discrepancies in the clustering of isolates. Afterwards, sets made of the nearest isolates to the potential recombinant strains were examined in RDP v.4.6 to figure out potential parental strains. To further investigate whether recombination occurred in ORF5 or could take place in different points of the viral genome two sets of ORF1-7 sequences (16 of genotype 1 and 64 of genotype 2) were analyzed similarly. The results indicate that most of the ORF5 sets of sequences contained at least one recombinant sequence. Interestingly, breakpoints were always located in first 200 nucleotides of the 5'end most commonly between nt 166 and 180. Parental strains of recombinant isolates were identified in several cases. When the whole sequences were examined, both genotype 1 and 2 presented a similar breakpoint in the nsp2-coding region of ORF1a. Mosaic genomes were detected in both genotypes. These results may have implications for the understanding of the molecular epidemiology of PRRSV.

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Evolution dynamics of highly pathogenic type 2 PRRSV: analysis of envelope protein coding genes

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The highly pathogenic type 2 PRRSV emerged in China in 2006 had been spreading widely in China swine herds, filtered out into pig populations in Vietnam, Laos, and Thailand, and placed neighboring countries at risk. The virus was the results of local diversification that lead increasing virulence and was shown continuously evolve. Applying the Bayesian Markov chain Monte Carlo and Maximum likelihood approaches, this study analyzed the evolutionary dynamics of type 2 PRRSV, focusing on the highly pathogenic clade and the typical clade. Inferred from the data best-fit model of nucleotide evolution, seven known structural genes (ORF2a-ORF6) of type 2 PRRSV were estimated to be fast evolving at the order of 10^{-3} nucleotide substitutions/site/year. Along internal branches of the viral phylogeny, the typical clade had higher rates (1.03 – 1.31 fold) in ORF2a-2b, and ORF5 genes, while it substituted at lower rates (1.12 – 1.45 fold) in ORF3, ORF4, ORF5a, and ORF6 genes than those of the highly pathogenic clade. Intriguingly, the estimations of absolute synonymous and non-synonymous substitution rates encountered a high level of non-synonymous than synonymous substitution divergence in ORF3, ORF5, and ORF6 genes, solely for the highly pathogenic clade. It reflected that the adaptive evolution of the highly pathogenic clade was in a qualitatively different manner from that of the typical clade. For conclusions, both the typical and the highly pathogenic clades of type 2 PRRSV are among fast evolving RNA viruses. Several structural genes of the highly pathogenic type 2 PRRSV displayed unique evolution dynamics to those of the typical type 2 PRRSV, thus suggested multigenic factors contribute to diversifying biological properties between the two clades.

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Phylogentic analysis and molecular characteristics of five variant Chinese field isolates of PRRSV

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Background: Porcine reproductive and respiratory syndrome (PRRS) has now been widely recognized as an economically important disease. The objective of this study was to compare the molecular and biological characteristics of porcine reproductive and respiratory syndrome virus (PRRSV) field isolates in China to those of the modified live virus (MLV) PRRS vaccine and its parent strain (ATCC VR2332).
Results: The two genes (GP5 and NSP2) of five isolates of PRRSV from China, designated AH4, GX5, ZJ1, ZJ2 and ZJ3, were sequenced and analyzed. Phylogentic analyses based on the nucleotide sequence of the ORF5 showed that the five Chinese isolates belonged to the same genetic subgroup and were related to the North American PRRS genotype. Comparative analysis with the relevant sequences of another Chinese isolate (NVDC-JXA1) and North American (VR2332 and MLV) viruses revealed that these isolates have 87.2-89.4% homology with VR2332, and 87.2-89.1% identity with MLV Resp PRRS/Repro vaccine and 89.9-99.2% with NVDC-JXA1. All NSP2 nonstructural protein of these five isolates exhibited variations (a 29 amino acids deletion) in comparison with other North American PRRSV isolates. Therefore, these isolates were novel strain with unique amino acid composition. However, they all share more than 96.8% identity with other highly pathogenic Chinese PRRS strains. Additionally, there are extensive amino acid (AA) mutations in the GP5 protein and the NSP2 protein when compared with the previous isolates.
Conclusions: These results might be useful to study the genetic diversity of PRRSV in China and to track the infection sources as well as for vaccines development.

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Phylogenetic analysis for PRRS evolution in Romania

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This is one of the few studies ever made on Romanian territory regarding the distribution and the phylogenetic relationships, since the detection of the virus in this country in 1998. The aim of this study was to evaluate the genetic diversity of PRRSV isolates from four most important pig farms in Romania by comparing the nucleotide sequences obtained for ORF5 gene and ORF7 gene with a wide range of sequences from GenBank belonging to the main types of PRRSV, the type 1. Molecular biology techniques were used to accomplish this study.

In order to investigate the PRRSV genetic variability in the four pig farms, 605 samples were collected from different individuals. From the total amount of samples, 33 were positive for PRRSV by PCR.

A number of 18 different sequences were obtained for ORF5 gene and 10 for ORF7 gene. One Romanian isolate (Genbank Accesion No. JX075094) was found in three of the four different investigated farms. The ORF5 and ORF7 specific PCR products were sequenced to confirm the occurrence of type 1 of PRRSV in Romania.

The phylogenetic analysis revealed that the Romanian PRRSV nucleotide sequences clustered in three groups within the subtype 1 of the virus. ORF5 and ORF7 corresponding sequences were compared with a set of reference sequences selected from GenBank to cover a wide range of genetic and geographic diversity from Europe.

This is the first extensive study on Romanian PRRSV isolates that provides information about the genetic diversity of this virus in the four most important pig farms in Romania. The results obtained from the phylogenetic trees together with the pairwise nucleotide sequence identity confirm the affiliation of all Romanian isolates to the subtype 1 of the virus.

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A cell culture-derived deletion in the nsp2 region of a North American PRRSV overlaps with a unique nsp2 deletion reported in a Chinese PRRSV isolate

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The nonstructural protein 2 (nsp2)-encoding region is one of the most variable regions in PRRSV genome, with various deletions (insertions) have been reported. However, the molecular mechanism which drives such hypervariability is still unknown. In this study, we characterized a cell-culture attenuated type 2 PRRSV isolate, NA 04-30. The virus was continuously passaged 80 times in MARC-145 cells. Full-length genome sequence analysis showed that NA 04-30 virus shares 98.4% nucleotide identity with VR-2332, the prototypic North American type 2 strain. In comparison to the P1 virus, the NA 04-30 P80 contains a large 123 amino acid (aa) deletion (nucleotide 3221 to 3593 of the viral genome) in the central region of nsp2. Most importantly, this 123 aa deletion region overlaps with a 144 aa deletion region recently reported in a Chinese field isolate, NJ1106 (Du et al., 2012, JVI, 86:13883-13884); and *in vivo* characterization in a nursery pig model demonstrated that the NJ-1106 was less virulent in comparison to HP-PRRSV strain JXA1. In our study, a swine cytokine assay showed that NA 04-30 P80 was able to induce higher level of interferon response in virus-infected swine alveolar macrophages, compared to that of NA04-30 P1, VR2332 and other typical PRRSV isolates. Sequence analysis showed that both 123 aa and 144 aa deletion regions contain predicted T-cell and B-cell epitopes. Results from this study suggested that NJ1106 might be initially derived from a cell-culture attenuated virus, and the unique deletion (123/144 aa) in nsp2 could contribute to the attenuation of viral pathogenesis. The selective pressure of the host immune system could be one of the driving forces for adaptation of the virus in the new environment, resulting in deletions (insertions) in the nsp2 region.

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A novel strain of PCV2 isolated from vaccinated pigs suffering PCVAD causes 40% mortality in CDCD pigs in the presence of PPV

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A unique strain of PCV2 was recently isolated at the Kansas State Veterinary Diagnostic Laboratory from a PCV2 vaccinated herd that was experiencing PCVAD. This novel PCV2 (nPCV2) isolate is only 94% homologous to both PCV2a and PCV2b and appears to be a novel strain of PCV2 that appears to be new to North America. It is very similar to PCV2 sequences found in banked serum of pigs suffering from PCVAD in China. The authors identifying this strain suggest that PCV2 in China appears to be evolving rapidly. In an attempt to characterize the pathogenicity of this novel PCV, virus laden serum was used to inoculate 10 cesarean-derived colostrum-deprived (CDCD) 6 week old pigs. Subsequent to the nPCV2 inoculation it was determined that the CDCD pigs were undergoing an acute porcine parvovirus outbreak. At seven days post exposure the pigs were nPCV2 viremic, as determined by both qPCR and virus isolation (VI). Twenty-one days post inoculation the mortality rate was 40% (4 of 10). The viral infection was accompanied by the clinical presence of fever in the animals. Pulmonary edema and lymphoid depletion were common in the pigs that died. Additionally, these four animals were jaundiced and suffered from portal hepatitis, liver fibrosis, and in two cases hepatic necrosis. Immunohistological staining of the lung and liver show very high concentrations of PCV2, greater than that seen in animals concurrently infected with PCV2b and PRRSV. The rate at which the PCV replicated in the animals was faster than that seen in animals co-inoculated with PCV2b and PRRSV based on previous experiments with a PCV2b and PRRSV co-challenge. Peak nPCV2 infectious viral titers in serum were found seven days post-exposure, while the peak qPCR serum concentration of nPCV2 DNA was found 14 days post-infection. This is much earlier than seen in controlled infections with PCV2b and PRSSV where PCV2b virus is first detected, by PCR at 14 days post-infection and peaks between 14 and 21 days. Three of the pigs that died and one surviving animal failed to produce a significant concentration of PCV2 antibodies as determined by an immunofluorescent antibody assay. The remaining animals all developed significant anti-PCV2 antibody titers. The results of this small scale animal study suggest that nPCV2 replicates more rapidly in infected animals and PPV can play an important role in the development of PCVAD. The presence of this novel PCV2 virus poses the question of whether the virus isolated from North American pigs is derived from a strain previously present in China and if so how was it transmitted? Further, will the emergence of this novel PCV2 lead to an increased incidence of PCVAD in North America?

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A monoclonal antibody against the N of a HP-PRRS vaccine strain (HuN4-F112) could not recognize its virulent strain(HuN4-F5) not due to their amino acid sequence difference

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Previous studies showed that a few, discontinuous amino acid mutations existed between HuN4-F112 and its virulent strain (HuN4-F5), while its biological characteristics had changed a lot. How to find the antigenic differences between them is of great scientific significance to investigate molecular mechanisms of its attenuation.

In this study, a hybridoma cell line against the HuN4-F112 not HuN4-F5 was generated, named 3-3D7, by using HuN4-F112 as immunogen and screened by IFA. The MAb could also recognize two other commercial vaccines(JXA1-R and TJM-F92). HuN4-F112 N protein could combine the MAb specifically by transient transfection and western blot. The further IFA results showed that 3-3D7 MAb could not recognize two HP-PRRSV strains (HNZJJ-F1 and HLJMZ-F2) in addition to HuN4-F5. ORF7 genes of the six strains mentioned above were sequenced. We found that HP-PRRSV had no identical difference point compared with vaccine strains. 3-3D7 MAb could not react with HuN4-F5 by either IFA or western blot. But N protein of HuN4-F112 and HuN4-F5 that had only one difference at position K11 to R11 were expressed in E.coli BL21, and both of them reacted with 3-3D7 MAb. Then, linear B-cell epitope recognized by 3-3D7 MAb was located in the N-terminal (1~42aa) by western blot.

On the whole, 3-3D7 MAb could only react with three vaccine strains not several HP-PRRSV. This phenomenon is not related to amino acid sequence difference. Is there any changes of protein modification in the passage or else? Maybe it is a respect of HP-PRRSV attenuation.

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Molecular basis associated with the pathogenicity of a genotype 1 PRRSV isolate

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Porcine reproductive and respiratory syndrome virus (PRRSV) is classified into two genotypes—European genotype 1 and North American genotype 2. Recent findings have revealed the emergence of genotype 1 PRRSV in China. In the present study, a strain of genotype 1 PRRSV (GZ11-G1) was characterized and the molecular basis associated with its pathogenicity was investigated. The full-length sequence of GZ11-G1 was determined. Its pathogenicity was analyzed in 6-week-old SPF piglets, together with the European vaccine strain (Amervac PRRS) and the Chinese genotype 2 PRRSV isolate (HB-1/3.9). The GZ11-G1 could cause obvious clinical symptoms, exhibiting higher pathogenicity than the vaccine virus in piglets. The genomic sequence analyses showed that GZ11-G1 was closely related to the European vaccine virus, with nucleotide homology of 98.6%, and its 4 amino acids within Nsp9-10-coding region (93 G in Nsp9, 281 P, 304 V, 401 K in Nsp10) and 3 amino acids in GP2-coding region (5 H, 120 G, 252 S) were conserved among pathogenic genotype 1 PRRSV. Based on these marked genomic difference, we constructed the full-length infectious clones of the two viruses (pWSK-GZ11-G1, pWSK-A) and generated six rescued mutant viruses by point mutagenesis (designated Rv-A-G-Nsp-9-10, Rv-A-G-GP2, Rv-A-G-GP2+Nsp-9-10, Rv-G-A-Nsp-9-10, Rv-G-A-GP2, Rv-G-A-GP2+Nsp-9-10 respectively) , and compared their pathogenicities in 6-week-old SPF piglets in order to analyze potential pathogenicity-associated determinants of GZ11-G1. Compared with parental rescued virus (Rv-A), Rv-A-G-Nsp-9-10 showed higher virus titer in sera and more severe pathogenicity for pigs. Oppositely, Rv-G-A-Nsp-9-10 exhibited lower replication capacity in vitro and milder pathogenicity than Rv-GZ11 for pigs. While the mutation of 3 conservative amino acids in GP2-coding region of GZ11-G1 did not affect viral growth ability. Our results show that GZ11-G1 is a pathogenic genotype 1 PRRSV, and its 4 amino acids in Nsp9-10 region (93 G in Nsp9, 281 P, 304 V, 401 K in Nsp10) are related to its growth ability both in vitro and in vivo and pathogenicity for piglets.

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Phylogenetic analysis and molecular characteristics of 17 porcine reproductive and respiratory syndrome virus isolates in Southern China from 2010-2011

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We analyzed the complete genomic sequences of 17 porcine reproductive and respiratory syndrome virus (PRRSV) isolates from Southern China obtained between 2010 to 2011 and found that four of seven isolates from 2011 were closely related to the JXA1-R strain (vaccine virus of JXA1). This close relationship between field isolates and China domestic vaccine viruses has not been reported to date. The occurrence of vaccine-like viruses potentially creates a threat for the pig breeding industry and brings difficulties for control of this disease.

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Non-structural protein 1-mediated interferon modulation is a common strategy for immune evasion in arteriviruses

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Type I interferons (IFNs) play a key role for the antiviral state of host but porcine reproductive and respiratory syndrome virus (PRRSV) has been shown to down-regulate the production of IFNs during infection. Non-structural protein (nsp) 1 of PRRSV has been identified as a viral antagonist for IFN production, and subsequently the nsp1-alpha subunit of nsp1 has been shown to degrade the CREB (cyclic AMP responsive element binding)-binding protein (CBP). CBP is a major component of the IFN enhanceosome, and thus the CBP degradation inhibits the formation of enhanceosome resulting in the suppression of IFN gene expression. The current study was conducted to determine whether the IFN modulation was a common strategy for other member viruses in the family Arteriviridae. The Arteriviridae family is consisted of PRRSV, equine arteritis virus (EAV), murine lactate dehydrogenase-elevating virus (LDV), and simian hemorrhagic fever virus (SHFV), and thus individual nsp1 genes were cloned from EAV, LDV, and SHFV and expressed in cells. LDV nsp1 was auto-cleaved into the nsp1-alpha and nsp1-beta subunits, whereas EAV nsp1 remained uncleaved. The SHFV nsp1 gene was larger in size than those of EAV, LDV, and PRRSV, and was predicted to generate three subunits. All subunits of nsp1 from all arteriviruses were localized in the nucleus, but their nuclear localization was predominant for PRRSV nsp1-beta, LDV nsp1-beta, SHFV nsp1-gamma, and EAV nsp1. When examined for their IFN modulation using luciferase reporter assays, all subunits of nsp1 including the uncleaved EAV nsp1 exhibited the suppression of IFN production, and the suppression was mediated via the interferon regulatory factor 3 (IRF3) and NF-kappa B pathways. The total amount of IRF3 remained unchanged and the IRF3 phosphorylation and nuclear localization occurred normally upon stimulation, suggesting that the IFN suppression by nsp1 was a nuclear event. The CBP degradation was evident in cells expressing LDV nsp1 and SHFV nsp1, whereas no degradation was identified for EAV nsp1. Our data demonstrate that the nsp1-mediated IFN modulation is a common strategy for immune evasion in arteriviruses, but their mechanisms of action differ.

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Suppression of type I interferon production mediated by PRRS virus non-structural protein 1-beta

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Production of type I interferons is essential for the establishment of anti-viral status and clearance of invading viruses. During infection of pigs with porcine reproductive and respiratory syndrome virus (PRRSV) however, only a low level of IFNs is detected and the disease is often associated with viral persistence. Non-structural protein (nsp) 1 is the first viral protein synthesized during infection and has been reported to be a viral IFN antagonist. Nsp1 is cleaved into two subunits, nsp1-alpha and nsp1-beta, and the nsp1-alpha subunit has been shown to degrade the CREB-binding protein in the nucleus, resulting in the suppression of IFN production. The present study was conducted to explore the molecular basis of the nsp1-beta subunit-based IFN modulation. As with nsp1-alpha, nsp1-beta was nuclear-cytoplasmic in PRRSV-infected cells. In gene-transfected cells however, nsp1-beta accumulated predominantly in the nucleus. The nsp1-beta-mediated IFN modulation was examined by targeting the IRF3 and NF-kB dependent production pathways. Expression of nsp1-beta resulted in the suppression of IFN production when stimulated by over-expressing the constitutively activated IRF3 and RIG-I adaptors. Nuclear translocation of IRF3 is required for IRF3 activation and was evident in cells expressing nsp1-beta, suggesting that inhibition of IFN production occurred in the nucleus. To identify the functional domain in nsp1-beta responsible for IFN suppression, mutations were introduced into the catalytic sites of the nuclease domain and/or the papain-like cysteine protease-beta (PCP-beta) domain in nsp1-beta. The IFN suppressive activity of nsp1-beta was not associated with the nuclease activity whereas it was partially affected by PCP-beta mutation H159Y. The subcellular distribution of PCP-beta mutants was altered in comparison with that of wild-type nsp1-beta and the mutant proteins mostly remained in the cytoplasm with a punctated pattern. The nsp1-beta-mediated IFN suppression was not limited to the production pathway only, but was also observed for the Janus kinase (JAK)-STAT (signal transducers and activators of transcription) pathway, indicating a multifunctional role of nsp1-beta as an IFN antagonist.

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Construction and biological characterization of recombinant porcine circovirus type 2 expressing the V5 epitope tag

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Porcine circovirus type 2 (PCV2) is a major causal agent of postweaning multisystemic wasting syndrome (PMWS) in piglets. To investigate the feasibility of PCV2 expressing an exogenous epitope, a 14 amino acid V5 epitope derived from simian parainfluenza virus type 5, was inserted into the C-terminus of the capsid protein. Recombinant PCV2 expressing the V5 epitope, recPCV2/CL-V5, was rescued by transfecting an infectious clone into PK-15 cells and was characterised by an immunoperoxidase monolayer assay (IPMA), a serum neutralisation assay (SNA), a capture enzyme-linked immunosorbent assay (ELISA) and immune electron microscopy. The V5 epitope could be detected in the recombinant marker virus by IPMA and the capture-ELISA. Furthermore, there was no detectable difference in the antigenicity of the recombinant marker virus compared with the parental virus by IPMA and SNA using PCV2 positive serum and the neutralizing monoclonal antibody (mAb) 1D2. However, recPCV2/CL-V5 marker virus could be differentiated from the parental virus by PCR, IPMA and the capture-ELISA. The recombinant marker virus was stable on multiplication through 10 passages in PK-15 cells; with a maximum titre of 106.25 the 50% tissue culture infective dose (TCID50)/ml. BALB/c mice were inoculated with the recombinant virus or parental virus via both the intranasal and intraperitoneal routes. Both parental and recombinant viruses could replicate in mice, cause microscopic pathological changes and induce mice to generate anti-PCV2 antibodies. Furthermore, the recombinant marker virus could also induce anti-V5 epitope tag antibodies. These results indicated that V5 epitope could be displayed on the surface of the capsid protein by inserting its gene just before stop codon of open reading frame 2 (ORF2). More importantly, insertion of the V5 epitope did not seem to interfere with biological characterization of the recPCV2/CL-V5 marker virus.

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Identification of one critical amino acid that determines a conformational neutralizing epitope in the capsid protein of porcine circovirus type 2

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Background: Porcine circovirus type 2 (PCV2) is associated with post-weaning multisystemic wasting syndrome (PMWS) in pigs. Currently, there is considerable interest in the immunology of PCV2, in particular the immunological properties of the capsid protein. This protein is involved in PCV2 immunogenicity, may play a role in viral immunosuppression and is a potential target for vaccine development.
Methods and results: One monoclonal antibody (mAbs; 8E4), against the capsid protein of PCV2, was generated and charactered in this study. MAb 8E4 reacted with PK-15 cells infected with the genotype PCV2a (CL strain) but not PCV2b (YJ strain) by an immunoperoxidase monolayer assay (IPMA) and a capture ELISA. Furthermore, the mAb had the capacity to neutralize the PCV2a-CL strain but not PCV2b-YJ strain. One critical amino acid that determines a conformational neutralizing epitope was identified using the mAbs 8E4 and PCV2 infectious clone technique. The amino acid residues 47 to 72 in the capsid protein of PCV2a-CL strain were replaced with the corresponding region of PCV2b-YJ strain, the reactivity of mAb 8E4 was lost. Further experiment demonstrated that one amino acid substitution, the alanine for arginine at position 59, A59R) in the capsid protein of PCV2a-CL strain, can inhibit completely the immuno-reactivity of PCV2a-CL strains with the mAb 8E4.
Conclusions: It is concluded that the alanine at position 59 in the capsid protein of PCV2a-CL strain is one critical amino acid, which determines the neutralizing epitope of PCV2a genotype. This study may provide valuable tool for further mapping the conformational neutralizing epitope of the virus capsid protein.

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Mutation in a conserved motif of porcine circovirus type 2 capsid protein reduces capsid protein expression, hampers colocalization between capsid and replication proteins and impacts viral fitness

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Background: The conserved motif ^{97}RIRKVKV^{103} in the porcine circovirus type 2 (PCV2) capsid protein (Cap) was initially presumed to be a binding site for cell surface proteoglycans. As this motif later appeared to be localized on the inner surface of Cap, its role as a binding region was excluded. Because this motif is highly conserved among PCV2 strains, it probably plays an important role during replication. Therefore, the present study aims to clarify the function of this conserved motif on PCV2 replication.

Methods: PCV2 strain 48285 (parental clone) was isolated from a PMWS-affected piglet. Mutation of positively charged amino acids in ^{97}RIRKVKV^{103} of PCV2 strain 48285 was carried out to substitute arginine or lysine for uncharged amino acids threonine by site-directed mutagenesis. Infectious clones of mutants and parental clone were transfected into PK-15 cells. After transfection, the transfected cells and the supernatants were collected at different time points. The number of PCV2 positive cells was quantified by an immunofluorescence assay (IFA). Sixteen monoclonal antibodies (mAbs) that recognize 48285 were used to test their reactivity to the mutants. The Cap expression of mutants and parental clone were quantified by western blot, a capture-ELISA and a reverse transcription quantitative PCR (RT-qPCR) assay. A double IFA for Cap and the replication protein (Rep) was performed and quantitative colocalization analysis were used to estimate the degree of colocalization of Cap and Rep. Virus titration and passaging were used to test the infectivity and fitness of mutants.

Results: After transfection in PK-15 cells, the Cap signal of mutants was less pronounced than that of the parental clone 48285 as revealed by IFA. Compared to the parental clone, a significant reduction in the expression of Cap was observed with the mutants by western blot analysis. The reactivity of mutants with 16 PCV2-Cap mAbs showed that the reduced signal of the mutants was mainly due to the lower Cap expression. Lower levels of Cap mRNA were detected with the mutants by RT-qPCR assay. Quantitative colocalization analyses demonstrated that the mutants showed a partial or even complete lack of colocalization of Cap and Rep. The decreased expression of Cap and the lack of colocalization abolished viral propagation and resulted in unfit viruses by virus multiplication and titration.

Conclusion: This study shows that the conserved motif is very crucial for PCV2 replication in PK-15 cells. Mutations in this motif resulted in (i) reduced expression of Cap mRNA and Cap, (ii) hindered Cap-Rep colocalization and (iii) finally unfit PCV2. This study provides new information on the interaction between Cap and Rep. We presume that interaction between Cap and Rep is necessary for genome encapsidation.

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Identification of porcine reproductive and respiratory syndrome virus ORF1a-encoded replicase proteins in virus-infected cells

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The porcine reproductive and respiratory syndrome virus (PRRSV) ORF1a-encoded replicase polyproteins (pp1a) is predicted to be proteolytically processed into ten nonstructural proteins (nsps), known as nsp1 to nsp8, with both nsp1 and nsp7 being cleaved internally (nsp1alpha/1beta and nsp7alpha/7beta). In this study, monoclonal and polyclonal antibodies were generated against seven of these proteins (nsp1alpha, nsp1beta, nsp2, nsp3, nsp4, nsp7 and nsp8). Using this panel of antibodies, we identified and characterized ORF1a-encoded nsps in PRRSV infected MARC-145 cells. Western blot and immunoprecipitation analysis of virus-infected cell lysates identified nsp1alpha, nsp1beta, nsp3 and nsp4 cleavage products of the predicted size, and revealed the association of nsp2 with nsp3. Protein microsequencing analysis identified the amino- and carboxyl-terminal sequences of nsp2. Transient expression of ORF1a in a recombinant vaccinia virus/T7 RNA polymerase system was used to analyze proteolytic processing in more details. In immunofluorescence assays, the majority of the PRRSV pp1a cleavage products can be detected as early as 6 hours post-infection. At early times of infection, nsp1alpha, nsp1beta, nsp2, nsp4, nsp7 and nsp8 localized to the perinuclear region as distinct punctate foci. Later in infection, nsp1alpha, nsp1beta and nsp4 were found to partially localize to the nucleus. Taken together, this study confirmed the actual existence of proteolytic processing products of PRRSV ORF1a-encoded polyprotein in virus infected cells. Our panel of antibodies against PRRSV nonstructural proteins will be important tools for studies into PRRSV replication and pathogenesis.

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Antigenic epitope mapping using the monoclonal antibody against the recombinant Rep' protein of porcine circovirus type 2

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Porcine circovirus type 2 (PCV2) is an important causative agent of postweaning multisystemic syndrome in pigs, and both replicase proteins (Rep' and Rep) encoded by PCV2-ORF1 are essential for the viral replication. To identify the antigenic epitopes, the monoclonal antibody (McAb) against the Rep' protein of porcine circovirus type 2 (PCV2), BALBc mice were immunized with the purified recombinant PCV2-rRep' protein expressed by baculovirus system. A cell line of hybridoma screening McAb to the Rep' protein were prepared by means of lymphocyte hybridoma technique. The obtained McAb was named as 3D1. The McAb isotypes was identified to be IgG1-kapa, and its antibody titer of ascites was 1: 819 200. Western blot analysis showed that the McAb could react with both of the recombinant PCV2-rRep' and PCV2-rRep proteins expressed by baculovirus. The Rep' and Rep expression dynamics in the PCV2-infected cells was detected by IPMA method using the McAb. The Rep' and Rep antigens could be detected as early as 24 h post-infection (p.i.) and reached the peak from 36 h to 72 h p.i., and up to 96 h p.i. downturn. The McAb was used for further accurately antigenic epitope mapping by the synthesized peptides scanning method. The epitope core sequence was determined to be 61FANFVKKQTFNKV73, which located at the nuclear localization signal region of the N terminal of Rep' protein. This suggests that the McAb against PCV2-Rep' protein and its corresponding epitope mapping can provide a basis for further research on the viral replication mechanism as well as differentiating diagnosis between PCV2 national infection and the recombinant PCV2-Cap protein subunit vaccine-immunized animals.

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Characterization of two chimeric viruses by exchanging ORF2 gene of porcine circovirus type 1 (PCV1) and type 2 (PCV2)

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The generation of chimeric viruses, typically by replacing a gene sequence with the similar sequence from a related virus, has been a useful genetic tool in various virus systems to analyze infectivity, replication, and virulence factors. Chimeric viruses are commonly used to compare the functional properties of genes from one virus with the homologous genes from a related virus. Information gained from these types of studies includes defining mechanisms of viral replication and pathology. In addition, chimeric viruses containing altered and optimized capsid protein epitopes could be generated and used as a tool in viral vaccine development. Two genotypes of porcine circovirus (PCV) have been identified. Porcine circovirus type 1 (PCV1) is non-pathogenic, whereas porcine circovirus type 2 (PCV2) causes post-weaning multi-systemic wasting syndrome (PWMS) in pigs. To investigate the probability of the functional interaction and the relatedness of ORF1 encoding viral replicase (Rep) and ORF2 encoding capsid (Cap) protein in the two different PCVs, two recombinant chimeric plasmids were constructed by exchanging the ORF2 genomic regions between PCV1 and PCV2. The two resulting constructs (PCV1R2C and PCV2R1C) produced infectious virus after transfection into swine kidney cells. The viral infectivity was determined using immunocytochemical staining and PCR analyses. The results revealed the presence of the capsid protein determined by serological reactivity with the two chimeric viruses. PCV1R2C and PCV2R1C viruses were harvested with initial titers of 104.4 and 103.8 TCID50/ml, respectively, although they showed poor growth properties relative to the parental viruses after a series of passages. This study suggests that the generated chimeric viruses may provide a useful tool for further understanding pathogenic mechanisms and genetic variation of these viruses.

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Natural recombinant mutants, resulting from replacement of an open reading frame 2 (ORF2)-containing genomic fragment of porcine circovirus (PCV) genotype 2b with that of genotype 2a, showed significantly enhanced viral replication and altered antigenicity in vitro

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Porcine circovirus type 2 (PCV2) is generally considered to be the primary causative agent of postweaning multisystemic wasting syndrome (PMWS), which has become a serious economic problem for the swine industry worldwide. Recently, a nomenclature was proposed for PCV2 which took into account the classification of Grau et al. (2008) and identified two major genotypes of the virus (PCV2a and PCV2b) circulating worldwide and a third genotype (PCV2c) that was reported sporadically in the 1980s. A number of studies have confirmed PCV2a and PCV2b as the predominant genotypes worldwide and PCV2 has been reported to have undergone much genetic variation in recent years. A recent report demonstrated the emergence of a new type of PCV, a recombinant between PCV1 and PCV2 (Gagnon et al., 2010). However, information regarding natural recombination between different genotypes of PCV2 remains limited. Two newly emerging natural recombinant porcine circovirus type 2 (PCV2) mutants, resulting from replacement of a genomic fragment containing open reading frame 2 (ORF2) of genotype PCV2b with that of genotype PCV2a, were first isolated from the co-infection of PCV2a and 2b genotype viruses in vitro. The two mutant viruses contained the ORF1 sequence from genotype PCV2b and the ORF2 sequence from genotype PCV2a. They were designated according to the nomenclature proposed by Grau et al., indicating the origin of the ORF1 sequence first and that of the ORF2 sequence second, i.e., PCV2b(JF11)/2a(CL1) and PCV2b(YJ)/2a(CL1). The replication efficiencies of the two newly emerging natural recombinant PCV2 mutants were significantly enhanced and their antigenicities were significantly altered in vitro compared with their parental strains.

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Discovery and characterization of the involvement of a cellular membrane fusion protein in PRRSV replication

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Elucidating protein-protein interactions between viral and host cellular proteins is critical to understanding viral pathogenesis. A yeast two-hybrid screen revealed that both major PRRSV envelope proteins GP5 and M interact with the cellular membrane fusion protein Snapin. Snapin is an accessory protein of the SNARE complex, the core intracellular membrane fusion mediator in eukaryotic cells. RNA interference (RNAi) technology was utilized to understand the potential involvement of the GP5/M interaction with Snapin in the PRRSV lifecycle. A synthetic short-interfering RNA (siRNA) that efficiently and consistently reduces Snapin expression in MARC-145 cells was developed. All cells were transfected with 50nM of either the Snapin-specific siRNA or a negative control siRNA. The Snapin-specific siRNA was utilized to reduce Snapin prior to or post- PRRSV infection. In the first experiment cells were transfected with either the siRNA or the negative control for 24h then mock-infected or infected with PRRSV isolate VR-2332 (M.O.I. 1) and collected at 12, 24, and 48 hpi. In the second experiment the cells were mock-infected or infected with VR-2332 (M.O.I. 1) for 12h and then transfected with the siRNAs and collected at 12, 24, and 48 hpt. All transfections were performed in triplicate. Snapin expression was reduced at both the mRNA and protein levels, as indicated by qRT-PCR and immunoblotting respectively. In both cases reduced Snapin expression resulted in diminished PRRSV replication as evidenced by reduced N expression levels, both mRNA and protein, and reduced viral titers. These results indicate that the interaction between GP5 and M with the cellular membrane fusion machinery via Snapin is a critical part of the PRRSV infection cycle and that this interaction may occur at more than one stage of infection. In an independent study undertaken to elucidate the involvement of small regulatory RNA termed microRNA (miRNA) in PRRSV pathogenesis and the cellular response to infection, a potential Snapin regulator, cellular *miR-147*, was down-regulated within 24 hour of PRRSV infection in swine alveolar macrophages (SAMs). Two potential *miR-147* binding sites within the 3'UTR of Snapin were validated using a retroviral based in vitro assay, supporting the legitimacy of these regulatory sites. It is possible that the down regulation of *miR-147* in PRRSV infected cells may serve to maintain Snapin levels for sufficient intracellular viral trafficking. In summary, the studies presented here demonstrate the potential involvement of the cellular membrane fusion machinery in PRRSV pathogenesis.

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Amino acid mutations in the capsid protein produce novel porcine circovirus type 2 neutralizing epitopes

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Background: Porcine circovirus type 2 (PCV2) is associated with postweaning multisystemic wasting syndrome in pigs. A monoclonal antibody (mAb) 8E4 against the PCV2 capsid protein has the capacity to neutralize the virus. However, this mAb can only react with some PCV2a strains (LG, CL, and JF2; mAb 8E4-positive strains), but does not cross-react with some PCV2b strains (YJ and JF; mAb 8E4-negative strains). Former experiments demonstrated that one amino acid substitution, alanine (A) for arginine (R) at position 59 (A59R) in the capsid protein of PCV2a (CL, LG, and JF2) strains, completely inhibited the reactivity of three PCV2a strains with mAb 8E4. However, an R→A substitution at position 59 (R59A) in the capsid protein of PCV2b/YJ did not induce recognition or neutralization by mAb 8E4. The objective of this study was to investigate which aa change(s) in the capsid protein of PCV2b (YJ and JF) strains caused the switch from mAb 8E4-negative to mAb 8E4-positive.
Method: In the present study, the capsid proteins of PCV2a strains (LG, CL, and JF2) were compared with PCV2b strains (YJ and JF). On the basis of the alignment of capsid protein sequences from different strains of PCV2 and the key aa at the position 59 of the capsid protein, as well as the crystal structure of PCV2 capsid protein (PDB accession no. 3R0R), 6–7 aa were chosen for construction of YJ and JF mutants. Fusion PCR or site-directed mutagenesis was performed targeting the external amino acids of the capsid proteins. Fourteen YJ mutant strains and seven JF mutant strains were constructed and rescued. Immunoperoxidase monolayer assay were used to test the mutant strains immunoreactivity with mAb 8E4.
Results: A mutation of arginine to alanine at position 59 in the capsid protein of strain JF allowed the mutant to be recognized and neutralized by mAb 8E4. Likewise, mutations of arginine to alanine at position 59 together with alanine to threonine at position 60 in the capsid protein of the YJ strain resulted in a gain of neutralization and recognition by mAb 8E4.
Conclusion: In summary, the present study demonstrated that the amino acids at positions 59 and 60 in the capsid protein of PCV2 participated in the formation of conformational neutralizing epitopes and mutations at positions 59 or 59/60 formed a novel neutralizing epitope for mAb 8E4-negative strains. Moreover, herein, we provided valuable information for further in-depth mapping of the conformational neutralizing epitope, clarified antigenic differences among PCV2 strains, and developed a useful vaccine candidate to control PCV2-associated diseases.

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RNA interference targeting nucleocapsid protein (N) inhibits porcine reproductive and respiratory syndrome virus replication in Marc-145 cells

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Background: Porcine reproductive and respiratory syndrome (PRRS) is an economically important disease, which leads to severe economic losses in swine-producing areas of the world. PRRSV ORF7 encodes the nucleocapsid (N) protein, which constitutes about 20–40% of the total protein content of the virion and is essential during the assembly and disassembly of the virion. Currently, the application of RNA interference (RNAi) to the inhibition of PRRSV replication in cell culture becomes a research hotspot, which would become a promising method for the treatment of PRRSV. Based on previous researches and because of the high conservation of PRRSV N gene, we exploit to apply the RNAi technique by targeting N gene to inhibit the PRRSV replication.
Materials and methods: According to the nucleocapsid protein (N) of porcine reproductive and respiratory syndrome virus, three interference target sites were designed and three siRNA-expressing plasmids were constructed. The recombinant RNAi plasmids were transfected into Marc-145 cells, and the cells were then infected with PRRSV (JL07/SW strain), the antiviral activity of the siRNA-expressing plasmids in Marc-145 cells were assessed by cytopathic effects, TCID50, indirect immunofluorescence and real-time quantitative PCR.
Results: Experimental results indicated that these three siRNA vectors could effectively inhibit the replication of PRRSV by 89.1%, 83.6% and 86.5% in Marc-145 cells. But among these three siRNA-expressing vectors, N71 was found to be most effective, while N144 and N218 displayed relatively weak ability. The results indicated that siRNA-expressing plasmids could efficiently inhibit PRRSV replication in Marc-145 cells by targeting N gene of PRRSV.
Discussion: As previously described in a report, the N gene can affect PRRSV function involved in both recognition of viral RNA, sub-cellular localization and interactions with other viral and cellular proteins, and even affect the virus assembly. Our results indicated that siRNA-expressing vector p2.1-N71 could significantly and most effectively inhibit the assembly and replication of PRRSV than siRNA-expressing vector p2.1-N144 and p2.1-N218, we have addressed a rational explanation for this is that N71 is closer to the N-terminal of N gene while N144 and N218 is relatively far from the N-terminal. The N-terminal of the N protein, rich in basic amino-acid residues (26%), may facilitate interaction of the N protein with the RNA genome and thus N71 may efficiently inhibit the interaction of the N protein with the RNA genome.

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Functional analysis of ORF4 protein encoded by porcine circovirus type 2

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Introduction: To date, three of eleven open reading frames (ORFs) within PCV2 genome have been well studied, including ORF1 encoding replicase Rep and Rep', ORF2 encoding the major immunogenic protein Cap, and ORF3 encoding protein ORF3 which plays a major role in virus-induced apoptosis. Our laboratory has demonstrated the novel viral gene (termed ORF4) at the transcriptional and translational levels. Here, we were dedicated to analysis this ORF4's function in vitro and in vivo.
Materials and methods: Wild-type PCV2 (wPCV2) and ORF4-deficient PCV2 (m4PCV2) were kept in our laboratory. PK-15 cells infected with wPCV2 or mPCV2 were collected for colorimetric caspase activity assay. SPF BALB/c mice were inoculated with wPCV2 or mPCV2. At 14, 21 and 42 days post infection (dpi), spleens were collected for hematoxylin-eosin (H&E) staining to observe microscopic changes, immunohistochemical (IHC) analysis to detect expression of viral proteins, and flow-cytometric analysis to determine T-lymphocyte subsets.
Result: Comparing to normal PK-15 cells, caspase-3, -8 and -9 activities of cells infected with wPCV2 or mPCV2 at 24 and 72 hpi were higher in varying degrees, suggesting caspase-3, -8 and -9 were involved in PCV2-induced apoptosis. At 24 and 72 hpi, the activities of caspase-3 and -8 induced by M4PCV2 were higher than those of wPCV2 ($P < 0.05$), suggesting that the ORF4 protein plays an important role in suppressing apoptosis in PCV2 infection. Mice inoculated with wPCV2 or mPCV2 didn't show any clinical signs. The degree of pathological changes was small. At 14 dpi, the mPCV2-infected mice revealed a higher frequency of lesions in splenic follicles ($P<0.05$) and more severe microscopic lesions than wPCV2-inoculated mice, but at late stage of infection (21 and 42 dpi), the situation was opposite. IHC assay showed that wPCV2 and mPCV2 could replicate in spleen cells, and the latter couldn't encode ORF4 protein. Using flow cytometry assay analyzing T-lymphocyte subsets, the relative proportions of splenic CD8+, CD4+CD8+, and CD4+ cell subsets at 14, 21, and 42 dpi of mPCV2-infected mice were all lower than these of wPCV2-infected mice.
Discussion: In this study, mPCV2 induced higher caspase-3 and -8 activities than wPCV2, indicating ORF4 protein may inhibit apoptosis through inhibiting extrinsic apoptotic pathway. Furthermore, mPCV2 exhibited a greater decrease of CD4 and CD8 T cells in infected mice, implicating a role for the PCV2 ORF4 protein in modulating the host immune system via regulation of T lymphocytes during infection. However, the mechanism of ORF4 protein involved in an antiapoptosis pathway, and, as a suspected antiapoptotic factor, whether ORF4 truly decreases lymphocyte depletion through antiapoptosis pathways still requires further investigation.

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ORF4 protein: a newly discovered virus protein in PCV2 replication

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Introduction: Porcine circovirus type 2 (PCV2) is the primary causative agent of porcine circovirus associated diseases (PCVAD). To date, three proteins encoded by ORF1, ORF2 and ORF3 have been identified. ORF1 directs the synthesis Rep and Rep', which are essential for replication, ORF2 encodes viral capsid, and ORF3 encodes an apoptosis-associated protein ORF3 protein. In present study, we used ORF4 specific DNA or RNA probes and antibody to identify a newly viral protein encoded by ORF4 gene and initially explored the role of ORF4 protein play in PCV2 replication.
Material and Methods: To detected ORF4 transcript, digoxigenin (DIG)-labeled ORF4 DNA and RNA probes were generated to hybridized with total RNAs from PK-15 cells infected with the wild-type PCV2 (wPCV2). To identify the expression of ORF4, prokaryotic expressed protein pGEX-4T-1-ORF4 and eukaryotic expressed plasmid PCI-neo-ORF4 were used as immunogen to generate specific mAbs and pAb to ORF4. Using these antibodies, we detected the expression of ORF4 gene in PCV2 infected PK15 cells, pEGFP-ORF4 transfected PK15 cells and splenocytes of PCV2 inoculated mice by dot-ELISA, IFA ,IPMA and IHC, respectively. To further identify the role of ORF4 in PCV2 replication, ORF4 deficient PCV2 (mPCV2) was rescued through change the initiation codon of ORF4 from ATG to GTG. PK-15 cells were inoculated with wPCV2 or mPCV2 and titers were determined at 18, 24, 36, 48, 72, and 96 h post-inoculation.
Result: Northern blot revealed an approximately 180bp bands corresponding to putative ORF4 transcript transcribe in the same direction of ORF3. IFA and IHC assay using anti-ORF4 mAbs and pAb revealed that ORF4 protein indeed expressed in PCV2 infected PK15 cells and PCV2 infected mouse splenocytes. Cross reaction with IPMA show that ORF4 mAb and pAb can specially react with PK15 cells transfected with pEGFP-ORF4 but not pEGFP-ORF1, 2 and 3. Finally, titer of wPCV2 and mPCV2 is similar when PCV2 replicated in PK15 cellls.
Conclusion and discussion: Northern blot revealed a newly discovered transcript which overlaps ORF3 in the same direction using different initiation codon. This transcript length was shorter than those of all confirmed viral RNAs but similar to that of the putative ORF4. The results demonstrated that ORF4 was transcribed during wPCV2 replication. IFA employed with ORF4 specific antibody reveals that ORF4 protein was expressed in PCV2 infected PK15 cells and mouse splenocytes but not in mPCV2 or PCV1 infected PK-15 cells, demonstrate that ORF4 indeed expressed in PCV2 infected cell and anti-PCV2 ORF4 mAbs generated in this study were type specific. Finally, the similar titers of wPCV2 and mPCV2 indicated that ORF4 is not essential to PCV2 replicated in PK15 cells.

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Co-infection study between porcine reproductive and respiratory syndrome virus and a bacteria led to the discovery of a possible new bacteria antiviral

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Main objective: Study the effect of a pre-viral infection with porcine reproductive and respiratory syndrome virus (PRRSV) on a bacterial infection by *Actinobacillus pleuropneumoniae* (App) *in vitro*.

Specific objectives:

- Compare adherence between mutant strain of *App ΔapxICΔapxIIC* (*AppΔ*), producing non-active ApxI and ApxII toxins, and *wild type App* (*AppWT*) strain in PRRSV co-infected SJPL cells.
- Compare toxicity of *AppΔ* and *AppWT* strain in PRRSV co-infected SJPL cells.
- Study the impact of *AppΔ* and PRRSV co-infection in different cell types, SJPL, MARC-145 and PAMs.

Brief methods: SJPL cells were infected with PRRSV and co-infected with *AppΔ* or *AppWT*, then adherence was measured (CFU/well) and cell cytotoxicity was measured using lactate dehydrogenase (LDH) assay. For PRRSV and *AppΔ* co-infections; SJPL, MARC-145 and PAM cells were infected with PRRSV and co-infected with *AppΔ*, UV inactivated *AppΔ* or LPS, then immunofluorescence assay (IFA) detection of PRRSV N viral protein and PRRSV titration on MARC-145 cells using the Kärber method was performed.

Summary of the results: Bacterial adherence is not modulated by PRRSV. *AppΔ* is not toxic to SJPL cells up to 6 hours post infection (PI). PRRSV/*AppΔ* co-infection significantly exacerbates cell cytotoxicity compare to PRRSV or *AppΔ* infected cells alone. Those results suggest that PRRSV/*App* co-infection does act in synergy to intensify cell mortality. On the other hand, in SJPL cells, PRRSV/*AppΔ* co-infection showed absence of PRRSV detection by IFA and replication compare to PRRSV infected cells and compare to MARC-145 cells, where both co-infected cells and PRRSV infected cells alone showed no difference in PRRSV. Those results suggest that *AppΔ* seems to interfere with PRRSV replication in SJPL and PAM cells but to a lesser extend in MARC-145 cells. Moreover, treatment of SJPL with UV inactivated *AppΔ* and LPS did not reduce PRRSV. Furthermore, treatment with *AppΔ* cell culture supernatant (*AppΔ* sup) also showed inhibition of PRRSV, demonstrating that it is a metabolite secreted from the bacteria that is mediating this effect. Moreover, to evaluate *AppΔ* antiviral effect specificity, other viruses have been tested. Results show that *AppΔ* antiviral effect is also observed against other viruses such as porcine influenza virus (H1N1 and H3N2) and equine herpes virus type 1 (EHV-1).

Conclusion: *AppΔ* antiviral effect can be observed in SJPL and PAM cells. It can inhibit PRRSV and other viruses such as influenza virus and EHV-1.

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Impact of co-infection by porcine circovirus (PCV) and porcine reproductive and respiratory syndrome virus (PRRSV) in porcine pulmonary epithelial cell line

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Main objective: The main goal of this research is to study the impact of PRRSV/PCV co-infections in a new cellular model, the NPTr-CD163 cells.
Specific objectives:
- Test NPTr-CD163 cells for permissivity to PCV (PCV2a, PCV2b, PCV1 and PCV1/2a) and PRRSV (IAF-Klop).
- Measure kinetic of replication of each viruses in co-infected cells.
- Test if the modulation in kinetic of replication observed in PRRSV and PCV is induce by cell mortality or apoptosis.
- Test if the modulation in kinetic of replication observed in each virus is dependent on cytokines production.

Brief methods: Porcine newborn pulmonary tracheal cells (NPTr cells) were known to be only permissive to PCV2 virus, and not to PRRSV. Thus, NPTr cells were genetically modified with CD163 gene, a major PRRSV's cell receptor, to obtain PRRSV permissivity. Thus, those cells were tested for permissivity with PRRSV and other PCV genotypes (PCV2a, PCV2b, PCV1 and PCV1/2a). Then, kinetic replication of each virus in co-infected cells was performed during a 72 hour time course and viral production was measure using the Kärber method. Cell mortality of co-infected cells was measured using lactate dehydrogenase (LDH) release and apoptosis was quantified using caspase 3-enzyme activity. Cytokines mRNA was quantified with qRT-PCR.

Summary of the results: NPTr-CD163 cells were found to allow complete viral replication of PRRSV and all tested PCV genotypes. Kinetic replication of each virus in co-infected cells show modulation of both virus replications depending on PCV genotype, as PCV1 replication is down regulated and PCV2b replication is up regulated in co-infection with PRRSV compared to PCV infection alone. Inversely, PCV1 increases PRRSV replication, whereas PCV1/2a, PCV2a and PCV2b significantly lower PRRSV replication. Also, cell mortality is increase in co-infected cells compared to both single infected cells. Moreover, caspase 3-enzyme activity is increase in PCV2a/PRRSV co-infected cells compared to cells infected with each single infection. RT-qPCR cytokines mRNA expression analyses showed that TNF-α and IFNβ were significantly increased in the PCV1-PRRSV co-infection model compared to single infections, whereas they remained low for the other PCV genotypes.

Conclusion: NPTr-CD163 cells are a good model to study PRRSV/PCV co-infections *in vitro*. Virus replication and cell metabolism are affected by PRRSV/PCV co-infections, and are PCV genotype dependent.

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The correlation of cyclin A expression level with porcine circovirus type 2 propagation efficiency

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Background: Porcine circovirus type 2 (PCV2) is a non-enveloped virus and has a circular, single-stranded DNA genome of ~1.7 kb that contains two major open reading frames (ORFs). The ORF1 encodes two replication proteins (Rep and Rep′) which are both essential for viral DNA replication, and ORF2 encodes the capsid protein (Cap), which is the only structural component of the virion. PCV2 genome is small and its coding capacity is limited; therefore, the viral life cycle depends on host enzymes. It is assumed that PCV2 replication is cell cycle-dependent (S-phase). However, the cellular molecules that regulate PCV2 replication have not been fully identified.
Method: Here, we cloned porcine cyclin A (CycA) and porcine cyclin-dependent kinase 2 (CDK2) gene, the major regulators of S-phase, and established CycA or CDK2 overexpression or lower-expression cell line. The propagation efficiency of PCV2a/CL or PCV2b/YJ in these cell lines were investigated by capture enzyme-linked immunosorbent assay (ELISA) or an immunoperoxidase monolayer assay (IPMA), and cell cycle was analyzed by flow cytometry. Subcellular localization of porcine CycA, CDK2, PCV2-Cap, and PCV2-Rep proteins were observed by confocal microscopy.
Results: The results showed that CycA overexpression suppressed PCV2 replication. In contrast, CycA down-regulation by shRNA induced increases during the S and G2/M phases and resulted in increased PCV2 propagation. Overexpression or lower expression of CDK2 exhibited no significant influence on PCV2 replication. Furthermore, CycA, rather than CDK2, overexpression altered normal nucleus localization of PCV2-Rep.
Discussion and conclusion: Overexpression of CycA, but not CDK2, suppressed PCV2 replication, in part because CycA overexpression altered the normal subcellular localization of PCV2-Rep. On the other hand, DNA replication-associated molecules were recruited by overexpression of CycA for CDK2 for DNA replication of the host cell and resulted in a reduction in cellular resources for PCV2 replication. Down-regulation of CycA by shRNA induced an increase in both the S and G2/M phases, and contributed to increased PCV2 propagation. In conlusion, PCV2 replication is both S and G2/M phase-dependent, and CycA expression level plays an important role in the PCV2 life cycle.

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Dissection for the structure and function of a 5'-proximal prominent stem-loop in Porcine Reproductive and Respiratory Syndrome virus

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Introduction

Like other arteriviruses, PRRSV 5' UTR is believed to carry a variety of cis-acting elements that regulate the discrete steps of viral life cycle. But the regulatory function and mechanism need to be addressed. PRRSV has been characterized into two distinct genotypes, type 1 and type 2. After alignment, the 5' UTRs of two genotypes are different in length and just share about 60% nucleotide identity. Nevertheless, the secondary structures of 5' UTR in two genotypes are quite similar and characterized by six major putative stem-loops, especially the prominent one which transcription-regulatory sequence (TRS) located in. Our previous research showed that the prominent stem-loop structure in SL5 is very important for viral infectivity and RNA synthesis, but the detailed regulatory function of each part of the structure is lack of experimental research. According to RNA structure model, we arbitrarily divide the prominent stem-loop structure in SL5 into three portions A/A' loop part, B/B' stem part, C (common) top stem-loop. In this study, we focused on the stem part B/B' in order to dissect its regulatory function in viral life cycle.

Materials and Methods

Based on type 2 full-length cDNA clone pAPRRS, a variety of B/B' stem part mutant clones were constructed. Then transfection was performed to detect the effect of the mutants on viral infectivity. IFA was used for evaluation of N protein translation level. The negative strand gRNA RT-PCR and Northern Blot were performed to analyze the viral genomic replication and sg mRNA transcription, respectively. Finally, plaque morphology and first cycle viral titration were conducted for mutant virologic characteristics.

Results

pCB209silt, pCB170U which destroyed the stem structure could be rescued and pCBCM which restored the B stem structure was still infectious. Otherwise, if primary sequences in left stem (169-173: CGGUC) were mutated in pCBL169, the viral infectivity could be totally disrupted. It was indicated that the B/B' stem part structure was not essential, but the primary sequences may be very important for viral infectivity. The right stem B' could be just silently mutated, which indicated that the two amino acids were crucial to the virus.

Among left stem (169-173: CGGUC) mutants, the infection of pCB169G, 172GA, 171CGA, 169GCGA, 170C, 170U were determined. Intriguingly, the single site mutants in cG^{170}guc were infectious, while accumulated mutations in this site would cause mutant lethal. This indicated that this site G^{170} was crucial for viral infectivity in the 5nt primary sequence.

Northern Blot showed that B/B' stem part structure had effect on sg mRNA transcription, although it is not essential for viral infectivity. The plaque phenotypes of WT and mutant viruses were indistinguishable but the plaque numbers were distinct when using the same MOI infection. The mutant which related to G^{170} developed decrease plaque numbers and low N protein translation level. The result was also consistent with that of viral titration. This indicated that G^{170} was crucial for viral replication.

Conclusions

The 5'-proximal prominent structure plays a key role in viral infectivity. The primary sequence of B/B' stem part, rather than its secondary structure is more important. The G^{170} is the crucial site for viral replication in this region. The key element is the top loop in which TRS located.

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Signal peptide cleavage from GP5 of PRRSV: Only a minor subfraction of molecules retains the decoy epitope, a presumed molecular cause for viral persistence

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GP5, the major glycoprotein of PRRSV, is considered as one possible target of neutralizing antibodies, which however appear only late in infection. This was attributed to the presence of a "decoy epitope" located near a hypervariable region in the N-terminal domain of GP5. This region also harbors the predicted signal peptide cleavage sites and (dependent on the virus strain) a variable number of potential N-glycosylation sites. However, molecular processing of GP5 has not been addressed experimentally so far. It was not known whether and where the signal peptide is cleaved and thus whether the "decoy epitope" is present in virus particles. In addition, since signal peptide cleavage and N-glycosylation happen co-translationally they could influence each other, i. e. insertion and deletion of glycosylation sites in the hypervariable region might affect signal peptide cleavage and hence the presence of the "decoy-epitope".

Here we show that the signal peptide of GP5 from the American type 2 reference strain VR-2332 is cleaved, both during in vitro translation in the presence of microsomes and in transfected cells. This was found to be independent of neighboring glycosylation sites and occurred in a variety of porcine cell types and for GP5 sequences derived from various type 2 PRRSV strains.

Next, the exact signal peptide cleavage site was elucidated by mass spectrometry of virus-derived as well as recombinant GP5 expressed in insect cells by the baculovirus expression system. The data revealed that the signal peptide of GP5 is cleaved at two sites, either between A26 and V27 (site 1) or between A31 and S32 (site 2). As a result, a mixture of GP5 proteins exists in virus particles, some of which still contain the "decoy epitope" that is located between both sites. Heterogeneity was also observed for the usage of glycosylation sites in the hypervariable region.

Since mass spectrometry is not quantitative, the results unfortunately do not tell whether the majority or only a small fraction of GP5 molecules present in virus particles contain the "decoy-epitope". To address this issue biochemically, we engineered GP5 mutants that contain bulky amino acids around either site 1 and site 2, respectively, to prevent signal peptide cleavage at that site. SDS-PAGE after deglycosylation of the expressed proteins showed a significant difference in mobility between both mutants. However, wild-type GP5 exhibited exactly the same SDS-PAGE mobility as the mutant that is presumed to be cleaved at site 2 only. This indicates that the overwhelming majority of all GP5 molecules do not contain the "decoy epitope".

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Non-structural protein 2 of the porcine reproductive and respiratory syndrome (PRRS) virus: a crucial protein in viral pathogenesis, immunity and diagnosis

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Porcine reproductive and respiratory syndrome (PRRS) is a swine disease of significant economic importance that causes reproductive and respiratory problems in pigs. The replicase non-structural protein 2 (Nsp2) of the porcine reproductive and respiratory syndrome virus (PRRSV) is recognized as the most variable region within the PRRSV genome. This review discusses the molecular characteristics and biological and immunological functions of the PRRSV Nsp2 and its involvement in the virus's pathogenesis. The role of Nsp2 in cell and tissue tropism, replication and growth, and variation and pathogenicity of PRRSV and the differences in virulence among different strains are described in the present review. Varied genetic alterations in the PRRSV Nsp2 gene are evident, such as point mutations, and nucleotide insertions or deletions. The variation of a specific section of Nsp2 could alter the cell and/or tissue tropism of PRRSV. Based on the discriminative virus titers of TJ F19 and attenuated TJM *in vivo* hypothesized that the cell tropism of F19 and TJM *in vivo* was altered, which further caused the decreased virulence. The Nsp2 protein has been thought to participate in multiprotein assembly during the formation of viral replication complexes. The Nsp2 gene interference test results demonstrated that the expression inhibition of Nsp2 significantly reduced the replication titer of HP-PRRSV TJ strain in Marc-145 cells and that the time of CPE was delayed. The relationship between Nsp2 gene deletion and virus virulence or pathogenicity is difficult to determine. A remarkable 120-aa deletion of TJM may play a role in the abated virulence. In addition, Nsp2 is multifunctional not only as a viral protease but also as a viral antagonist of host defenses and appears to be a highly immunogenic protein. Nsp2 is an ideal marker for monitoring genetic variation and for developing differential diagnostic tests. The continuous 120-amino acid deletion in the Nsp2 region of PRRSV TJM has been developed for differentiating the vaccine strain TJM from the wild-type strain.

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Role of non-structural protein 2 in the regulation of the replication of the porcine reproductive and respiratory syndrome virus in MARC-145 cells: effect of gene silencing and over expression

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Porcine reproductive and respiratory syndrome (PRRS) is an economically important disease in swine-producing areas. Many vaccine strategies have been developed to control the disease, but none have yet been completely successful. The development of a cell line that can produce large yields of PRRSV vaccine is very necessary. In order to determine the role of Nsp2 in the replication of the highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) in MARC-145 cells, we used an RNA interference-based short hairpin RNA of Nsp2 and constructed cell lines expressing the HP-PRRSV Nsp2 gene. Conserved HP-PRRSV Nsp2 sequences were used to design short interfering RNAs and test their ability to silence PRRSV transcript expression and replication in cells in vitro transfection. Nsp2, ORF7, and beta-actin mRNA expression were determined using semi-quantitative real-time PCR. Infection with siRNA targeting Nsp2 was found to reduce the Nsp2 expression in MARC-145 cells infected with PRRSV. Both MARC-145-TJ Nsp2 and MARC-145-TJM Nsp2 cell lines were screened by G418, which were infected with HP-PRRSV, normal MARC-145 cells for mock, and then virus titers were calculated by TCID50 after the CPE showing up. The downregulation of Nsp2 induced a remarkable decrease in PRRSV replication, causing the reduction of structural protein. The Nsp2-targeted siRNA was found to downregulate the expression of Nsp2 in MARC-145 cells and inducing replication reduce of PRRSV in MARC-145 cells. The shRNA vectors S-1 and S-2 could effectively induce the inhibition of viral replication in MARC-145. Results showed that cells expressing the Nsp2 gene of the highly pathogenic PRRSV TJ and attenuated TJM remained stable. PRRSV replication was faster in these cells than in MARC-145 cells, especially during the early stage. This shows that Nsp2 plays a positive role in PRRSV proliferation.

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The effect of the position of N-linked glycosylation sites within hypervariable region of GP5 on virus neutralization

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Introduction

N-linked glycosylation of the GP5 protein is involved in diverse functions such as virus infectivity, receptor binding and immune response. Potential N-glycosylation sites were observed at different positions: N30, N32, N33, N34, N35, N44, N51. N-glycosylation sites variations were located mainly within the hypervariable region (HVR) (aa 30–35) upstream of principal neutralizing epitope (PNE). The N44 and N51 N-glycosylation sites were conserved in the PNE region except for a few sequence. It has been shown that engineered and natural field viruses that have fewer glycans in the GP5 ectodomain have an increased neutralizing antibody response compared to highly glycosylated viruses. The effect of variation in N-glycosylation site within HV on antibody neutralization is still unknown. The aim of this study was to analyze the association between the variation in glycosylation site (N32, N33, N34, N35) in HVR of GP5 and its effects on virus neutralization.

Materials and Methods

The infectious PRRSV cDNA clone pAJXM was the backbone for mutagenesis to introduce mutations at the potential N-linked glycosylation sites (N32, N33, N34, N35) using overlap extension PCR with synthetic primers. The amount of infectious particles was determined by calculation of the $TCID_{50}$. Neutralization assays were performed using the fluorescent focus neutralization assay to detect the presence of anti-PRRSV neutralizing antibodies.

Results

1. Various glycosylation GP5 mutant clones were constructed based on an infectious PRRSV cDNA clone pAJXM backbone. Mutants pJGP5N32, pJGP5N33, pJGP5N34, pJGP5N35 in which GP5 have glycosylation sites pattern N32/44/51, N33/44/51, N34/44/51, N35/44/51, respectively. Mutant pJGP5G32, pJGP5G33, pJGP5G34, pJGP5G35 with GP5 only have a glycosylation site at N32, N33, N34, N35, respectively.
2. The growth kinetics of the mutants vJGP5N32, vJGP5N33, vJGP5N34, vJGP5N35 were similar to that of wt virus. The overall yields were not significantly different between wt and mutant viruses. However, the overall yields of the mutants vJGP5G32, vJGP5G33, vJGP5G34, vJGP5G35 were nearly 1 log less than that of wt virus.
3. Western-blot analysis showed that introduced glycosylation sites in GP5 were indeed glycosylated.
4. The change in glycosylation site from N32 to N35 in HV of GP5 did not render the viruses (vJGP5N32, vJGP5N33, vJGP5N34, vJGP5N35) more or less susceptible to antibody neutralization. On the other hand, vJGP5G35 was less sensitive to the sera neutralization than vJGP5G32, vJGP5G33 or vJGP5G34. But the geometric mean titers of neutralization antibodies were not significantly different between vJGP5G35 and vJGP5G32, vJGP5G33 or vJGP5G34.

Conclusions

The variation (N32, N33, N34, N35) in N-linked glycosylation sites within HV region of GP5 did not affect the sensitivity of PRRSV to antibody neutralization.

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The role of antibody in PRRSV infection PAM

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Introduction: The virus into the target cell by Fc receptor-mediated with specific antibody, the specific antibody enhanced virus infection, this phenomenon is known as the antibody dependent enhancement of virus infection. A lot of research data shows that the PRRSV entry PAM cells is through specific receptors or receptor complex, the study also found that certain doses antibodies specific can enhance PRRSV infection in the process of PRRSV infection of host cells. But in pigs also exist some PRRS non-specific antibodies and immune complexes, and the few studies about these nonspecific antibodies and immune complexes can enhance the infectivity of PRRSV. This experiment mainly through the effect of antibodies of different characteristics on the proliferation of PRRSV, to further explore the antibody dependent enhancement mechanism of PRRSV infection.

Abstract: (Objective)This study aimed to discuss the role of specific and non-specific antibody in antibody-dependent enhancement of PRRSV infection by studying the effect of IgG on the proliferation of PRRSV.(Methods and Material)PRRS specific IgG (2.64 mg/ml) and non-specific IgG (2.63 mg/ml) were diluted by a 1⁄2 dilution series method , and then an equal volume of PRRSV Hn-1/06 virus with a titer of 200 TCID50/100 μ L was added to each dilution ,and a series of infectious immune complex (PRRSV - IgG) and negative antibody - virus mixture (PRRSV + IgG) were prepared for inoculating PAM cells; At the same time ,we mixed purified swine negative IgG (2.63 mg/ml) with rabbit anti -swine IgG (2.64 mg/ml)by equal volume to make immune complex(IC),and an equal volume of PRRSV Hn-1/06 virus with a titer of 200 TCID50/100 μ L prepared immune complex- the virus mixture (PRRSV + IC) for inoculating PAM cells. after cultured for 24h, we detected the level of mRNA by real-time fluorescent quantitative PCR .In the same method, we inoculated PAM cells with 2 times diluted PRRSV-IgG, PRRSV+IgG, and 4 times diluted PRRSV + IC,then detected different periods of the level of mRNA of PRRSV after infecting12, 24, 36 and 48 h with the real-time fluorescent quantitative PCR. (Results)The results indicated that a certain concentration of PRRS specifically sub-neutralizing level antibody and nonspecific antibody all could significantly promote the proliferation of PRRSV in PAM cells during the period of infecting 48 h ;While the right dose of immune complex also can promote the proliferation of PRRSV in PAM cells during the period of infecting 48 h. (Conclusion)The results indicated that a certain concentration of PRRS specifically sub -neutralizing level antibody and nonspecific antibody all could promote the proliferation of PRRSV in PAM cells, but different characteristic of antibody had some differences in the ability of proliferation of PRRSV.

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Regulation of host cell gene expression and cell cycle progression by nsp11 of PRRS virus

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Non-structural protein 11 (nsp11) of porcine reproductive and respiratory syndrome virus (PRRSV) is a vial endoribonuclease that is essential for infectivity with an unknown mechanism. To examine the cellular gene expression profiles regulated by nsp11, MARC-nsp11 cells were established by retrovirus-mediated gene transfer to constitutively express the nsp11 protein. Gene integration and protein expression were confirmed by PCR, RT-PCR, and western blot analysis using anti-nsp11 antiserum raised in a rabbit. Subsequently, RNA microarrays were conducted using these cells. The luciferase reporter activities for interferon (IFN)-β, interferon regulatory factor 3, and nuclear factor-kB promoters were suppressed compared to those of parental MARC-145 cells, suggesting that nsp11 may be a viral antagonist for IFN production. Differential cellular transcription profiles regulated by nsp11 were then examined using Affymatrix exon chips representing 28,536 human gene transcripts. After statistical analyses, 66 cellular genes were shown to be up-regulated and 104 genes were down-regulated. These genes were grouped into 5 major cellular pathways according to their functional relations; histone-related, cell cycle and DNA replication, mitogen activated protein kinase signaling, complement, and ubiquitin-proteasome pathways. Of these 5 pathways, the modulation of cell cycle by nsp11 was examined in particular. Flow cytometry showed that nsp11 caused the delay of cell cycle progression at the S phase and the BrdU staining confirmed the cell cycle arrest in nsp11-expressing cells. This study provides insights into specific cellular responses to nsp11 during PRRSV infection.

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Concurrent highly pathogenic porcine reproductive and respiratory syndrome virus infection accelerates Haemophilus parasuis infection in conventional pigs

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The study was aimed at determining the effect of HP-PRRSV on HPS in co-infection. The infB gene, which is conserved among different HPS serotypes and different species, was amplified using PCR from extracted DNA of HPS culture by boiling. The PCR product of target gene was cloned into pMD18-T vector and then transferred into DH5α. Positive recombinant plasmid was selected and used as a positive quantitative template to establish a standard curve. The method of quantitative real-time PCR targeting infB gene improved the accuracy and speed of the detection of HPS. A total of 32 four-week-old conventional pigs were distributed randomly into four groups: pigs in group I were intranasally infected with HP-PRRSV first, and were then intraperitoneally inoculated with HPS on 5 days after HP-PRRSV infection; pigs in group II were intranasally inoculated with HP-PRRSV alone; pigs in group III were intraperitoneally inoculated with HPS alone; pigs in group IV were intraperitoneally inoculated with physiological saline. Higher mean rectal temperatures ($p<0.05$) were observed in pigs of group I and group III than group II, and group IV after HPS infection. However, group I showed higher mean temperatures than group III during 1-3 dpi with HPS ($p<0.05$). The amount of HPS in serum on 0, 3, 6, 9 and 12 days post-inoculation (dpi) with HPS were detected using the established quantitative real-time PCR. Clinical signs, pathological changes and histopathological lesions were observed. The amount of HPS in serum reached 106 copies/ml at 3 dpi with HPS in pigs of group I, while it arrived 105.7 copies/ml at 9 dpi with HPS in pigs of group III. The HPS loads in hearts and lungs were much higher than in other tissues. Samples from the hearts, lungs and lymph nodes were proceeded pathological observation. The results showed the issues had different degree damages among group I-III, while there were no changes in group IV. In conclusion, HP-PRRSV could increase the proliferation rate of HPS in blood and tissues confirmed by the established quantitative real-time PCR in the study. Further studies should be focused on how to control the co-infection of PRRSV and HPS in the process of pig production.

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Mutagenesis analysis of porcine reproductive and respiratory syndrome virus nonstructural protein 7

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Nonstructural protein7 (nsp7), which is flanked by nsp6 and nsp8, is one of the most conserved nonstructural proteins of porcine reproductive and respiratory syndrome virus (PRRSV). Nonstructural protein- (nsp) specific antibodies are produced in high titers in response to virus replication, especially against nsp1a, nsp1b, nsp2, and nsp7. However, many regional aspects of nsp7 are still veiled, such as its impact on viral replication and virulence or the immunological mechanism between virus and host. Based on the structure of the predicted nsp7 domain, we have constructed a series of large mutations and deletions. We ultimately demonstrated all mutations (nsp7, nsp7α/nspβ) and the majority of substitutions of nsp7 affected the PRRSV replicative cycle in some ways and were fatal for viral recovery, which indicates that these are significant to structure or function of the nsp7. What's more, the mutant vOKXH-nsp7 (F40A) indeed caused some of the variation compared with the parental virus vOKXH-GD, which shortens the amount of time needed to reach its highest viral titer, and decreases the concentration of the highest viral titer, obstructing viral mRNA and protein synthesis. Consequently, these valuable results possibly provide the first direct evidence the nsp7 is really a critical protein domain for the RNA synthesis and the translation of viral protein of PRRSV.

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The host miR-26a inhibits porcine reproductive and respiratory syndrome virus replication by activating innate antiviral immunity

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Porcine reproductive and respiratory syndrome (PRRS) has caused large economic losses in the swine industry in recent years. Current PRRS vaccines not effectively prevent and control this disease. Consequently, there is a need to develop a new antiviral strategy. MicroRNAs play critical roles in intricate host-pathogen interaction networks, but the involvement of miRNAs during PRRS virus (PRRSV) infection is not well understood. In this study, pretreatment with miR-26a induced a significant inhibition of PRRSV replication and remission of the cytopathic effect in Marc-145 cells infected with either the PRRSV VR2332 strain or with the highly pathogenic PRRSV JXwn06 strain. The antiviral effect was dose-dependent and sustained for at least 120 h. In addition, at 8 h post-PRRSV infection, the over-expression of miR-26a could still efficaciously inhibit PRRSV replication. The 3' untranslated region luciferase reporter analysis showed that the PRRSV genome was not the target of miRNA-26a. Instead, RNA-seq analysis demonstrated that miR-26a significantly up-regulated the innate anti-viral responses, including activating the type I interferon (IFN) signaling pathway and promoting the production of IFN-induced and antigen presenting proteins. These findings suggest that delivery of miR-26a may provide a general strategy for anti-PRRSV therapies.

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The application of deep sequencing for the analysis of mutations within hypervariable regions of PRRSV

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Genetic variation in both structural and non-structural genes is a key factor in the capacity of PRRSV to maintain persistence within an animal, and within a production system. However, the exact mechanisms of how genetic variation contributes to persistence remain unclear. As part of a study to understand the role of host genetics in disease resistance, we identified pigs that were still viremic at 42 days after PRRSV infection. We took advantage of this subpopulation to identify changes in the genomes of viruses circulating in pigs. The study focused on sequencing nine regions of the genome that show sequence hypervariability. Specific and universal primers were designed for the hypervariable regions within nsp1 (297-1366), nsp2 (1311-2101, 2701-3040, 3535-3921), ORF3 (12761-13154) and ORF5 (13718-14091). Total RNA were extracted from 100μL of virus suspension of the parental virus NVSL 97-7895, as well as sera collected from four infected pigs at 4dpi, 28dpi and 42dpi and tonsils (42dpi) using TRIzol reagent. cDNA was generated by reverse transcription using random hexamer primers. The first round of PCR was performed using sequence-specific primers. A second amplification was performed using 454 adaptor multiple identifier (MID) primers. For unidirectional sequencing, MID were included only on the forward primers. Amplicons were normalized and pooled together then subjected to emPCR before 454 pyrosequencing. The range in depth of coverage was from 205 to 2180. Mutations were identified in all genomic regions, including differences between pigs, between different samples from the same pig, and at different times. When compared to the inoculum, sera collected at 28dpi had the greatest number of nucleotide changes ($1.08\text{-}10.26\times10^{-5}$ change/nucleotide). Evidence for positive selection of amino acids was the appearance of $L_{852}S$ in nsp2, $D_{85}E$ in GP3, $A_{27}V$ and $N_{32}S$ in GP5. The disappearance of $P_{96}L$ in GP3 was evidence for negative selection. Remarkably, the $N_{32}S$ substitution in GP5 created a new N-glycosylation site.

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The effect of knockdown of non-muscle myosin II-A on Porcine Reproductive and Respiratory Syndrome virus infection

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Objective: Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, positive-sense single-stranded RNA virus belonging to the Arteriviridae family. PRRSV requires cellular mediators to infect host cells. At present, several cellular proteins have been identified to interact with PRRSV proteins or RNA. Heparin sulfate proteoglycans, heparin-like molecules on PAM cells, bind to the PRRSV M protein, sialoadhesin binds to M/GP5 dimmer through the mediation of sialic acids, CD163 interacts with both GP2a and GP4, vimentin binds to N protein, and CD151 interacts with the 3' untranslated region (UTR) of PRRSV RNA. Previously, we generated a monoclonal anti-idiotypic antibody (designated Mab2-5G2) that is specific for the syngenic mouse antibodies against PRRSV GP5, binds Marc-145 cells and porcine alveolar macrophages (PAM) and immunoprecipitated a 230kDa soluble protein from these cells. Based on the amino acid sequence by MS, the 230kDa protein was identified as non-muscle myosin heavy chain II-A (NMHC II-A). The objective of this study was to examine the effect of knockdown of NMHC II-A gene expression by RNAi on PRRSV infection of Marc-145 cells.
Method: To knockdown NMHC II-A gene expression, five artificial microRNAs (amiRNAs) oligonucleotides were designed including four specific amiRNAs targeting NMHC II-A gene and one control amiRNA. The efficient amiRNA was selected by real time RT-PCR and Western blot. Marc-145 cell line stable expressing efficient amiRNA was generated by Blasticidin screening and PRRSV infection was determined on this cells. To investigate whether down regulated NMHC II-A expression would interfere Marc-145 cell viability, cell cycle and cell division, the effect was tested by using WST-8, FACS and confocal micro- scopy.
Results and Discussion: One of four amiRNA targeting NMHC II-A was selected and approximately 47% of NMHC II-A expression was down reduced. Marc-145 cell line stably expressing amiRNA against NMHC II-A gene was established. PRRSV binding to these cells was not affected, but the amount of virus propagation was decreased to 42.1%, 22.3%, 13.2% and 12.6% at 24, 48, 72 and 96hpi compared with the cells expressing the control RNAi. Cells proliferation, cell cycle progression and cell mitosis assay revealed that Marc-145 cell line with the down regulated NMHC II-A expression did not affect cells proliferation and division.
Conclusion: In summary, the results demonstrated that down regulation of NMHC II-A expression decreased the PRRSV internalization and replication but not the binding of PRRSV to the cells without interfere proliferation and division of the cells.
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MicroRNA-181 suppresses PRRSV infection by targeting its receptor CD163

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Emerging evidence indicates that the host microRNAs (miRNAs) are involved in host-pathogen interactions. We previously showed that microRNA-181 (miR-181) could inhibit PRRSV replication by directly targeting its genomic RNA. Here, we investigated whether miR-181 could down-regulate PRRSV receptor CD163 in porcine alveolar macrophages (PAMs), and subsequently suppresses PRRSV infection. We first examined the relationship of CD163 and miR-181c expression levels in cultured blood monocytes (BMos) in vitro. Our results showed that there was an inverse correlation between the expressions of miR-181c and CD163 during monocyte-macrophage differentiation. We then confirmed that aberrant higher expression of miR-181c mimics impaired the up-regulation of CD163. These results suggested that miR-181c down-regulated CD163 expression in differentiated BMos. Next, we investigated whether over-expression of miR-181c reduced CD163 level in PAMs. As expected, additional expression of miR-181c resulted in less CD163 mRNA dose-dependently in PAMs. Immunoblot analysis showed that CD163 level was lower in a dose-dependent manner at 72 hours after miR-181c mimics transfection. The reduction of CD163 protein was also confirmed by flow cytometry. The mean fluorescence intensity of stained CD163 in miR-181c transfected PAMs was reduced to about 57% of that in control cells, and the percentage of CD163-positive cells in miR-181c transfected group was down to ~43% from 68% in mock or NC control group. These results demonstrated that increasing expression of miR-181c in PAMs reduced CD163 expression through both mRNA degradation and translation inhibition. To investigate whether the diminished CD163 is due to direct targeting by miR-181c, we first did bioinformatic analysis of the 3' UTR of CD163 with the RegRNA to identify the potential target sites in the 3' UTR of CD163 mRNA, and found 1 seed binding site. We constructed firefly luciferase reporter pGL3-CD163 containing the wild-type sequence of CD163 3' UTR, and also mutated four nucleotides in the predicted seed binding site and yielded pGL3-CD163 (mut). In addition, we synthesized miR-181c (mut) with mutations in the seed sequence complementary to the mutation in CD163 3'UTR. We observed that miR-181c robustly down-regulated the activity of firefly luciferase fused to the CD163 wild-type 3'UTR, whereas miR-181c (mut) did not. In the reciprocal experiment, we showed that transfection of miR-181c (mut) resulted in significantly less luciferase activity, while miR-181c had no effect on it. To obtain further evidence for the targeting of CD163 by miR-181c, we examined the physical interaction between CD163 mRNA and miR-181c by an RNA-induced silencing complex (RISC) immunoprecipitation assay. We found that there was about 3-fold enrichment for CD163 mRNA in AGO-bound mRNAs when miR-181c was over-expressed. As expected, no enrichment for CD163 mRNA was observed when miR-181c (mut) was transfected. Together, these results demonstrated that miR-181c could down-regulate CD163 expression through directly targeting its mRNA.

Given that CD163 functions as a cellular receptor for PRRSV, we next investigated whether down-regulation of CD163 in PAMs leads to the inhibition of PRRSV infection. To rule out the influence of miR-181c on PRRSV genome, we constructed PRRSV-JXwn06 (mut) which had mutations in the target sequence of miR-181c in its genome, and then evaluated the effect of miR-181c on PRRSV infection in PAMs. We inoculated the cells with PRRSV-JXwn06 (wt) or PRRSV-JXwn06 (mut) at an MOI of 5 at 72 hours post transfection of PAMs with miR-181c mimics, and then quantified intracellular PRRSV RNAs using quantitative real-time PCR at 3 hours after virus inoculation at 37°C. We showed that PRRSV RNAs in PAMs transfected with miR-181c were about 70% less than that in mock or NC control PAMs. However, no significant difference in RNA quantity existed between PRRSV-JXwn06 (wt) and PRRSV-JXwn06 (mut) infected cells. These results indicated that miR-181c remarkably blocked both PRRSV-JXwn06 (wt) and PRRSV-JXwn06 (mut) entry into PAMs.

Our data here showed that in addition to inhibit PRRSV replication by directly targeting PRRSV genomic RNAs, miR-181 could also suppress PRRSV infection through targeting its cell receptor CD163, implicating that miR-181 has the potential to be used for anti-PRRSV therapeutics.

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Nonstructural proteins 1beta and 11 play an important role in differential TNF-alpha production induced by different PRRSV strains

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An unparalleled large-scale, atypical clinical outbreak of PRRS in China in 2006 was caused by a highly pathogenic PRRSV (HP-PRRSV). TNF-alpha has been shown to have an antiviral effect by inducing apoptosis in virus-infected cells and promoting inflammatory response. Previous studies have shown that different PRRSV stains exhibited distinct ability in TNF-alpha induction. In this study, we first confirmed differential TNF-alpha expression in pulmonary alveolar macrophages (PAMs) induced by different pathogenic PRRSV strains and then indentified the viral proteins involved in such effect, and furthermore, verified the role of such viral proteins using reverse genetic technology to construct chimeric viruses by exchanging the determinant viral proteins-coding region. The results showed that the HP-PRRSV JXwn06 could induce higher level of TNF-alpha mRNA expression than the low-virulence PRRSV HB-1/3.9, whereas the level of TNF-alpha protein induced by JXwn06 was significantly lower than that induced by HB-1/3.9. Thus, the HP-PRRSV is a weak inducer of TNF-alpha than the low-virulence PRRSV. Using a TNF-alpha promoter reporter system, Nsp7, Nsp11 and Nsp12 were found to be the determinant proteins involved in differential TNF-alpha expression at mRNA level. Nsp1beta and Nsp11 of HP-PRRSV were found to be more capable of inhibiting ERK signaling pathway, which might result in greater ability in suppression of TNF-alpha production, by investigating the effect that each Nsp exerted on the ERK signaling pathway. By exchanging both Nsp1beta- and Nsp11-coding region between JXwn06 and HB-1/3.9, the chimeric viruses could almost reverse the phenomenon of differential TNF-alpha production. Our results suggest that the Nsp1beta and Nsp11 play an important role of in differential TNF-alpha production induced by different pathogenic PRRSV strains. The property that HP-PRRSV is not able to effectively induce TNF-alpha production at protein level is beneficial for its replication in vivo, which may contribute to its high virulence for piglets.

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Using high throughput proteomics to understand the functional interaction of PRRSV and the nucleocapsid protein with the host cell and comparison with the coronavirus infectious bronchitis virus

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High throughput quantitative proteomics and bioinformatics were used to determine the interaction of porcine reproductive and respiratory syndrome virus (PRRSV) and infectious bronchitis virus (IBV) with the host cell. Porcine alveolar macrophages (PAMS) were infected with a highly pathogenic isolate of PRRSV and Vero cells were infected with IBV. The viral and cellular interactomes were determined at several time points using quantitative proteomics using different approaches. A label free approach was used to quantify proteins in the primary PAM cells whereas stable isotope labeling with amino acids in cell culture (SILAC) was used in the continuous Vero cell line. Both cellular and viral proteins were identified and quantified using LC-MS/MS. This allowed relative abundance differences between the same cellular proteins in mock and infected cells to be determined as well as with time of infection. Bioinformatics was used to construct interactome/network pathway maps of dynamic changes in the cellular proteome as infection progressed for both viruses. In order to validate the LS-MS/MS analysis changes in the abundance and localization of cellular proteins were investigated using western blot and indirect immunofluorescence microscopy. The data indicated that changes in the cell were confined to specific pathways and signaling activities.

In addition, both SILAC and label free approaches were used to map the cellular interactome of several viral proteins including the nucleocapsid (N) protein for both viruses. For the PRRSV N protein two immune-precipitation approaches were used and compared. In the first approach, an EGFP-N fusion protein was expressed in 293T cells and coupled to an EGFP trap for immune-precipitation. SILAC was used to distinguish between cellular proteins that specifically interacted with N protein versus the EGFP moiety and the background matrix. In the second approach, N protein was expressed and purified from E.coli and used as a bait to capture proteins from extracts purified from 293T cells. Label free LC-MS/MS was used to distinguish bait versus control samples. With the PRRSV N protein some 80 cellular proteins were identified which interacted with this viral protein including translation initiation factors and proteins involved in RNA modification. This was in contrast to the IBV N protein that additionally bound more ribosomal proteins. Ablation of cellular mRNAs using siRNA and small molecule inhibitors were used to investigate the role of the identified cellular proteins in virus biology.

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Fetal preservation and virus concentration following type 2 PRRSV infection in pregnant gilts

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Objective: We have undertaken a large-scale project investigating the genomic and phenotypic predictors of PRRS resistance in pregnant gilts. One of the objectives of this project was to evaluate the susceptibility of gilts by assessing variation in preservation and viral levels in late term fetuses.

Methods: At gestation day 85 (±1), 114 gilts were inoculated with a Type 2 PRRSV strain, 50% IM and 50% IN (10^5 $TCID_{50}$ total dose). Nineteen gilts were similarly sham inoculated (control).
On 21 dpi, dams and their litters were humanely euthanized and fetal preservation scored as: viable (VIA), live meconium stained (MEC), decomposed (DEC), or autolysed (AUT). PRRSV RNA concentration in fetal thymus, endometrium adjacent the umbilical stump, and serum (from VIA and MEC only) were quantified by in-house RT-qPCR. Mummified fetuses (crown-rump length < 20cm), considered dead prior to inoculation, were excluded from analysis.

Results: Two PRRS-infected gilts aborted (17 and 20 dpi) and 1gilt died (11 dpi); data from those gilts were excluded. In PRRSV challenged litters, 59 ±23% of piglets survived until termination. The within litter survival rate ranged from 5% to 100% (Table 1). MEC fetuses were mainly observed in infected gilts suggesting a PRRS-related pathologic process. PRRSV RNA was detected in over 90% of MEC, DEC and AUT, but in fewer VIA fetuses (Table 2). In ~10% of fetuses, PRRS virus was not detected in either serum or thymus, even though moderate to high PRRS virus concentration was detected in the endometrium adjacent the umbilical attachment. Conversely, ~7% of autolysed and decomposed fetuses had no detectable levels of PRRS virus in fetal thymus, possibly due to degradation of virus RNA within 21 days or death of the fetus prior to infection of the fetal thymus. Of the PRRS-infected fetuses that were live at termination, the viral levels were clearly higher in both tissues and sera of MEC than in VIA (data not shown).

Table 1. Fetal preservation in infected and control gilts at 106 days of gestation (21 dpi)

Fetal preservation	***% VIA***	***% MEC***	***% DEC***	***% AUT***
Control (n=227)	97.8	0.88	0	1.32
Infected (n=1392)	50.07	8.98	7.97	32.97

Table 2. Positive PCR results (%) in fetal thymus, placenta/uterus and fetal serum of fetuses from infected gilts according to preservation status

PCR	***VIA***	***MEC***	***DEC***	***AUT***
% pos fetal thymus	53.08	93.6	97.3	91.92
% pos PLC/UTR	77.04	98.4	94.59	92.16
% pos fetal serum	60.95	96.0		

Conclusion/Discussion: Fetal preservation and PRRS virus levels were highly variable among litters and suggest differences in PRRS resistance at the fetal level. The mechanisms associated with fetal death are presently unclear but given all gilts were viremic by 2 dpi, and 7% of dead fetuses had no detectable PRRS virus, these results support previous research that fetal death is independent of PRRS replication in fetal tissue. Further analysis is underway to determine if the variations in PRRS susceptibility are associated with single nucleotide polymorphisms.

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PRRS RNA concentration in serum does not predict clinical severity in pregnant gilts

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Objective: We have undertaken a large-scale project investigating the genomic and phenotypic predictors of PRRS resistance in pregnant gilts. One of the objectives was to determine if PRRS severity is associated with temporal changes in PRRS RNA levels in serum or tissues.
Methods: At gestation day 85 (±1), 114 gilts were inoculated with a Type 2 PRRSV strain, 50% IM and 50% IN (10^5 $TCID_{50}$ total dose). Serum was collected on 0, 2, 6 and 21 dpi. On 21 dpi, gilts were humanely euthanized and tissue samples were collected from lung, tonsil, reproductive and tracheobronchial lymph node. Fetal preservation was recorded. PRRS RNA was quantified in sera and tissues using an in-house RT-qPCR. General estimating equations were used to compare fetal survival to PRRS levels in gilt sera and tissues.
Results: Data of 2 PRRS-infected gilts that aborted (17 and 20 dpi) and 1gilt that died (11 dpi) were excluded. PRRS RNA concentration was categorized as: 0=not detected; 1=low viral load (positive, too low to quantify); 2=medium viral load (quantifiable; lower than mean); 3=high viral load (quantifiable; higher than mean). All infected gilts had quantifiable PRRS levels on 2 dpi (mean 2.9 ± 0.5 log_{10} copies/µl) and PRRS RNA concentration in serum peaked at 6 dpi (mean 4.3 ±0.7 log_{10} copies/µl). At 21 dpi PRRS RNA levels were higher in lymphoid tissues than in sera or lung (Table 1). No gilt cleared the virus from investigated lymph nodes. Percent fetal survival was negatively associated with PRRS RNA concentration in reproductive lymph node ($P = 0.04$).

Table 1. PRRSV levels in serum and tissues of 111 infected gilts at 21 dpi*

PRRS concentration	**serum**	**lung**	**tonsil**	**reprod. LN**	**tracheobr. LN**
0 (not detected)	13.5	9.9	0.9	0	0
1 (low)	41.4	29.7	0	0	2.7
2 (medium)	44.1	31.5	47.8	42.3	45.1
Mean (SD)	*1.35 (0.51)*	*3.34 (0.48)*	*4.92 (0.69)*	*5.15 (0.72)*	*4.11 (0.76)*
3 (high)	0.9	28.8	51.4	57.7	52.3
Mean (SD)	*3.28*	*4.95 (0.62)*	*6.15 (0.42)*	*6.34 (0.32)*	*5.46 (0.41)*

*Data shows the percentage of gilts in each PRRS virus category within a column. Italicized values in medium and high categories represent mean (SD) RNA concentration (log_{10} copies per µl/mg).

Conclusion/Discussion: Virus levels in all gilt sera peaked by day 6. A small proportion of gilts cleared virus from serum. The positive detection rate was highest in lymphatic tissues and 100% of gilts were PRRS RNA positive in both investigated lymph nodes at 21 dpi. Only PRRS RNA concentration in reproductive lymph node at 21 dpi was predictive of fetal survival which decreased 5.4% for each 1 log increase in viral load. PRRS levels in gilt serum can therefore not be used to assess the clinical severity of PRRS.

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Temporal changes in white blood cell counts and PBMC subsets following type 2 PRRSV in pregnant gilts

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Objective: We have undertaken a large-scale project investigating the genomic and phenotypic predictors of PRRS resistance in pregnant gilts. One of the objectives was to evaluate temporal changes in white blood cell counts (WBCs) and peripheral blood mononuclear cell (PBMC) subsets following infection to determine if any predict PRRS severity.
Methods: At gestation day 85 (±1), 114 gilts were inoculated with a Type 2 PRRSV strain, 50% IM and 50% IN (10^5 $TCID_{50}$ total dose). Nineteen gilts were similarly sham inoculated (control). All gilts were euthanized 21dpi and the fetal preservation recorded. Heparinized blood was collected on 0, 2, 6 and 19 dpi. Automated WBC counts (Cell-Dyn 3500, Abbott Diagnostics) and manual differential counts were performed (300 cells total). PBMC isolated from whole blood were stained with fluorochrome-conjugated, monoclonal antibodies to define cell subsets using flow cytometry. General estimating equations were used to relate fetal mortality to cell subsets.
Results: Two PRRS-infected gilts aborted (17 and 20 dpi) and 1gilt died (11 dpi); data from these were excluded. WBC and lymphocyte subsets decreased by 2 dpi in PRRS infected gilts, while neutrophil numbers were unaffected by PRRS infection (Table 1). All except B-lymphocytes began to recover by 6 dpi. Fetal mortality (%) was negatively associated with WBC on 2 dpi (*P*=0.03; beta= -2.0) and T-helper cells on 0 (*P*=0.06; beta= -8.8) and 2 dpi (*P*=0.06; beta=-15.3).

Table 1. Mean (SD) cell counts in 111 inoculated and 19 control gilts

		0 dpi		2 dpi			6 dpi			19 dpi		
Cells ($x10^9$/L)	Group	mean	SD	mean	SD	% chg	mean	SD	% chg	mean	SD	% chg
WBC	control	10.4	2.4	10.8	2.8	104	11.1	2.3	107	10.2	2.1	98
	PRRS	11.0	1.9	6.1	2.3	55	8.4	1.7	76	11.7	3.0	106
Seg.	control	2.62	1.5	3.14	1.7	120	3.17	1.3	121	3.00	1.6	115
Neutrophils	PRRS	3.05	1.3	3.04	2.0	100	2.30	0.9	75	4.10	2.0	134
T-helper cells	control	1.79	0.5	1.82	0.3	102	1.79	0.4	100	1.69	0.4	94
($CD3^+CD4^+$)	PRRS	1.88	0.4	0.58	0.3	31	1.58	0.4	84	1.53	0.4	81
CTL	control	1.48	0.5	1.54	0.5	104	1.43	0.5	97	1.33	0.4	90
($CD3^+CD8b^+$)	PRRS	1.43	0.5	0.33	0.2	23	1.15	0.4	80	1.45	0.6	101
B-lymphocytes	control	1.09	0.3	1.15	0.3	106	1.05	0.3	96	0.97	0.4	89
($CD79a^+$)	PRRS	1.12	0.4	0.66	0.3	59	0.56	0.2	50	1.00	0.4	89

Legend: % change (%chg) = number of cells at 2, 6 or 19 dpi divided by the number of cells at D0

Conclusion/Discussion: PRRS infection caused a profound, transient decrease in most leukocyte subsets on 2 dpi that was most prominent in cytotoxic T lymphocytes (CTL). While T lymphocytes began to rebound by 6 dpi, B lymphocytes showed a further decrease on day 6. The mechanisms associated with these changes are not clearly understood, however, it is most likely the decrease reflects migration of leukocytes into systemic tissues following infection. Total leukocyte numbers at 2 dpi, and T-helper cells at 0 and 2 dpi time were significant predictors of fetal mortality. This finding could be exploited to further enhance control strategies.

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A porcine circovirus type 2 (PCV2) mutant with 234 amino acids in capsid protein showed more virulence in vivo, compared with classical PCV2a/b strains

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Background: Porcine circovirus type 2 (PCV2) is considered to be the primary causative agent of postweaning multisystemic wasting syndrome (PMWS), which has become a serious economic problem for the swine industry worldwide. The major genotypes, PCV2a and PCV2b, are highly prevalent in the pig population and are present worldwide. However, another newly emerging PCV2b genotype mutant, which has a mutation in its ORF2-encoded capsid protein, has been sporadically present in China, as well as in other countries. It is therefore important to determine the relative virulence of the newly emerging PCV2b genotype mutant, compared with the existing PCV2a and PCV2b genotypes, and to investigate whether the newly emerging mutant virus induces more severe illness.
Methods: Twenty healthy, 30-day-old, commercial piglets served as controls or were challenged with PCV2a, PCV2b and the newly emerging mutant virus. A series of indexes representing different parameters were adopted to evaluate virulence, including clinical signs, serological detection, viral load and distribution, changes in immune cell subsets in the peripheral blood, and evaluation of pathological lesions.
Results: The newly emerging PCV2 mutant demonstrated more severe signs compatible with PMWS, characterized by wasting, coughing, dyspnea, diarrhea, rough hair-coat and depression. Moreover, the pathological lesions and viremia, as well as the viral loads in lymph nodes, tonsils and spleen, were significantly more severe ($p<0.05$) for piglets challenged with the newly emerging mutant compared with those in the groups challenged with PCV2a and PCV2b. In addition, a significantly lower average daily weight gain ($p<0.05$) was recorded in the group challenged with the newly emerging PCV2 mutant than in the groups challenged with the prevailing PCV2a and PCV2b.
Conclusions: This is believed to be the first report to confirm the enhanced virulence of the newly emerging PCV2 mutant in vivo.

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The role of Hsp70 in the replication of porcine circovirus type 2 in vitro

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Porcine circovirus type 2 (PCV2) has been identified as the essential causal agent of postweaning multisystemic wasting syndrome, which has spread worldwide. Infection of pigs with PCV2 and other infectious/noninfectious triggers are required for PMWS to develop. Several studies have linked PMWS to management measures, presence of concurrent viral infections, immune system stimulation, nutrition, male castration and lower piglet weight at weaning. However, the pathogenic mechanism of PCV2 remains poorly understood.

To discover cellular protein responses of porcine alveolar macrophages (PAM) to PCV2 infection, two-dimensional liquid chromatography–tandem mass spectrometry coupled with isobaric tags for relative and absolute quantification (iTRAQ) labeling was employed to quantitatively identify the proteins that were differentially expressed in PAMs from the PCV2-infected group compared to the uninfected control group. A total of 145 cellular proteins in PAMs that were significantly altered at different time periods post-infection were identified. These differentially expressed proteins were related to the biological processes of binding, cell structure, signal transduction, cell adhesion, etc., and their interactions. Among them, some heat shock proteins, such as hsp70 and hsp27 expression was increased in the cells post infection of the virus, indicating they might affect the virus replication. Furthermore, the positive effects of Hsp on the replication of PCV2 were investigated in a PAM cell line 3D4/31. Downregulation of Hsp70 by the specific chaperone inhibitor Quercetin or RNA interference and upregulation of Hsp70 by expression from a recombinant adenovirus showed that Hsp70 enhanced PCV2 genome replication and virion production. A specific interaction between Hsp70 and PCV2 Cap was confirmed by colocalization by confocal microscopy and co-immunoprecipitation. In addition, the NF-kappaB pathway was activated and caspase-3 activity was reduced when Hsp70 was overexpressed in PCV2-infected 3D4/31 cells. These data suggested that Hsp70 was important for PCV2 replication. The results provide a basis for understanding the molecular mechanism of PCV2 infection.

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Porcine reproductive and respiratory syndrome virus (PRRSV)-mediated NF-κB activation is dependent on TLR2, TLR4 and MAPK signaling pathway

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Nuclear factor kappa B (NF-κB) is a critical transcription factor in innate and adaptive immune response as well as cell proliferation and survival. Previous studies have demonstrated that porcine reproductive and respiratory syndrome virus (PRRSV) infection activated NF-κB pathways through IκB degradation in MARC-145 cells and alveolar macrophages. To evaluate the mechanisms behind this, we investigated the role of TLR, RIG-I and MAPK signaling pathway in the regulation of NF-κB. Using small interfering RNA, we demonstrated that PRRSV sensing by the TLR2 and TLR4 receptors triggers a signaling cascade involving the MyD88, TRIF and TRAF6 adaptors that ultimately leads to p65 phosphorylation. Furthermore, we found that the PRRSV infection trigger activation of signaling pathways mediated by mitogen-activate protein kinases (MAPK), including p38, extracellular signal-regulated kinases 1 and 2 (ERK1/2) and c-Jun N-terminal kinase. Treatment of cells with pharmacological inhibitors against p38 and ERK1/2, markedly diminished the PRRSV-induced NF-κB activation and such inhibitory effect on NF-κB activation is in a dose-dependent manner. Taken together, these results indicated that TLR2/TLR4-MyD88/TRIF-TRAF6-p65 phosphorylation pathway plays an important role in NF-κB activation induced by PRRSV as well as p38 and ERK1/2 pathways. The results presented here provide a basis for understanding molecular mechanism of PRRSV infection and NF-κB-driven proinflammatory response.

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Quantitative mitochondrial proteomics by isobaric tags for relative and absolute quantification (iTRAQ) labeling in PRRSV infected pulmonary alveolar macrophages

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Porcine reproductive and respiratory syndrome virus (PRRSV) is the cause of an economically important swine disease that has been devastating the swine industry since the late 1980s. The swelling of mitochondrion in PRRSV-infected pulmonary alveolar macrophages (PAMs) was observed by electron microscope. To discover mitochondrial protein responses in PRRSV-infected PAMs, mitochondrial fractions were isolated and labeled with isobaric tags for relative and absolute quantification (iTRAQ) and subsequently liquid chromatography-tandem mass spectrometry (LC-MS/MS) was employed to quantitatively identify the differentially expressed proteins between the PRRSV-infected cells and the controls. A total of 176 cellular proteins isolated from mitochondrion that were significantly altered post-infection were identified. These modulated proteins involved in apoptosis, oxidative stress, calcium ion transport, signal transduction. Selected data were validated by Western blot. This is the first report that analyzed the mitochondrial protein profile of PRRSV-infected PAMs using iTRAQ approach, and the obtained data could provides insights into the complexity and dynamics of virus-host cell interactions and may help elucidate the underlying mechanism of PRRSV pathogenesis.

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PRRSV infection change Bax/Bcl-2 ratio in primary porcine alveolar macrophages

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Objective: In mitochondrial pathway and death receptor pathway of cell apoptosis, the composition of Bcl-2 family members, especially the ratio of Bcl-2/Bax, is "molecular switch" which starts the cell apoptosis. It was found that American type porcine productive and respiratory syndrome virus(PRRSV), represented by strain ATCC VR-2332, and Europe type PRRSV, represented by strain LV, all could induce cell apoptosis both in vitro and in vivo, and the inducing mechanism was related with virus pathogenesis. In order to accumulate data for PRRSV's pathogenesis research, Bcl-2 and Bax gene expression in PAM infected by PRRSV were detected in this study.

Method: Primary porcine alveolar macrophages (PAM) cells were isolated from PRRSV negative piglets and relative quantitative real-time RT-PCR methods for detection Bcl-2 and Bax gene expression of PAM were established. Then PAM were seeded in 6-well plate with $1x10^6$ cells /well. 2h later, the cells were divided into two groups, control and inoculation. Cells of inoculation group were inoculated PRRSV strain SD16 with 0.01 MOI. At 6h, 12h, 24h, and 48h after inoculation, cells were collected and RNA was extracted to detect Bax and Bcl2 mRNA relative expression level with the established real-time RT-PCR methods. The mRNA of beta-actin was served as an internal reference, genes in control group was set at 1.

Results and Discussion: The results showed that at 6h and 12h after inoculation, Bax expression decreased while Bcl-2 expression increased compared with these of control PAM. What's more, those of 12h inoculation group had significant difference compared with these of control group. At 24h after inoculation, Bax expression had no noticeable change, but Bcl2 expression reduced and had significant difference compared with these of control group. At 48h after inoculation, Bax expression improved and Bcl2 expression reduced and all had significant difference compared with these of control group.

Conclusion: Those results suggested that the cells were in condition of suppression apoptosis at the beginning of PRRSV infection and it was conducive to PRRSV replication. However, cell apoptosis was increased at late stage of PRRSV infection.

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Porcine, murine and human sialoadhesin: portals for PRRSV entry into target cells

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PRRSV entry in alveolar macrophages has already been extensively studied, and a number of entry mediators have been identified. Two of these molecules appear to be crucial for efficient infection of porcine alveolar macrophages: sialoadhesin (Sn; siglec-1; CD169) and CD163. Sn mediates efficient binding and internalization of the virus, whereas CD163 is most likely involved in genome release. Ample data have shown that sialic acids on the virion surface interact with the N-terminal sialic acid-binding domain of Sn, upon which the virus-receptor complex is internalized via a process of clathrin-mediated endocytosis. To complement our knowledge on Sn, this study aimed to evaluate if Sn homologs from other mammalian species also show PRRSV entry mediator activity and to assess the importance of a functional sialic acid-binding ectodomain and an intact endodomain for their role as virus receptors.
ORFs encoding porcine (pSn; pSn_{WT}), murine (mSn; mSn_{WT}) and human (hSn; hSn_{WT}) sialoadhesin were cloned into mammalian expression vectors. Non-sialic acid-binding (Sn_{RE}; $R^{116}E$ mutants) and endodomain-deletion (Sn_{-endo}) mutant forms of pSn, mSn and hSn were obtained via site-directed mutagenesis. Expression analysis via Sn-specific immunofluorescence staining showed that Sn_{RE} proteins were expressed equally well or better than their respective Sn_{WT}. In contrast, the Sn_{-endo} proteins were expressed less well than their respective Sn_{WT}. Erythrocyte binding assays confirmed that the Sn_{RE} proteins lack sialic acid-binding capacity, while all Sn_{WT} and Sn_{-endo} proteins efficiently bind sialic acids. Finally, infection assays were performed to evaluate the functionality of porcine, murine and human Sn_{WT}, Sn_{RE} and Sn_{-endo} as PRRSV entry mediators. PK-15 cells were transiently transfected with an expression vector encoding porcine CD163 (pCD163), or co-transfected with the Sn-encoding constructs and the pCD163-encoding vector. Cells were inoculated with PRRSV LV and infection was evaluated after 24h via PRRSV-specific immunofluorescence staining and titration assays. In $pCD163^+$ transfected cells, only few cells were virus-positive and production of new virions was limited. In $pCD163^+pSn_{WT}^+$, $pCD163^+mSn_{WT}^+$ and $pCD163^+hSn_{WT}^+$ cells, the number of virus-positive cells was markedly higher and the titrations revealed a much more efficient production of new PRRSV virions. These data indicate that mSn_{WT} and hSn_{WT}, like pSn_{WT}, can promote PRRSV infection of target cells by binding and shuttling the virus to the correct endosomal compartment for pCD163-mediated genome release. In $pCD163^+Sn_{RE}^+$ cells, infection and virus production were significantly lower than in $pCD163^+Sn_{WT}^+$ cells, approximating levels observed for $pCD163^+$ cells. In $pCD163^+Sn_{-endo}^+$, cells, the number of virus-positive cells was lower than in $pCD163^+Sn_{WT}^+$ cells, and virus production was significantly reduced. Nevertheless, infection and virus production levels were higher than those observed for $pCD163^+$ cells, indicating that the Sn_{-endo} molecules retain a capacity to promote infection.

In conclusion, we showed that, apart from pSn, also mSn and hSn have PRRSV receptor activity. These data, in combination with other published data, suggest that species differences in Sn and CD163 do not account for the strict host species specificity displayed by PRRSV. Sialic acid-binding capacity appears to be crucial for the functionality of the different sialoadhesins as PRRSV receptors. Specific involvement of the Sn endodomains remains currently unclear.

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PRRSV infection induces Sn expression on local endometrial and placental CD163-positive cells

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Reproductive failure due to PRRSV infection is characterized by late-term abortions, early farrowing, increased number of dead and mummified fetuses and weak-born piglets. PRRSV efficiently replicates in the endometrial and placental sialoadhesin (Sn)-positive macrophages that is most probably a prerequisite for reproductive disorders. Decidual and placental macrophages play a crucial role to maintain a relatively steady state situation in the maternal-fetal interface that is essential for normal human pregnancy. A differentiated and inflammatory status of decidual/placental macrophages has been associated with reproductive disorders. The purpose of the present study was to examine if PRRSV infection is able to alter Sn mRNA and protein expression in endometrial/placental macrophages. Tissue samples were obtained from three PRRSV-inoculated (inoculation at 90 days of gestation) and three non-inoculated control sows at 100 days of gestation. Several pieces of uteri with placenta adjacent to all fetuses were randomly excised and frozen. Endometrium with placenta tissues were mechanically separated from the underling myometrium on the cryosection. Afterwards, PRRSV-positive and -negative tissues from infected and control sows, respectively, were subjected to total RNA extraction, cDNA synthesis and Sn gene expression analyses. Prior to RNA extraction, immunofluorescence (IF) staining was performed to detect PRRSV antigens in tissue cryosections. Moreover, IF staining specific for Sn, CD163 and CD14 were also performed. Real-time RT-PCR analysis revealed Sn mRNA up-regulation in PRRSV-positive endometrium/placenta samples ($p<0.04$). The mean number of Sn-positive cells was significantly higher in the endometrium (PRRSV-positive tissues: 93/0.5 mm^2; PRRSV-negative tissues: 41/0.5 mm^2) and in the placentae (PRRSV-positive tissues: 87/0.5 mm^2; PRRSV-negative tissues: 19/0.5 mm^2) from virus-inoculated sows than from control sows. It is already known that only some subset of endometrial/placental CD163-positive macrophages expresses Sn and that all Sn-positive macrophages are positive for CD163. The number of CD163-positive and CD14-positive cells was quite similar between PRRSV-positive and -negative tissues and in the PRRSV-positive placentae, the numbers of CD163-positive and CD14-positive cells were slightly lower than in the negative control placentae. The decrease of CD163-positive and CD14-positive cells in the placenta can be attributed to massive PRRSV-induced apoptosis. In summary, *de novo* Sn expression on local endometrial/placental CD163-positive cells was demonstrated upon PRRSV infection. The molecular mechanisms involved in the induction of Sn overexpression and its pathophysiological role in PRRSV-related reproductive disorders remain to be examined.

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Phenotypic modulation and cytokine profiles of antigen presenting cells by European subtype 1 and 3 porcine reproductive and respiratory syndrome virus strains *in vitro* and *in vivo*

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Porcine reproductive and respiratory syndrome virus (PRRSV) causes continuous problems in the pig industry, due to high costs of outbreaks and reduced welfare of diseased pigs. The severity of infection is, partly, dependent on the virus strain. Recently isolated Eastern-European subtype 3 strains are more virulent than the widespread subtype 1 strains. There is, however, almost no information available about the mechanisms involved in the virulence of these subtype 3 strains. The objective of the present study was to characterize the *in vitro* and *in vivo* response of two European subtype 1 strains, Belgium A and Lelystad-*Ter Huurne* (LV), and a virulent subtype 3 strain, Lena, in dendritic cells (*in vitro*) and alveolar macrophages (*in vitro* and *in vivo*). It was shown that infection with the Lena strain resulted in a higher apoptosis of cells *in vitro* and a higher level of infectivity *in vitro* and *in vivo* than the other virus strains. Furthermore, infection with Lena resulted in the highest down regulation of the immunologically relevant cell surface molecules SLA-I, SLA-II and CD80/86 *in vitro*, and SLA-II *in vivo*. In spite of these differences, *in vitro* cytokine responses did not differ significantly between strains except for a down regulation of IL-10 by Lena in dendritic cells. The higher infectivity, apoptosis and down regulation of the cell surface molecules, may have contributed to the increased pathogenicity of Lena, and have dampened specific immune responses. This could explain the delayed and decreased adaptive immune responses observed after infections with this strain.

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Genome-wide analysis of marker genes related to antiviral regulation in PRRSV-infected porcine macrophages at different activation statuses

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Specific objectives: Previously, we showed that porcine monocytic cells could be skewed to various activation statuses similar to those described in mice and humans, including M1 and M2. We hypothesize that the activation status of porcine monocytic cells, during PRRSV infection, provides a framework for optimizing antiviral immunity and immune homeostasis. The specific objectives of this study include: (1) genome-wide profiling of differentially expressed genes (DEGs) in PRRSV-infected porcine alveolar macrophages (PAMs); (2) compare the expression of classic marker genes associated with different activation statuses in PRRSV-infected PAMs; (3) perform a family-wide analysis of innate immunity and lipid metabolism genes during PRRSV infection; (4) define significant marker genes at different activation statuses for antiviral/homeostatic regulation in porcine monocytic cells.
Methods: Monocytic cells of known activation states, M1, M2a, M2b and M2c were prepared from PAMs using standard methods and confirmed by cytokine secretion patterns. PAMs were infected with a GFP-expressing PRRSV isolate for 5 h. Total RNA was extracted from 3×10^7 pooled cells and used for construction of sequencing libraries containing 200-bp inserts. RNA-seq was performed with Illumina HiSeq2000 technology to achieve approximately 30,000,000 reads/sample and the edgeR package was used to profile DEGs. Authentic expression of some DEGs was confirmed using real time RT-PCR. In addition, a proteomic procedure was used to screen marker genes at the protein level.
Results and conclusions: Of the approximately 30M clean reads per pooled sample, about 80% could be mapped to current genome or gene databases, shown by the relatively uniform distribution of reads mapped to most scaffolds of reference genome and random distribution along reference genes. In addition, reads mapped to more than 60% of transcripts flanked at least 50% of their full-length sequence. Therefore, our RNA-seq reads resulted in good genome-wide coverage. For comparison between any two activation statuses, we normalized >20,000 DEGs and filtered out 150-5,000 significant DEGs (FDR≤0.001, fold change>2) in each comparison. For example, the highest 5,303 significant DEGs were revealed between M2b (LPS) and M2a (IFNγ) cells with 4,257 up-regulated and 1,046 down-regulated, respectively; whereas, only 153 significant DEGs were detected between M2c (IL10) and non-stimulated PRRSV-infected PAMs. To confirm classic marker genes revealed in mice and humans and to obtain species-specific marker genes in pigs, we have determined a set of DEGs per activation status that only show significant up-regulation in one but not other activation states. Collectively, we have determined DEGs classified into pathways including innate immunity, lipid metabolism, antigen presentation and hypothetical functional unknowns. Our data revealed family-wide differential expression of inflammatory cytokines, chemokines, Toll-like receptors, interferon (IFN)-regulatory factors (IRFs) and IFN-stimulated genes (ISGs). Further studies will functionally characterize some significant DEGs for their roles in antiviral/homeostatic regulation in porcine monocytic cells.

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In vivo impact of deoxynivalenol naturally contaminated feed on porcine reproductive and respiratory virus infection

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Animal feeds are frequently contaminated with various mycotoxins produced by the secondary metabolism of diverse fungal contaminants. Among these contaminants, deoxynivalenol (DON), also known as vomitoxin, is the most prevalent type B trichothecene mycotoxin worldwide. Pigs show a great sensivity to DON, and because of the high proportion of grains in their diets, they are frequently exposed to this mycotoxin. The objective of this study was to determine the impact of DON naturally contaminated feed on porcine reproductive and respiratory syndrome virus (PRRSV) infection, the most important viral pathogens in swine. Experimental infections were performed with 30 commercial animals. Pigs were randomly divided into three experimental groups of 10 animals based on DON content of served diets (0, 1.5 and 3.5 ppm of DON/Kg). They were fed these rations for 2 weeks prior to experimental infection. All experimental groups were further divided into subgroups of 6 pigs and were with 1 ml of $1x10^4$ $TCID_{50}$ PRRSV intra-muscularly and 1 ml of the same inoculums in each nostril. The remaining pigs (control) were sham-inoculated with PBS. Pigs were daily monitored for temperature, weight and clinical signs for 21 days. Blood samples were collected and tested for PRRSV RNA by qPCR and for virus specific antibodies. During necropsy, lung macroscopic lesions were observed and sections of lung and assorted lymph nodes were collected for evaluation of specific microscopic lesions. Results of PRRSV infection showed that ingestion of diet highly contaminated with DON greatly increases the effect of PRRSV infection on weight gain, lung lesions and mortality, without increasing significantly viral replication, for which tendency was rather directed towards a decrease of replication. These results suggest that PRRSV infection could exacerbate anorectic effect of DON, when ingested in large doses. Results also demonstrate DON negative effect on humoral response. DON could therefore potentially undermine the efficacy of live attenuated vaccines by interfering with the humoral response of pigs.

In conclusion, this study demonstrate that high concentrations of DON contaminated feed decreased immune response against PRRSV and can influence the course of infection.

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The susceptibility of porcine myeloid cells to Porcine Reproductive and Respiratory Syndrome Virus 1

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Scavenger receptor CD163 and sialic-acid binding lectin CD169 were identified as key receptor molecules for Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), an important *arterivirus* which replicates in porcine myeloid cells expressing the above. Porcine alveolar macrophages (PAMs) are generally used for propagating virus, but being primary tissue cells are difficult to obtain, vary in susceptibility and are thus rather unreliable. $CD14^+$ monocytes are considered unsuitable for PRRSV infection, but initial experiments in our laboratory showed that at least some monocytes could propagate PRRSV.

The aim of this study was to compare PRRSV-1 replication (Lena strain) across different porcine myeloid cells. The first objective was to maximise PRRSV infection in monocytes, alongside the modulation of CD163 and CD169 expression. Secondly we aimed to characterise monocyte derived macrophages and dendritic cells (MoMØ and MoDC) according to their morphologies, phenotypes, and activation/maturation states, before assessing PRRSV replication in these cells.

Monocytes were cultured for 2 days with a panel of cytokines and macrophage activating factors (some known to modulate CD163/CD169), before being infected with PRRSV. CD163/CD169 expression was measured by flow cytometry and viral replication was measured using qPCR. IL-10 and dexamethasone (Dex) caused the most significant up-regulation of CD163/CD169 and significantly increased PRRSV replication was observed.

MoMØ were stimulated with factors identified as activators of human macrophage subsets M1 (LPS + IFN-γ) and M2 (IL-4), and also with IL-10 and Dex. By flow cytometric analysis of markers such as CD25, CD206 and CD209, preliminary data suggests that porcine macrophage subsets are different to the human macrophage lineage. Furthermore, all subsets are susceptible to PRRSV-1 infection.

MoDC were generated via GM-CSF & IL-4 treatment as previously described and activated with a maturation cocktail containing LPS, IFN-γ, IL1β, IL-6, TNFα and PGE_2, which increased the expression of MHCII, CD80/86 and CD83 as expected from the literature in other species. IL-10 and Dex were added for comparison. MoDCs failed to express CD163/CD169, even following Dex and IL-10 treatment. Despite this, MoDCs were susceptible to PRRSV-1 Lena infection. There were, however, notable differences in the replication of PRRSV-1 across the populations of myeloid cells.

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PRRSV type 1 can induce aggravation of infection with a mild virulent Actinobacillus serotype 2 strain

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PRRSV is endemic in most pig producing countries of the world and causes disease characterized by abortions and stillbirth, increased pre-weaning mortality and respiratory disorders in growing pigs. The genotype 1 strains of PRRSV are primary pathogens for reproductive failure and abortion. However, infections with this EU-type of PRRSV, only, do not lead to respiratory disease, but can induce a change in lung immune cell composition. In recent experiments we have been able to show, that the experimental infection had an effect on cellular immunity parameters and also induced mild, interstitial, inflammatory lung alterations, which can be predisposing for pneumonia. In the field situation, it has been shown that PRRSV predisposes for respiratory disease in pigs and pneumonia is correlated with co-infections with bacterial and viral pathogens. Fablet et al. (2011) have shown that PRRSV and *Actinobacillus pleuropneumoniae (A.pp)* serotype 2 are significantly associated with pneumonia and pleuritis. In experimental studies *A.pp* serotypes strongly differ in virulence depending on the expression of APX toxins, but although *A.pp* serotype 2 belongs to a mild virulent serotype, this serotype is currently the most diagnosed *A.pp* serotype in western European swine herds.

To be able to study predisposing and aggravating pathogenesis mechanisms an experimental co-infection study with PRRSV genotype 1, subtype 1 (Lelystad strain) followed by a *A.pp* serotype 2 infection was performed. In this study, pigs were allocated to three groups (group 1:PRRSV/*A.pp* 2; group 2: PBS/*A.pp*2; group3: PBS/PBS). Pigs of group 1 were infected with PRRSV by intranasal inoculation on day -7 and pigs of group 1 and 2 exposed to an aerosol during 20 minutes in an aerosol chamber. Aerosols were generated by an Aeroneb™Pro micropump nebulizer and 5 ml of the inoculum containing 1 x 10^9 cfu/ml was placed in the nebulizer vessel and dispersed by an airstream of oxygen. Pigs were followed clinically for 7 days after *A.pp* challenge and changes in haematology and leucocyte populations were addressed and pathological and microbiological examinations performed at the end of the study. Infection with PRRSV resulted in a significantly more cases with pneumonia than *A.pp* infection alone (Group 1: 6/10 x pneumonia; group 2: 1/10 x pneumonia, group 3: 0/6 x pneumonia). Strikingly all PRRSV infected pigs developed febrile body temperatures 4 hours after *A.pp* infection, whereas pigs infected with *A.pp* alone did not show increased body temperatures. From this results, we conclude, that PRRSV predisposes for an aggravation of a by nature mild A. pp infection. The observed early raise in body temperature might be associated with an earlier reported increase in CD14 expressing cells in the lungs after PRRSV infection and explain an increased susceptibility for gram-negative infections in the lungs of PRRSV infected pigs.

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Identification of differentially expressed proteins in porcine alveolar macrophages infected with virulent/attenuated strains of porcine reproductive and respiratory syndrome virus

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Introduction

The highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) is still a serious threat to the swine industry. However, the pathogenic mechanism of virulent/attenuated PRRSV strains remains unclear. In the study, we infected host porcine alveolar macrophages (PAMs) with the virulent HuN4 strain and the attenuated HuN4-F112 strain and then utilized fluorescent two-dimensional difference gel electrophoresis (2D-DIGE) and mass spectrometry (MS) to screen for intracellular proteins that were differentially expressed in host cells. Analyze the relation between differentially expressed proteins and pathogenicity, which provides important information to understand the mechanism of HP-PRRSV replication and pathogenesis.

Materials and Methods

Virulent strain HuN4 and attenuated vaccine strain HuN4-F112; Newborn piglets for preparation of PAMs. PAMs were inoculated with either the HuN4 strain or the HuN4-F112 strain and collected the total protein of PAMs at 48hpi. Utilize 2D-DIGE to screen for differentially expressed proteins in host cells infected with the two strains and identify the differentially expressed proteins by MS. Gene Ontology (GO) annotation of the identified proteins was performed with Blast2GO and protein-protein interaction network was built with String software.

Results

1. A total of 1492 Protein spots were resolved in a single master gel image and Image analysis revealed 153 differentially expressed cellular protein spots between two strains. We picked and analyzed the 153 spots, of which 27 spots were successfully identified to 23 cellular proteins by mass spectrometry.
2. GO annotation was performed for the 23 proteins, which were related to the biological processes of virus binding, signal transduction, cell adhesion, etc.. The protein-protein interaction network was also constructed and PKM2 was located in the most center of the network.
3. The differential expression of PKM2, HSPB1, and PSMA6 in PAMs infected with either HuN4 strain or HuN4-F112 strain was further identified by Real-time PCR analysis.

Conclusions

We revealed 153 differentially expressed cellular protein spots and identified 23 differentially expressed cellular proteins between PAMs infected with either the virulent HuN4 strain or the attenuated HuN4-F112 vaccine strain, which were correlated with the degree of infectivity of the virulent/attenuated strains in the target PAM cells and suggested that these differentially expressed proteins are involved in the pathogenic mechanisms of PRRSV strains with different pathogenicity.

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Porcine SAMHD1 blocks HP-PRRSV replication in Marc-145 cells

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Introduction

A highly pathogenic PRRSV named as HP-PRRSV, emerged in China in 2006 and posed disastrous losses to the swine production and the farmers. At present, HP-PRRSV is a major concern for the veterinary worldwide and is sustaining evolution. Most recently, Sterile alpha motif and HD domain 1 (SAMHD1) is identified as a restriction factor that blocks HIV-1 replication in myeloid-lineage cells. However, the biological characteristic of SAMHD1 is largely unknown. Are there other viruses interacted with SAMHD1 except HIV-1? To investigate the impact of SAMHD1 on the replication of PRRSV, we cloned complete gene sequence and CDS of porcine SAMHD1 by RACE. Expression vector pFLAG-pSAMHD1 was constructed and which was heterologously expressed in Marc-145 cells. Then the cells were infected with HP-PRRSV. The results demonstrated that porcine SAMHD1 expressed in Marc-145 cells significantly inhibited proliferation of HP-PRRSV.

Materials and Methods

A stock of highly pathogenic PRRSV HuN4 at passage 5 was prepared and titrated. The total RNA of peripheral blood lymphocytes (PBMCs) and PAMs was extracted from a 4-week-old piglet free of PRRSV. The yield and purity of the extracted RNA was confirmed.

RACE PCR was performed using a SMARTer™ RACE cDNA amplification kit from PBMCs cDNA. The 5’ and 3’ PCR amplification products were sequenced. The open reading frame of SAMHD1 gene was analyzed by sequence alignment.

The CDS of SAMHD1 was amplified from cDNA of porcine alveolar macrophages (PAMs) using specific primers containing specific restriction sites. The PCR product was cloned into p3×FLAG CMV 7.1 vector to construct the expression vector pFLAG- pSAMHD1. Recombinant plasmid was transfected into Marc-145 cells. Empty p3×FLAG CMV 7.1 vector and transfection reagent were used as control. After 48h transfection, cells were infected with 0.1 MOI of HP-PRRSV. After 24h post infection, cell cultural supernatant was collected and used to viral titration. Marc-145 cells were washed with phosphate-buffered saline (PBS, pH 7.4) and then collected for western blot analysis. The expression of the target protein was confirmed by FLAG tag antibody.

Results

1. The full-length gene sequence of porcine SAMHD1 is 3981 nucleotides containing a ORF, including the 3' poly(A) tail. The ORF is 1884 nucleotides between nucleotide positions 68 and 1951, encoded 627aa.

2. The western blotting results indicated that recombinant protein of porcine SAMHD1 expressed in Marc-145 cells was about 72kDa, similar to the natural state. HP-PRRSV replication was efficiently inhibited in the cells transfected with the pFLAG- pSAMHD1, confirmed by monoclonal antibody of PRRSV N protein. The virus titer in cell cultural supernatant was about 1.5-log-unit reduction.

Conclusions

Here, the full-length of porcine SAMHD1 gene was first cloned. Virus titer and western blot assays indicated that HP-PRRSV replication was efficiently blocked in porcine SAMHD1 heterologously expressed cells.

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Reduction of PRRSV infection in Marc-145 cells that express the exogenous C-terminal domain of non-muscle myosin heavy chain II-A

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Objective: We have identified, by a monoclonal anti-idiotypic antibody (Mab2-5G2) which functionally mimics PRRSV GP5 protein, non-muscle myosin heavy chain II-A (NMHC II-A) as a novel cellular receptor on Marc-145 cells and porcine alveolar macrophages (PAM) for PRRSV. The functional domain in NMHC II-A has been identified at its C-terminus and the corresponding recombinant protein (designated PRA) was produced. The objective of this study was to investigate the role of PRA in PRRSV infection in Marc-145 cells stably expressing exogenous PRA gene (GenBank No. HM490008).
Methods: To construct the PiggyBac (PB) donor plasmid containing PRA gene, PRA gene was amplified by RT-PCR from Marc-145 cells and cloned into PB donor vector via restriction enzyme sites XbaI and NotI. Based on the restriction digestion and sequencing, recombinant plasmid carrying PRA gene with an HA tag at its N-terminus was selected and named pB-CMV-PRA-EF1α-GFP-Puro. Marc-145 cells were co-transfected with pB-CMV-PRA-EF1α-GFP-Puro plasmid and PB helper vector plasmid and subjected for single cell cloning with puromycin. The presence of PRA gene and its expression in Marc-145 cells were detected by RT-PCR, indirect immunofluorescence assay (IFA) and Western blot with mAbs to HA and PRA and Marc-145 cell stably expressing PRA was named Marc-145-PRA. To determine the effect of PRA on PRRSV infection of Marc-145 cells, Marc-145-PRA cells were incubated with HP-PRRSV SD16 strain at 0.01 MOI 1 h at 4℃ for the detection of virus attachment or 1 h at 37℃ for virus internalization by using mAb to PRRSV N protein, FITC-goat anti-mouse IgG and confocal laser scanning microscopy. HP-PRRSV SD16 RNA was extracted from these cells and N gene was detected by qRT-PCR.
Results and discussion: PRA gene was detected in Marc-145-PRA cells by RT-PCR, indicating that the exogenous PRA gene was integrated in host genome and transcribed. PRA protein with MW of approximately 45-kDa was expressed in the cells as indicated by the specific fluorescent signals and binding with mAbs to HA and PRA in Western blot. HP-PRRSV attached to and internalized in Marc-145-PRA cells were much less than that in Marc-145 cells as observed by immunofluorescence, indicating that Marc-145-PRA cells could inhibit HP-PRRSV attachment and internalization. The amount of virus attached to Marc-145-PRA cells was decreased by 66% and that internalized by 78% by qRT-PCR in comparison to that of Marc-145 cells.
Conclusion: Marc-145-PRA cells expressing C-terminus region gene of NMHC II-A by PB transposon system were established. The ability of HP-PRRSV attachment to and internalization in these cells were significantly reduced suggesting that C-terminus region gene of NMHC II-A play important roles in HP-PRRSV infection.
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Experimental infection and comparative genomic analysis of a highly pathogenic PRRSV-HBR strain at different passage levels

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Background: In China, PRRS is first recognized in 1996 and has become one of the most severe diseases affect swine production. Moreover, an outbreak of highly pathogenic PRRS (HP-PRRS), a highly virulent form of PRRS whose principal clinical sign is high fever and high mortality in pigs of all ages, which occurs in 2006, inflicts severe damage on the Chinese pork industry. Highly pathogenic PRRSV (HP-PRRSV) is identified as the main pathogen of HP-PRRS.
Methods: A highly pathogenic strain of porcine reproductive and respiratory syndrome virus (PRRSV-HBR) was passaged on Marc-145 cells for 125 passages. In order to elucidate the change in virulence of PRRSV-HBR strain during the process of passage in vitro, swine infection experiment was performed with the viruses of low (F5 and F10) and high passage (F125). In addition, to identify the mutations related to the change in virulence of PRRSV-HBR strain, we compared and analyzed the genomic sequences of the F5, F10 and F125 of the strain.
Results: The virulence of F125 was significantly lower than that of F5 in the virus-infected pigs. In comparison with F5 and F125, there were 45 amino acid (aa) mutations and a deletion of 2 continuous aa by means of the virus genome sequence analysis. For these mutations, 33 aa (73.3%) occurred in the viral nonstructural proteins and the other 12 aa (26.7%) were contained in the viral structural proteins. Of the mutations, only 15 aa (33.3%) appeared in F10 and 30 aa (66.7%) occurred during passage from F10 to F125.
Conclusion: The latter 30 aa mutations were probably associated with attenuation of PRRSV-HBR strain, and that the change in virulence of the virus was determined by multiple alterations both in the structural and nonstructural genes. The virulence of PRRSV-HBR strain was remarkably attenuated after serial passages, and it can be used as vaccine candidate for control of the PRRS.

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Synergistic effects of sequential infection with highly pathogenic porcine reproductive and respiratory syndrome virus and porcine circovirus type 2 in piglets

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Background: Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of porcine reproductive and respiratory syndrome (PRRS) and porcine circovirus type 2 (PCV2) is associated with post-weaning multisystemic wasting syndrome (PMWS) in piglets. Recently, coinfection with highly pathogenic PRRSV (HP-PRRSV) and PCV2 in the field has become global. As previously reported, there is a synergistic pathogenicity in PRRSV and PCV2 coinfection; however, the consequences of sequential infection of pigs with these two viruses have remained unknown.

Methods: Thirty 35-day-old piglets were randomly divided into six groups (n = 5 each): HP-PRRSV/PCV2 (group 1, inoculated with HP-PRRSV then inoculated with PCV2 1 week later), PCV2/HP-PRRSV (group 2, inoculated with PCV2 then inoculated with HP-PRRSV 1 week later), HP-PRRSV+PCV2 (group 3, inoculated with HP-PRRSV and PCV2 concurrently, HP-PRRSV (group 4, inoculated with HP-PRRSV), PCV2 (group 5, inoculated with PCV2), and control (group 6, uninfected). Clinical symptoms and rectal temperatures were recorded on each day post-inoculation, body weight was recorded weekly, and serum samples were obtained for viral nucleic acid quantification and antibody titration. Additionally, variations in CD3+, CD4+ CD8-, CD3+, CD4-, and CD8+ cells, γδ T cells, natural killer (NK) cells, granulocytes, and mononuclear cells were determined by flow cytometry. The serum concentrations of interferon γ (IFN-γ), tumor necrosis factor α (TNF-α), interleukin 10 (IL-10), and macrophage granulocyte colony stimulating factor (GM-CSF) were determined. Pathological changes were observed in different tissues from the experimentally infected pigs.

Results: Piglets in group 1 had the highest viral loads, the lowest antibody titers, and the most severe clinical signs and highest mortality (3/5, 60%), and interstitial pneumonia was more severe in this group than in the others infected with HP-PRRSV. The levels of IFN-γ, TNF-α, IL-10, and GM-CSF in serum varied (increased or decreased) most widely in group 1, as well as each immunocyte subgroup.

Conclusions: HP-PRRSV infection followed by PCV2 infection enhanced replication of both viruses in the experimental piglets and led to more severe clinical signs and lesions, indicating a more effective synergistic effect during sequential infection of piglets with HP-PRRSV earlier than PCV 2.

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Swine immunoglobulin lambda-like polypeptide 5, an adaptor of RIG-1 and MDA5, regulates type I Interferon production and PRRSV replication through interaction with PRRSV nonstructural proteins

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Porcine reproductive and respiratory syndrome virus (PRRSV) infection appears to elicit a week innate immune response. Several nonstructural proteins (NSPs) encoded by PRRSV genome antagonize type I interferon (IFN) system in PRRSV-infected PAMs. However, the nonstructural proteins-interacting partners and their influence on the outcome of antivirus infection are poorly defined.In this study, yeast two-hybrid was used to screen PAM cDNA library to identify the binding protein(s) of nonstructural proteins encoded by PRRSV genome. Swine immunoglobulin lambda-like peptide 5 (sIGLL5), was identified as a novel interacting partner of NSP4 and NSP11. sIGLL5 interacts and colocalizes with NSP4 and NSP11 in cytoplasm was confirmed by using Glutathione S-transferase (GST) pulldown, coimmunoprecipitation and immunofluorescence microscopy assay. Silencing of sIGLL5 in PAM and Marc-145 cells with small interfering RNAs suppressed PRRSV growth and replication, whereas overexpression of sIGLL5 promoted PRRSV growth and replication. Interestingly, sIGLL5 was also identified as a novel adaptor of swine RIG-I (sRIG-I) and swine MDA5 (sMDA5). sIGLL5 interacted with sRIG-I and sMDA5 and overexpression of sIGLL5 inhibited sRIG-I and sMDA5-induced type I interferon production. Upon Sendai and dsRNA stimulation, IFN-ß production was shown to significantly decrease with increasing levels of sIGLL5 expression. In addition, sIGLL5 significantly interfered dsRNA-induced phosphorylation and nuclear translocation of IRF3 in vitro. Overall, our results suggest that the interaction of nonstructural proteins and sIGLL5 promotes PRRSV growth and genomic replication probably by suppressing IFN signaling. sIGLL5 represents a novel binding partner by PRRSV nonstructural proteins. This study reports for the first time the novel role of sIGLL5 in innate immunity and in PRRSV replication.

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Heme oxygenase 1 inhibition of HP-PRRSV infection in Marc-145 cell lines

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Introduction/Objective: Porcine reproductive and respiratory syndrome(PRRS) is one of the most economically important swine viral infectious diseases affecting swine industry worldwide. However, the mechanisms of anti-PRRSV infection of host are still poorly understood. Our recent studies have demonstrated that pig infected with highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) showed the significant higher expression levels of heme oxygenase 1(HO-1), which indicated that HO-1 may play important roles in host responses against HP-PRRSV infection. However, the role of HO-1 in the HP-PRRSV infection is unknown. This study demonstrated for the first time, that HO-1 inhibited PRRSV infection of Marc-145 cells.

Material and Methods: SD-16, a HP-PRRSV strain was isolated and stored in our laboratory. The cDNA clone stably expressing enhanced green fluorescent protein, rHP-PRRSV/EGFP was constructed. HO-1 activators cobalt protophorphyrin (CoPP) was purchased from Sigma. The gene expression of HO-1 from Marc-145 cells and N from PRRSV were tested by employing real-time fluorescent quantitative PCR (qPCR) and western blot. The HP-PRRSV-positive cell ratio was tested by employing indirect immunofluorescence (IFA) and flow cytometry (FACS).

Results and Discussion: Results showed that infection with HP-PRRSV strain SD-16 significantly up-regulated the expression of HO-1 in Marc-145 cell lines indicating that HO-1 play an important role in HP-PRRSV infection. However, the role of HO-1 in the HP-PRRSV infection is unknown. However, previous in vitro studies have demonstrated that HO-1 and its activators inhibited replication of hepatitis C virus, human immunodeficiency virus, and hepatitis B virus, down-regulation of cellular protein HO-1 inhibits proliferation of classical swine fever virus in PK-15 cells, and HO-1 promotes murine plasmodium liver infection. These results showed that HO-1 plays different roles in the infection of hosts with various pathogens. What role of HO-1 plays in the HP-PRRSV infection? Our results showed that CoPP induced higher expression level of HO-1 which inhibited PRRSV infection of Marc-145 cells. It inhibited significantly HP-PRRSV replication and nucleocapsid (N) protein expression and reduced significantly the virus titer. By employing the cDNA clone rHP-PRRSV/EGFP, IFA and FACS, we found that HP-PRRSV-positive cells were reduced from 57.5% to 2.4% at 0 to 100 micromole CoPP, respectively.

Conclusion: HO-1 induced by CoPP inhibited PRRSV infection of Marc-145 cells. This finding may pave the way for the development of the novel approach for the prevention and control of PRRS.

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USP18 restricts PRRSV growth through alteration of nuclear translocation of NF-κB p65 and p50 in MARC-145 cells

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Although the functions of porcine respiratory and reproductive syndrome virus (PRRSV) proteins are increasingly understood, the roles of host factors in modifying infection are less well understood. Growing evidence places deubiquitylation at the core of a multitude of regulatory processes, ranging from cell growth to innate immune response and health, such as cancer, degenerative and infectious diseases. The ubiquitin-specific peptidase 18 encoded by the USP18 gene has been shown to be a negative regulator of type-I interferon signaling (IFN) in both murien and porcine systems. More recently genetic and biochemical evidence has suggested important roles for USP18 in immune function. The full length USP18 cDNA harboured a Cys-box and a His-box. The Cys-box has putative transmembrane helices and a conserved putative active-site cysteine at cys64, which is essential for the catalytic property of USP18. The mutant version of USP18 (USP18DN) lacks the Cys-box and is thus unlikely to retain any significant proteolytic activity as demonstrated previously. This report provides further information on the functional role of the porcine ubiquitin-specific peptidase 18 (USP18) during innate immune responses to PRRSV. USP18 and USP18DN cDNAs were PCR-amplified and cloned into the pLenti6V5-D- TOPO expression vector to generate the expression constructs USP18 and USP18DN tagged with V5-epitope. MARC-145 cells were transduced with lentivirus expressing USP18 or USP18DN under the control of a constitutive CMV promoter. The accumulation of transcripts encoded by the transgene(s) was measured by semi-quantitative RT-PCR. 104 recombinant MARC-145 cells were subjected to PRRSV infection with the MARC-145-adapted PRRSV Olot/91 strain at multiplicity of infection (moi) of 0.5. For PRRSV, experimental evidences suggest that the nucleocapsid N protein and nsp2 protein may be involved in NF-kappaB activation. Thus, we also tested if USP18 could affect the cellular translocation of NF-kappaB p65 and p50 monomers 24 hours post infection with PRRSV Olot/91 using immunolocalization and confocal microscopy. The results shown that constitutive overexpression of the porcine USP18 in MARC-145 cells restricts PRRSV growth, at least in part, via alteration of nuclear localization of important regulators of the innate immune response NF-kappaB p65 and p50 during viral infection. Our data highlight USP18 as a host restriction factor during innate immune response to PRRSV.

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Induction of ROS generation and NF-kappa B activation in MARC-145 cells by a novel Porcine Reproductive and Respiratory Syndrome virus isolated in the southwest of China

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Introduction: Viral infection is often accompanied by alteration of the intracellular redox state. Reactive oxygen species (ROS) have been shown to function as cellular signaling molecules, influencing a variety of molecular and biochemical processes, such as NF-kappa B. The aim of the present study was to investigate the interaction between reactive oxygen species (ROS) and NF-kappa B during PRRSV infection. **Methods:** In this study MARC-145 cells were infected with a novel PRRSV isolate from the Yunnan Provence in southwest China (YN-2011, GenBank accession no. JX 857698). Cells and supernatants from PRRSV infected and non-infected controls were collected at different time points after infection (12, 24, 36, 48, 60 hpi). Samples were assayed for ROS and Maleic Dialdehyde (MDA) level by Chemoluminescence, NF-kappa B protein expression by Western Blot, and I kappa B alpha mRNA transcription by Real-time RT-PCR. **Results:** Compared with control groups, a significant production of ROS was observed in PRRSV infected MARC-145 cells, peaking at 24 hpi. After 24 hpi, ROS levels had declined from the maximum level but were still elevated above those in untreated cells. Similarly, there was a remarkable time-dependent increase in MDA, a byproduct during the production of ROS. Accordingly, PRRSV infection led to accumulation of NF-kappa B protein in the nucleus, which was apparent at 48 hpi. In addition, degradation of I kappa B mRNA was detected at 36, 48 and 60 hpi. Overexpression of the dominant negative form of I kappa B alpha significantly suppressed NF-kappa B induced by PRRSV. **Discussion:** Interestingly, in contrast to the HP-PRRSV strain previously reported in China, the YN-2011 strain lacks the 30-aa deletion in Nsp2. However, YN-2011 possesses several new NSP2 aa substitutions, such as 110-T/I, 284-S/F, 344,345-EV/QL, 474-P/S, 490-T/A, 565-Y/H, 567-D/N, 599-H/R, 683-R/H, 767-M/K, and 925-F/L. The role of the new substitutions remains to be determined in the future. The results of this study indicate that the generation of ROS during PRRSV infection plays a role in up regulating NF-kappa B. The observation that PRRSV induced I kappa B alpha degradation suggests that ROS affects the expression of I kappa B alpha, which would prevent the formation of the NF-kappa B-I kappa B complex. The results of this study may contribute to a better understanding of the interaction between PRRSV and the cell from an oxidative stress point.

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Nonstructural proteins 4 and 10 of highly pathogenic PRRSV induce apoptosis in virus-infected MARC-145 cells

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Apoptosis has been considered as a critical component of innate immune responses and contributes to the anti-viral defense of the host cells by interfering the viral replication and exposing the virus to extracellular immune surveillance. Previous studies have shown that apoptosis could be induced during porcine reproductive and respiratory syndrome virus (PRRSV) infection both in vitro and in vivo, and glycoprotein 5 (GP5), a structural protein of PRRSV, was identified as an apoptosis inducer. In this study, the effect of nonstructural proteins of highly pathogenic (HP)-PRRSV on inducing apoptosis in MARC-145 cells was investigated. In order to analyze the induction of apoptosis, flowcytometric analysis, western blot assay and laser scanning confocal microscopy were applied. During early of PRRSV infection, chemical-stimuli induced apoptosis in MARC-145 cells was inhibited by PRRSV infection, and this inhibition was maximized at 6 h post-infection (pi), a time point that is consistent with the initiation of viral non-structural proteins (nsps) synthesis. Our results indicated that the activation of the apoptosis occurred at 24 h pi, and with a significant activation at 36 h pi, considering the release of cytochrome c, activity of caspase-3 and cleavage of PARP, suggesting the HP-PRRSV-induced apoptosis occurred at late of infection. Pathways related to the apoptosis activation were studied as well, and death receptor pathway was the first pathway for apoptosis activation and the death receptor-induced activation of the initiator caspase-8 could be detected as early as 16 h pi, while significant activation of caspase-8/-9/-12 appeared at 36 h pi which represent the activation of death receptor pathway, mitochondrial pathway and ER (endoplasmic reticulum) stress pathway, respectively. Meanwhile, apoptosis and degradation of procaspase-8 was detected in HP-PRRSV-infected pulmonary alveolar macrophages (PAMs) as well, which was earlier than HP-PRRSV-infected MARC-145 cells. Nonstructural proteins involving in the apoptosis were screened, and found that two proteins, nsp4 and nsp10, were capable of inducing the apoptosis in MARC-145 cells dramatically. Nsp10 could initiate the apoptosis by inducing degradation of procaspase-8 and promoting the expression of the pro-apoptotic protein, Bid protein, which contributes to the following activation of caspase-9 and the cleavage of PARP. Our data indicate that the nsp4 and nsp10 of HP-PRRSV participates in the regulation of apoptosis in a two-phase model and the nsp10 plays an important role in the apoptosis induction by PRRSV. However, the effect of the apoptosis induced by the nsps of PRRSV on the viral pathogenicity needs to be further explored.

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Porcine reproductive and respiratory syndrome virus activates inflammasomes of porcine alveolar macrophages via its small envelope protein E

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Porcine reproductive and respiratory syndrome virus (PRRSV) infection results in extensive tissue inflammation and damage, which are believed to be responsible for increased susceptibility to secondary infection and even for death. However, its pathogenic mechanisms are not fully understood. To explore the mechanism underlying the PRRSV-induced tissue inflammation and damage, we investigated whether PRRSV activates porcine alveolar macrophage (PAM) inflammasomes which mediate por-IL-1β maturation/release and subsequently induce tissue inflammation and injury. Our results showed that PRRSV and its small envelope protein E significantly increased IL-1β release from LPS-primed macrophages, however, only PRRSV not protein E significantly increased IL-1β release from no-LPS-primed PAMs, which indicates that PRRSV can activate inflammasomes of PAMs by its encoded protein E. These results provide a molecular basis for the pathogenic mechanism of PRRSV on inducing extensive tissue inflammation and damage, and suggest that the inflammasome may provide a potential therapeutic target for PRRS prevention and treatment.

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Non-muscle myosin heavy chain II-A is a novel cellular receptor for PRRSV

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Objective: Previously, we generated a monoclonal anti-idiotypic antibody (designated Mab2-5G2) that is specific for the syngenic mouse antibodies against PRRSV GP5, binds Marc-145 cells and porcine alveolar macrophages (PAM) and immunoprecipitated a 230kDa soluble protein from these cells. Based on the amino acid sequence by MS, the 230kDa protein was identified as non-muscle myosin heavy chain II-A (NMHC II-A) with the functional region located in its carboxyl terminus. The objective of this study was to determine the association of PRRSV GP5 with NMHC II-A.
Methods: The potential interaction between PRRSV GP5 and NMHC II-A was detected by expression of GP5-Flag in Marc-145, PK-15 and COS-7 cells. It was examined by confocal microscope and co-immunoprecipitated using anti-Flag antibody. The truncated recombinant protein (designated PRA) from the C-terminus domain of NMHC II-A was produced and examined for its binding to PK-15-GP5 cells to determine the interaction between GP5 and NMHC II-A on the cell surface. PRA expressing Marc-145 cells and 7 synthetic peptides in PRA region were used to examine the role of PRA in PRRSV infection Marc-145 cells with HP-PRRSV SD16 strain expressing a GFP marker (HP-PRRSV-GFP). To determine the role of endogenous NMHC II-A in PRRSV infection, polyclonal antisera against NMHC II-A were produced and used to inhibit the infection of Marc-145 with PRRSV-GFP. Marc-145 cells stably expressing artificial microRNA against NMHC-IIA were generated and PRRSV infection of these cells was examined. Furthermore blebbistatin that specifically binds to NMHC II-A but not inhibit cytogenesis of the cell was used to block PRRSV infection of Marc-145 cells.
Results and Discussion: The Co-IP results clearly showed that NMHC II-A was co-precipitated with GP5-FLAG from Marc-145 and PK-15 cells, but not from COS-7 cells (naturally lacking of NMHC II-A). NMHC II-A co-localized with GP5 in the cells as determined by confocal microscope. PRA protein interacted with GP5 since it bound PK-15 cells transfected with GP5 expression plasmid, but did not bind that transfected with GP4 expression plasmid. HP-PRRSV infection of Marc-145 cells expressing PRA was significantly decreased indicating that NMHC II-A C-terminus region protein plays important role in HP-PRRSV infection. Three of 7 peptides, antisera to NMHC II-A and blebbistatin blocked HP-PRRSV infection of Marc-145 cells in a dose dependent manner. Knockdown of endogenous NMHC II-A expression with amiRNA decreased the PRRSV infection.
Conclusion: In summary, the results presented in this report provide solid evidence that NMHC II-A functions as a novel entry cellular receptor for PRRSV by binding to GP5 protein.
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