

Technical University of Denmark



Clinical, Pathological and Immunological Aspects of Transplacental PRRS Virus Infection

Results from Danish Experiments

Nielsen, Jens

Published in:
Slovenian Veterinary Research

Publication date:
2011

[Link back to DTU Orbit](#)

Citation (APA):
Nielsen, J. (2011). Clinical, Pathological and Immunological Aspects of Transplacental PRRS Virus Infection: Results from Danish Experiments. *Slovenian Veterinary Research*, 48(Suppl. 13), 171-177.

DTU Library

Technical Information Center of Denmark

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

**Clinical, pathological and immunological aspects of transplacental PRRS virus infection
– results from Danish experiments**

Jens Nielsen

Address of author:

Jens Nielsen, Technical University of Denmark, National Veterinary Institute, Division of
Virology, Lindholm, DK-4771 Kalvehave, Denmark

Corresponding author:

Jens Nielsen, Technical University of Denmark, National Veterinary Institute, Division of
Virology, Lindholm, DK-4771 Kalvehave, Denmark

E-mail: jeni@vet.dtu.dk

Phone: +45 35887836

Summary

The present paper describes Danish research activities on porcine reproductive and respiratory syndrome (PRRS) with emphasis on experimental infections in pregnant swine. The first case of PRRS was diagnosed in Denmark in 1992 and subsequently the disease spread to most other parts of the country. The first animal experiments elucidated the pathogenicity of Danish PRRS virus (PRRSV) isolates in pregnant sows together with the effects of infection at various stages of gestation. In 1996, the introduction of a vaccination program using an attenuated live PRRS vaccine led to an epidemic of American type PRRSV in the previously unaffected Danish pig population. Acute PRRS like disease was observed in non-vaccinated as well as in vaccinated herds, and it was demonstrated that the vaccine strain had reverted to virulence. By experimental infection of late term pregnant sows, we demonstrated that a field isolate of PRRS vaccine-derived virus (VDV) could cause disease in swine consistent with PRRS, thus confirming the etiological role of VDV. Since the complex pathology following in utero infection with PRRSV indicates impairment of the immune system of congenitally infected pigs, we studied various aspect of the host defence in piglets surviving transplacental infection with PRRSV. Leukocyte subpopulations in peripheral blood and bronchoalveolar fluid (BALF) were modulated, viability of lung macrophages was reduced, phagocytosis against Salmonella in blood monocytes as well as oxidative burst capacity of alveolar macrophages was inhibited, there was an over-expression of cytokine IL-10 in BALF cells, and ciliary disruption in the airways was observed. Altogether, our findings supported the hypothesis of the existence of immunosuppression in piglets congenitally infected with PRRSV.

Keywords: PRRSV; pigs; Denmark; field isolate; vaccine-derived virus; animal experiments; intrauterine infection; immune modulation; leukocyte subpopulations; bronchial ciliar disruption

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a severe disease in pigs characterized by reproductive failure in sows and gilts, increased preweaning mortality and pneumonia in growing pigs. The syndrome was initially observed in the USA in 1987 (1). In 1990, similar clinical outbreaks were reported in Germany (2) with further spread throughout Europe in the following years (3,4). The causal agent of the disease is a positive-strand enveloped RNA virus (5) designated PRRS virus (PRRSV), which primarily replicates in macrophages. The clinical signs of PRRS vary widely. Already early after the appearance of the disease, these variations were suggested to be the result of a range of modifying factors, e.g. animal age, differences in genetic susceptibility, environmental factors including sanitary status of pigs at the time of infection, management, immune status, virus strain differences (6-9).

PRRSV isolates are divided into two main genotypes: European type PRRSV and North American type PRRSV, representing Type I and Type II, respectively (10,11). Initially, the European strains were only isolated in Europe and the American strains in the US and Canada but now they are mixed

During 2 decades, PRRS has constituted an economically significant viral disease in most major pig-producing areas throughout the world. In spite of the fact that tremendous efforts have been initiated to combat the disease, there is still a lack of crucial knowledge of disease mechanisms in PRRS, thus hampering the possibilities of effectively controlling this disease. Thus, more than 20 years after the appearance of the disease, PRRSV still continues to be an important threat for the pig industry.

In Denmark, the first case of PRRS was diagnosed in 1992 (12). Subsequently, the disease spread to most other parts of the country and became a major challenge to the Danish swine

population with considerable implications for animal welfare and economical sustainability. This paper will present some of the various research activities initiated at our institute to contribute to the international battle against the disease. However, since major efforts have related to PRRS in pregnant sows, this review will primarily describe work on experimental transplacental infections.

Animal experiments with PRRSV –European type

The appearance of PRRS in Germany and in the Netherlands in the winter of 1990 - 1991 and the further spread through Western Europe during 1991 made clear the risk of introduction of the disease to Denmark. The identification of the etiologic agent as PRRSV paved the way for the development of diagnostic procedures to confirm the disease. By the end of 1991, virological and serological analyses had been established in our laboratory. In March 1992, the first case of PRRS in Denmark was diagnosed by the detection of specific antibodies against PRRSV in serum samples from a sow herd in the Southern part of Denmark, close to the German border. Subsequently, European type PRRSV with only minor antigenic differences to a Dutch isolate, Lelystad Virus (5), was isolated from a sow farrow to finish herd with clinical signs consistent with PRRS. In order to test the pathogenicity of the isolate, we carried out an animal experiment in pregnant dams (12). Transplacental infection was demonstrated but neither significant reproductive disorders nor marked clinical signs were observed. This was in contrast to the typical signs of PRRS demonstrated in previous studies (13-16). The result of our experiment was speculated to reflect that this early isolated Danish strain was of a lower virulence. However, substantial evidence of the variety of factors affecting the clinical outcome of PRRSV infection leaves the explanation open.

Early observations revealed that exposure of pregnant sows and gilts to PRRSV in the last trimester typically results in reproductive failure and increased preweaning mortality under

field as well as experimental conditions (5,14-16). Only few studies focused on the reproduction of the disease at earlier stages of gestation (17-20) and they did not elucidate putative consequences of intrauterine infection on perinatal disease and mortality of pigs. Therefore, we initiated an experiment to study the effect of PRRSV infection at various stages of gestation (21). The demonstration of transplacental infection of PRRSV in 6 out of 8 litters inoculated on day 72 of gestation or later but not at earlier stages of gestation supported the hypothesis that PRRSV infections late in pregnancy had the greatest likelihood of transplacental infection of fetuses, as previously described (5,14-16). Pronounced reproductive disorders with high numbers of stillborn pigs together with high piglet mortality during an observation period of 2 weeks were observed for dams inoculated around 85 days of gestation and - although less pronounced - for dams inoculated at 72 day of gestation. The above findings together with the histopathological demonstration of PRRS typical focal non-suppurative inflammatory conditions in the lung occasionally associated with hyperplastic lymphadenopathy (7,20) provided a valuable contribution to the knowledge of the importance of stage of pregnancy for the outcome of infection.

Problems associated with the introduction of vaccination of Danish pigs with an attenuated live PRRS vaccine

Around 1995, the herd prevalence of PRRS in finishing pigs in Denmark was 33% (22). In order to prevent or at least reduce further spread of the disease in the Danish pig population, a voluntary PRRS control program was started early 1996. One of the objectives of the program was to define the PRRS serological status of all swine herds followed by vaccination of 3- to 18-week-old piglets in seropositive herds using the attenuated live “Ingelvac[®] PRRS MLV” vaccine (Boehringer Ingelheim Animal Health, MO, USA). Furthermore, all boars entering the artificial insemination centres had been vaccinated with the vaccine since October 1995. Based on the examination of more than 2000 sow sera originating from all

regions in Denmark, the serological screening, using the immunoperoxidase monolayer assay (IPMA) carried out as a double test with a Danish strain of PRRSV and the PRRS vaccine virus (23), did not indicate the occurrence of vaccine/American PRRSV strains in Denmark at that time (24). The PRRS vaccination was carried out almost simultaneously in more than 1000 herds, many of which had no clinical symptoms and displayed a serological herd profile indicating that PRRSV did not circulate among sows and nursing piglets.

Two to 3 months after the implementation of the vaccination program, acute PRRS-like problems continuously developed in about 500 herds (25). At the same time, a sudden increase in the number of samples (fetuses, weakborn and stillborn piglets) submitted to our laboratory for PRRS examination was observed. In the vast majority of the herds, the appearance of the clinical problems coincided with the recent vaccination using “Ingelvac® PRRS MLV”, which is based on the pathogenic American PRRSV field isolate VR-2332 (26). American type PRRSV, identified as vaccine-derived virus (VDV) by the use of monoclonal antibodies, RT-PCR and nucleotide sequencing, was isolated from more than 100 of the herds (25). This was the first time American type PRRSV was isolated from clinical cases in Denmark, and the outbreak of acute PRRS was epidemiologically closely linked to the use of the live vaccine (27,28), and linked to reversion of the vaccine virus to a pathogenic phenotype (25,29), which again was proven to be a direct derivative of the live attenuated vaccine virus (30). In order to confirm the etiological role of PRRSV-VDV for the clinical outbreaks, we studied the pathogenicity of one of the field isolates by experimental infection of late term pregnant sows (31).

Intranasal inoculation of PRRS-naïve sows with the PRRSV-VDV isolate in the last trimester resulted in congenital infection, fetal death and increased preweaning mortality. The reproductive signs and losses, the gross lesions in the fetuses, and the generally unhealthy appearance of many of the live-born pigs were comparable with previous observations, and

the interstitial pneumonia demonstrated in the vast majority of the infected piglets were compatible with lesions typical for PRRS (7,13-16,20,21,32). As such, the present study showed that vaccine-derived PRRSV can cause disease in swine consistent with PRRS.

The attenuated vaccine was widely used in a number of other countries, apparently without displaying any problems. Since, at that time, PRRS vaccine problems were only reported to occur in Denmark, parts of the scientific community initially considered the interpretation of our results as controversial. As an explanation of the Danish situation, however, we suggested that a number of unique circumstances existed regarding the use of the live vaccine in Denmark, e.g. the use of serologic tests which enabled distinction between infection with European and American strains of PRRSV (31). Altogether, the situation in Denmark made it very simple to observe vaccine reversion, which might go unnoticed in other countries, and later studies have also supported vaccine reversion as the cause of clinical problems (33-35).

In utero infection with PRRSV affects immune functions of surviving piglets

It is well known that piglets congenitally infected with PRRSV can be viremic at birth (12,21,31), and that preweaning mortality due to secondary infections often increases during outbreaks of PRRS (36-38). An underlying mechanism for increased susceptibility to other pathogens following PRRSV infection could be suppression of the immune system in infected pigs as a result of interaction between PRRSV and the immune system. The majority of studies dealing with the influence of PRRSV on immune functions of the pig were carried out either in vitro or in vivo using young pigs infected at various ages (39) and the experimental evidence for any interaction of PRRSV with other pathogens was ambiguous. Thus, we infected more than 100 young piglets with European type PRRSV without seeing neither clinical signs nor significant immunological changes (40), - and this observation challenged our view of angle.

In 2001, Feng et al. (41) suggested an immunosuppressive effect of in utero infection with PRRSV. Since the hypothesis was supported by our own observations (31), we carried out a number of experiments to elucidate the interaction of PRRSV with various immune mechanisms in piglets surviving transplacental infection (39,42-45). After inoculation with PRRSV on day 90 of gestation, two sows delivered 23 liveborn, congenitally infected pigs, which were examined over a period of 6 weeks after birth (39). The significantly increased postnatal levels of cells with CD2, CD3 and CD8 phenotypes in peripheral blood and broncho-alveolar lavage fluid (BALF) indicated that in utero infection causes a long lasting activation of the immune system. Furthermore, our results supported the hypothesis that CD8⁺ cells play a role in the control of PRRSV replication (46,47,48), as also indicated by the detection of cytotoxic T lymphocytes close to PRRSV infected macrophages in the lungs of infected pigs (45). The cytokine profiles for peripheral blood mononuclear cells and lymph node cells showing increased levels of IL-4, TNF- α and IFN γ in infected pigs up to 6 weeks after birth (42) are believed to reflect a general lymphocyte activation stage supporting the assumption of a prolonged activation of immune mechanisms after in utero infection, as also suggested above. The detection of PRRSV in BALF cells (especially macrophages) of infected pigs for a period up to 6 weeks after birth, when virus could no longer be detected in blood (39), indicated functional alterations of these cells. In addition, the occurrence of immunomodulatory events could also be confirmed by the detection of increased levels of cytokine (TNF- α , IL-10, IL-12, IL-8 and IFN γ) expression in BALF cells obtained from 2- and 4-week-old infected piglets (43). As also later demonstrated in a substantial number of reports (49-51), our study suggested an important role for a number of cytokines, e.g. TNF- α , IL-10, and IFN γ , in the immune response to PRRSV. However, further studies are required to enlighten the complicated cytokine puzzle during PRRSV infection.

None of the findings described above provided unequivocal support of the existence of immunosuppression after in utero infection. However, it has been observed that pigs surviving congenital PRRSV infection can exhibit the severest form of respiratory disease with mortality sometimes reaching 100% within 3 weeks after birth (41,52,53). The complex pathology following in utero infection with PRRSV is described to represent a unique form of the disease referred to as congenital PRRS (54). This is in accordance with our findings that *i*) intrauterine infection with PRRSV resulted in high preweaning mortality of liveborn piglets associated with severe pathological lesions characterized by varying levels of pneumonia, including severe pleuritis and necrotizing pneumonia, pericarditis, enteritis and multiple abscesses (31), and that *ii*) pigs congenitally infected with PRRSV were significantly more susceptible to infection with *Salmonella Typhimurium* at 4 weeks of age than non-infected control pigs (55). Altogether, these observations indicated that the hypothesis of the existence of PRRSV induced suppression of the host immune defence in pigs congenitally infected with PRRSV should be sustained. This was also supported by our demonstration of decreased phagocytic activity (56) and reduction (57) of the total number of alveolar lung macrophages after PRRSV infection in vivo and in vitro, respectively, reduced viability of lung macrophages in 2-week-old congenitally infected piglets (39), inhibition of phagocytosis against *Salmonella* in blood monocytes as well as oxidative burst capacity of alveolar macrophages (44), over-expression of the immunosuppressive cytokine IL-10 in BALF cells (43), and ciliary disruption in the bronchi (Figure 1) after intrauterine infection with PRRSV. Altogether, these findings indicate impairment of the host defence in congenitally PRRSV infected pigs. In particular, the reduced capacity of alveolar lung macrophages to ingest and kill bacteria combined with the hampered mucociliary clearance mechanisms, caused by the disruption of cilia in the airways, is likely to play an important role in the pathogenesis of respiratory disease in pigs infected in utero.

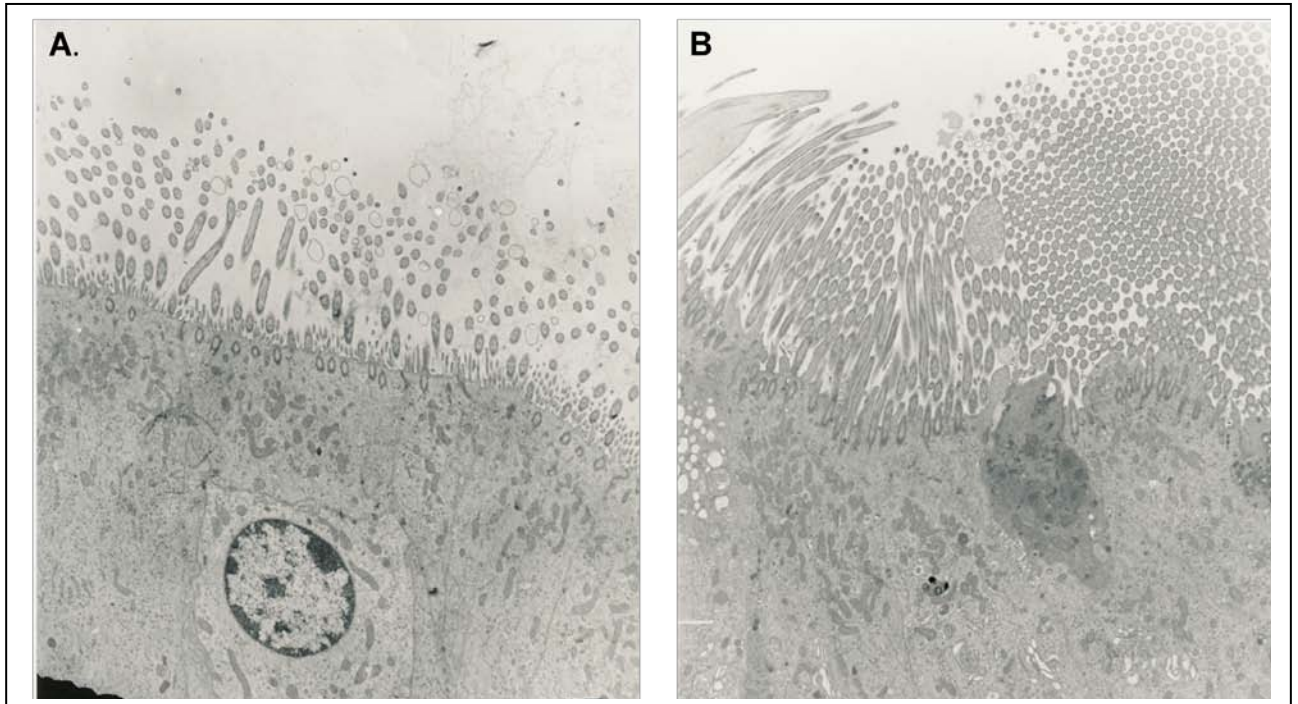


Figure 1: Destruction and loss of bronchial cilia in a pig congenitally infected with PRRSV (A). Normal structure and numbers of cilia are present in an age-matched non-infected control pig (B). Electron microscopic examination.

References

1. Keffaber, K. K., 1989. Reproductive failure of unknown etiology. *Am. Assoc. Swine Pract. News*, 1989 1: 1-10.
2. Leyk, W., 1991. 3-case studies of affected herds. In: EEC Seminar reports on the “new” pig disease: Porcine Reproductive and Respiratory Syndrome (PRRS). April 1991, Brussels.
3. de Jong, M.F., Cromwijk, W. and Van ‘t Veld, P., 1991. The “new” pig disease: Epidemiology and production losses in The Netherlands. In: EEC Seminar reports on the “new” pig disease: Porcine Reproductive and Respiratory Syndrome (PRRS). November 1991, Brussels.
4. Baron, T., Albina, E., Leforban, Y. et al., 1992. Report on the first outbreaks of the porcine reproductive and respiratory syndrome (PRRS) in France. Diagnosis and viral isolation. *Ann. Rech. Vet.* 23: 161-166.
5. Wensvoort, G., Terpstra, C., Pol, J.M. et al., 1991. Mystery swine disease in The Netherlands: the isolation of Lelystad virus. *Vet Q.* 13:121–130
6. Robertson, I.B., 1992. Porcine reproductive and respiratory syndrome (blue-eared pig disease): some aspects of its epidemiology. In: Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, Edinburgh, UK, 1–3 April, pp. 24–38.
7. Halbur, P.G., Paul, P.S., Frey, M.L. et al., 1995. Comparison of the pathogenicity of two US porcine reproductive and respiratory syndrome virus isolates with that of the Lelystad virus. *Vet. Pathol.* 32: 648–660.
8. Polson, D.D., 1995. Assessing the cost of endemic reproductive and sucklet pig disease in the swine breeding herd. In: Proceedings of the Second International Symposium on

- Porcine Reproductive and Respiratory Syndrome (PRRS), Copenhagen, Denmark, 9–10 August, pp. 47–48.
9. Klinge, K.L., Vaughn, E.M., Roof, M.B., Bautista, E.M. and Murtaugh, M.P., 2009. Age-dependent resistance to porcine reproductive and respiratory syndrome virus replication in swine. *Virology Journal* 6: 177-187.
 10. Nelsen, C.J., Murtaugh, M.P., and Faaborg, K. S., 1999. Porcine reproductive and respiratory syndrome virus comparison: divergent evolution on two continents. *J. Virol.* 73: 270-280.
 11. Meng, X.J., 2000. Heterogeneity of porcine reproductive and respiratory syndrome virus: implications for current vaccine efficacy and future vaccine development. *Vet. Microbiol.* 7: 309–329.
 12. Bøtner, A. , Nielsen, J. and Bille-Hansen, V. 1994. Isolation of porcine reproductive and respiratory syndrome (PRRS) virus in a Danish swine herd and experimental infection of pregnant gilts with the virus. *Vet. Microbiol.*,40: 351-360.
 13. Terpstra, C., Wensvoort, G. and Pol, J.M.A., 1991. Experimental reproduction of porcine epidemic abortion and respiratory syndrome (mystery swine disease) by infection with Lelystad virus: Koch's postulates fulfilled. *Vet. Q.* 13: 131–136.
 14. Christianson, W.T., Collins, J.E., Benfield, D.A. et al., 1992. Experimental reproduction of swine infertility syndrome in pregnant sows. *Am. J. Vet. Res.* 53: 485–488.
 15. Plana, J., Vayreda, M., Villarrasa, J. et al., 1992. Porcine epidemic abortion and respiratory syndrome (mystery swine disease). Isolation in Spain of the causative agent and experimental reproduction of the disease. *Vet. Microbiol.* 33: 203-211.
 16. Collins, J.E., Benfield, D.A., Christianson, W.T. et al., 1992. Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) in North America and

- experimental reproduction of the disease in gnotobiotic pigs. *J. Vet. Diagn. Invest.* 4: 117–126.
17. Prieto, C., Sánchez, R., Martín-Rillo, S. et al., 1996. Exposure of gilts in early gestation to porcine reproductive and respiratory syndrome virus. *Vet. Rec.* 138: 536-539.
18. Christianson, W.T., Choi, C.S., Collins, J.E., Morrison, R.B. and Joo, H.S., 1993. Pathogenesis of porcine reproductive and respiratory syndrome virus infection in mid-gestation sows and fetuses. *Can. J. Vet. Res.* 57: 262–268.
19. Mengeling, W.L., Lager, K.M. and Vorwald, A.C., 1994. Temporal characterization of transplacental infection of porcine fetuses with porcine reproductive and respiratory syndrome virus. *Am. J. Vet. Res.* 55: 1391–1398.
20. Lager, K.M. and Halbur, P.G., 1996. Gross and microscopic lesions in porcine fetuses infected with porcine reproductive and respiratory syndrome virus. *J. Vet. Diagn. Invest.* 8: 275–282.
21. Kranker, S., Nielsen, J., Bille-Hansen, V. and Bøtner, A., 1998. Experimental inoculation of swine at various stages of gestation with a Danish isolate of porcine reproductive and respiratory syndrome virus (PRRSV). *Vet. Microbiol.* 61: 21-31.
22. Mortensen, S., Strandbygaard, B., 1996. PRRS spredes nu langsommere. *Veterinærinformation* 4: 35–36 (in Danish).
23. Sørensen, K.J., Strandbygaard, B., Bøtner, A., Madsen, E.S., Nielsen, J. and Have, P. 1998. Blocking ELISA's for the distinction between antibodies against European and American strains of porcine reproductive and respiratory syndrome (PRRS) virus. *Vet. Microbiol.* 60: 169-177.
24. Mortensen, S. and Bøtner, A., 1996. Ugeudsendelse til konsulenter. Landsudvalget for Svin, Copenhagen, January 1996 (in Danish).

25. Bøtner, A., Strandbygaard, B., Sørensen, K.J., Have, P., Madsen, K.G., Madsen, E., Smedegaard, Alexandersen, S., 1997. Appearance of acute PRRS-like symptoms in sow herds after vaccination with a modified live PRRS-vaccine. *Vet. Rec.* 141: 497–499.
26. Chladek, D.W., Gorczyza, D.E., Harris, L.L., 1995. US Patent 5 476 778. US Department of Commerce, Patent and Trademark Office, Washington, DC, December 19.
27. Bøtner, A., 1998. PRRS — the Danish experience. In: Proceedings of the XVIII Nordic Veterinary Congress, Helsinki, Finland, August 1998, pp. 122–125.
28. Mortensen, S., 2000. Herd testing and disease spread models in swine populations. Ph.D. Thesis. DSR Forlag, Copenhagen.
29. Storgaard, T., Oleksiewicz, M.B., Bøtner, A., 1999. Examination of the selective pressures on a live PRRS vaccine virus. *Arch. Virol.* 144: 2389-2401
30. Nielsen, H.S., Oleksiewicz, M.B., Forsberg, R., Stadejek, T., Bøtner, A., Storgaard, T., 2001. Reversion of a live porcine reproductive and respiratory virus vaccine investigated by parallel mutations. *J. Gen. Virol.*, 82: 1263-72.
31. Nielsen, J., Bøtner, A., Bille-Hansen, V., Oleksiewicz, M. B., and Storgaard, T., 2002. Experimental inoculation of late-term pregnant sows with a field isolate of porcine reproductive and respiratory syndrome vaccine-derived virus. *Vet. Microbiol.* 84: 1-13.
32. Allende, R., Kutish, G.F., Laegreid, W. et al., 2000. Mutations in the genome of porcine reproductive and respiratory syndrome virus responsible for the attenuation phenotype. *Arch. Virol.* 145: 1149–1161.
33. Key, K.F., Guenette, D.K., Yoon, K.J., Halbur, P.G., Toth, T.E., Meng, X.J., 2003. Development of a heteroduplex mobility assay to identify field isolates of porcine reproductive and respiratory syndrome virus with nucleotide sequences closely

- related to those of modified live-attenuated vaccines. *J. Clin. Microbiol.* 41: 2433–2439.
34. Zimmerman, J.J., Benfield, D.A., Murtaugh, M.P., Osorio, F., Stevenson, G.W., Torremorell, M., 2006. Porcine reproductive and respiratory syndrome virus (porcine arterivirus). In: Straw, B.J., et al. (Eds.), *Diseases of Swine*, 9th edition. Blackwell Publishing Ltd, Oxford, pp. 387–418.
35. Opriessnig, T., Halbur, P.G., Yoon, K.J. et al., 2002. Comparison of molecular and biological characteristics of a modified live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine (Ingelvac PRRS MLV), the parent strain of the vaccine (ATCC VR2332), ATCC VR2385, and two recent field isolates of PRRSV. *J. Virol.* 76: 11837–11844.
36. Keffaber, K., Stevenson, G., Van Alstine, W et al., 1992. SIRS virus infection in nursery/grower pigs. *Am. Assoc. Swine Pract. Newslett.* 4: 38–40.
37. Collins, J.E., Rossow, K.D., 1993. Pathogenesis of PRRS. In: *Proceedings of the Allen D. Lemans Swine Conference*, University of Minnesota, USA, pp. 47–48.
38. Done, S.H. and Paton, D.J., 1995. Porcine reproductive and respiratory syndrome: clinical disease, pathology and immunosuppression. *Vet. Rec.* 136: 32–35.
39. Nielsen, J., Bøtner, A., Tingstedt, J.E., Aasted, B., Johnsen, C.K, Riber, U., Lind, P., 2003. In utero infection with porcine reproductive and respiratory syndrome virus modulates leukocyte subpopulations in peripheral blood and bronchoalveolar fluid of surviving piglets. *Vet.Immunol.Immunopathol.* 93: 135-151
40. Nielsen, J., Kristensen, B., Bøtner, A. Unpublished results
41. Feng, W., Laster, S.M., Tompkins, M., 2001. In utero infection by porcine reproductive and respiratory syndrome virus is sufficient to increase susceptibility to challenge by *Streptococcus suis* Type II. *J. Virol.* 75: 4889–4895.

42. Aasted, B., Bach, P., Nielsen, J., Lind, P., 2002. Cytokine profiles in peripheral blood mononuclear cells and lymph node cells from piglets infected in utero with porcine reproductive and respiratory syndrome virus. *Clin. Diagn. Lab. Immunol.* 9: 1229-1234.
43. Johnsen C. K., Bøtner, A., Kamstrup, S. Lind P., Nielsen J., 2002. Cytokine mRNA profiles bronchoalveolar cells of piglets experimentally infected in utero with porcine reproductive and respiratory syndrome virus: association of sustained expression of IFN- γ and IL-10 after viral clearance. *Viral Immunol.* 15: 549-556.
44. Riber, U., Nielsen, J., Lind, P., 2004. In utero infection with PRRS virus modulates cellular functions of blood monocytes and alveolar lung macrophages in piglets. *Vet Immunol Immunopathol.* 99: 169-77.
45. Tingstedt, J.-E., Nielsen, J., 2004. Cellular immune responses in the lungs of pigs infected in utero with PRRSV. An immunohistochemical study. *Viral Immunol.* 17: 558-564.
46. Albina, E., L. Piriou, E. Hutet, R. Cariolet, R. L'Hospitalier. 1998. Immune responses in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV). *Vet. Immunol. Immunopathol.* 61: 49-66.
47. López-Fuertes, L., Doménech, N, Alvarez, B. et al., 1999. Analysis of cellular immune response in pigs recovered from porcine respiratory and reproductive syndrome infection. *Virus Res.* 64: 33-42.
48. Samsom, J.N., de Bruin, T.G.M., Voermans, J.J.M. et al., 2000. Changes of leukocyte phenotype and function in the broncho-alveolar lavage fluid of pigs infected with porcine reproductive and respiratory syndrome virus: a role for CD8⁺ cells. *J. Gen. Virol.* 81: 497–505.

49. Liu, C.H., Chaung, H.C., Chang, H.L., Peng, Y.T., Chung, W.B., 2009. Expression of Toll-like receptor mRNA and cytokines in pigs infected with porcine reproductive and respiratory syndrome virus. *Vet. Microbiol.* 136: 266-76
50. Lunney, J.K., Fritz, E.R., Reecy, J.M. et al., 2010. Interleukin-8, Interleukin-1 β , and Interferon- γ levels are linked to PRRS virus clearance. *Viral Immunol.* 23: 127-134.
51. Wang, G., Song, T., Yu, Y. et al., 2011. Immune responses in piglets infected with highly pathogenic porcine reproductive and respiratory syndrome virus. *Vet. Immunol. Immunopathol.* 142: 170-8
52. Rossow, K.D., Morrison, R.B., Goyal, S.M., Singh, G.S., Collins, J.E., 1994. Lymph node lesions in neonatal pigs congenitally infected with porcine reproductive and respiratory syndrome virus. *J. Vet. Diagn. Invest.* 6: 368-371.
53. Rossow, K.D., 1998. Porcine reproductive and respiratory syndrome. *Vet. Pathol.* 35: 1-20.
54. Rowland, R.R., Lawson, S., Rossow, K., Benfield, D.A., 2003. Lymphoid tissue tropism of porcine reproductive and respiratory syndrome virus replication during persistent infection of pigs originally exposed to virus in utero. *Vet. Microbiol.* 96: 219-235.
55. Nielsen, J., Riber, U., Bøtner, A. Unpublished results.
56. Nielsen, H.S., Oleksiewicz, M., Nielsen, J., 1999. Reduced phagocytic activity of porcine pulmonary alveolar macrophages following infection with PRRS. In: 3rd International Symposium on PRRS and Aujeszky's disease. Ploufragan,, June 21-24, 1999: 97.
57. Oleksiewicz, M.B, Nielsen, J., 1999. Effect of porcine reproductive and respiratory syndrome virus (PRRSV) on alveolar lung macrophage survival and function. *Vet. Microbiol.*, 66: 15-27.

