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Editorial

Porcine reproductive and respiratory syndrome virus: An update on an emerging and re-emerging viral disease of swine

ABSTRACT

Recognized in the late 1980s in North America and Europe the syndrome that caused reproductive and respiratory problems in swine was initially called "mystery swine disease" and is now termed "porcine reproductive and respiratory syndrome (PRRS)". In the early 1990s an arterivirus, referred to as PRRS virus (PRRSV), was determined to be the etiologic agent of this disease. Since then research has progressed substantially. Most recently "porcine high fever disease" was reported in China starting in 2006 with PRRSV being a critical virus associated with high morbidity and mortality (20%) associated with this syndrome which in 2010 is still causing severe pathology in pigs in China, with spread to Vietnam and Cambodia. This volume contains a series of reviews that highlight the virus, its pathogenesis, epidemiology, immunology, vaccinology and host genetic control. This paper provides a brief historical review of PRRS and the associated PRRSV. It presents areas of research gaps that inhibit current progress towards PRRS elimination through production of effective vaccines and current plans for PRRS elimination or eradication programs. It is hoped that this discussion will stimulate further collaboration between researchers and swine veterinarians throughout the world to provide answers that enhance our understanding of PRRS and PRRSV in an effort to eliminate this economically important disease.

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1. Introduction

1.1. Historical perspective

Veterinary clinicians noted in the late 1980s the appearance in U.S. swine herds of a previously unrecognized disease with a clinical presentation of severe reproductive losses in late gestational sows, increases in the number of weak live-born pigs, severe pneumonia in neonatal and nursery pigs, reductions in growth performances and increased mortality (Keffaber, 1989; Loula, 1991; Hill, 1990). A similar clinical syndrome was initially described in Germany in 1990 and spread to other countries in Europe over the next few years. Despite several reports describing a number of different agents as the cause of this disease, the causative agent remained unknown leading to the name "mystery swine disease" in the U.S. and either "porcine epidemic abortion and respiratory syndrome" or "blue-ear pig disease" in Europe. The latter name refers to the cyanosis or blue-reddish coloration of ears of pigs with this disease. Once the etiology of this "mystery" disease was established the name swine infertility and respiratory syndrome (SIRS) was suggested by North American investigators while the Europeans preferred porcine reproductive and respiratory syndrome (PRRS). The latter name is now the accepted name for the syndrome.

Researchers at the Central Veterinary Institute (Lelystad, The Netherlands) were the first group to fulfill Koch's postulates describing a small, enveloped RNA virus (Lelystad Virus) as the causative agent (Wensvoort et al., 1991; Terpstra et al., 1991). A similar virus (VR-2332) was isolated in the U.S. at the same time (Collins et al., 1992; Benfield et al., 1992) and one-year later in Canada (Dea et al., 1992). Comparison of the sequence of the Lelystad virus, now termed type 1 PRRSV, and North American, or type 2, virus isolates indicated that these viruses shared properties similar to the family *Arteriviridae*, genus *Arterivirus*, that includes lactate-dehydrogenase elevating virus (LDH), equine arteritis virus (EAV) and simian hemorrhagic fever virus (Benfield et al., 1992; Dea et al., 1992; Meulenberg et al., 1993; Balasuriya and MacLachlan, 2004).

PRRS has now emerged as the most prevalent disease of swine in the world. In the U.S. annual losses to PRRS are estimated at $560 million (Neumann et al., 2005). The virus and the syndrome continue to evolve with clinical variations of the disease, such as swine mortality and abortion syndrome or atypical PRRS (Epperson and Holler, 1997; Halbur and Bush, 1997; Bush et al., 1999); the recent pandemics in China of "blue ear disease" or "pig high fever disease" that has resulted in severe losses, affecting millions of pigs with high (~20%) mortality (Tong et al., 2007; An et al., 2010; Li et al., this issue); and the highly virulent 1-18-2 strain that emerged in the North Central U.S. in 2007 (Murtaugh, 2009).

Since the original discovery of PRRSV, much has been learned about the disease and the virus. This paper is a brief historical review of these accomplishments. It also serves as an introduction to the set of extensive reviews and articles included in this special issue of Virus Research.

2. The virus

The etiological agent of PRRS is an enveloped, single stranded, positive sense RNA virus with properties similar to the *Arteriviridae* family and placed with the *Coronaviridae* in the order *Nidovirales*, based on similarities in genome organization and expression strat-

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egy (Cavananagh, 1997). The 15 kb genome consists of two large open reading frames ORF 1a and 1b that comprise 75% of the viral genome and are translated and processed into 14 non-structural proteins (nsp) that exhibit replicase, protease and polymerase activities required for viral replication (Meulenberg et al., 1993; Snijder and Meulenberg, 1998). As described in several articles in this issue, reverse genetics and infectious virus clones have become the predominate tools for understanding nsp function (Faaberg et al., this issue; Fang and Snijder, this issue; Sun et al., this issue; Yoo et al., this issue). In this issue, Fang and Snijder (this issue) explore the functional characterization of the key nsp encoded enzymes for arterivirus RNA synthesis, the nsp9 RNA polymerase and nsp10 helicase, the induction of replication-associated membrane rearrangements (nsp2 and nsp3), and a replicative endoribonuclease (nsp11). Yoo et al. (this issue) note that at least four viral proteins have been studied as viral antagonists of host defenses: N as a structural protein and three non-structural proteins, nsp1 (nsp1α and nsp1β), nsp2, and nsp11. Fang and Snijder (this issue) identified the nsp3–8 region as possibly containing PRRS virulence factors. An important element in virus replication, the 5′ and 3′ untranslated regions are explored by Sun et al. (this issue) using infectious clones. This set of reviews affirms how the technical advancements offered by reverse genetics are already leading to important developments for the understanding of the mechanism of pathogenesis and for the design of novel vaccine approaches.

The seven viral structural proteins are encoded by ORFs 2–7 and expressed from six subgenomic mRNAs that are synthesized as a 3′-coterminal nested set of mRNAs with a common leader sequence at the 5′ end (Snijder and Meulenberg, 1998). They include three N-glycosylated minor envelope proteins (GP2a, GP3 and GP4), ORF 2b that encodes a non-glycosylated minor protein 2b (Wu et al., 2001) and the major envelope glycoprotein GP5 that forms a heterodimer with the membrane non-glycosylated protein M (Mardassi et al., 1996; Snijder et al., 2003). The heterodimer consisting of the GP5 and M proteins is required for infectivity of arteriviruses (Snijder et al., 2003); 2b is also essential for virus infectivity and likely functions as an ion channel to facilitate uncoating of the virus (Lee and Yoo, 2006). The three main structural proteins N, M and GP5 are required for particle formation and viral infectivity, whereas the minor proteins (GP2a, GP3 and GP4) are essential only for viral infectivity (Wissink et al., 2005). One additional structural protein N, or nucleocapsid protein, is encoded by ORF7 and is highly immunogenic and has served as the main diagnostic protein to detect antibodies to PRRSV (Dea et al., 2000). The N protein is found in both the cytoplasm (viral particle assembly) and the nucleus where it has an important role in antagonizing cellular gene function. Dokland (this issue) explored the structural biology of PRRSV and affirmed that based on detailed structural analyses this arterivirus is more closely related to coronaviruses than previously thought.

2.1. Pathogenesis, virulence and attenuation

The pathogenesis of PRRSV was first reviewed by Zimmerman et al. (1997) and Rossow (1998). Infection begins with the interaction between PRRSV and CD163, which is reviewed in this issue by Welch and Calvert (this issue). Primary targets for replication are alveolar macrophages of the lung and other cells of the monocyte/macrophage lineage including pulmonary intravascular macrophages, subsets of macrophages in lymph nodes and spleen and intravascular macrophages of the placenta and umbilical cord (Duan et al., 1997; Lawson et al., 1997; Thanawongnuwech et al., 2000). In this issue, Li et al. (this issue) report that the highly pathogenic PRRSV isolate, JXwn06, may possess an expanded tropism to include epithelial cells.

The virus can persist in pigs for long periods of time. The first reported evidence of persistence was transmission of PRRSV from an infected sow to susceptible pigs 99 days post-infection (Zimmerman et al., 1992). Others have detected (either by virus isolation or swine bioassay) virus in tonsil and lymph nodes up to 132 days after birth in pigs infected in utero (Benfield et al., 2000; Rowland et al., 2003; Rowland, this issue); and from 105 to 157 days post-infection in young pigs (Allende et al., 2000; Horter et al., 2002; Wills et al., 1997b). High levels of viral RNA are present in lymphoid tissues in acutely infected pigs and over time viral loads decrease by 1000-fold or more in tonsil and lymph nodes the primary sites of viral persistence (Xiao et al., 2004). The mechanism of persistence is not completely understood but likely includes the emergence of viral variants which can escape host defenses (Rowland et al., 1999).

Studies using mutations in viral genes or the use of chimeras have established potential roles for certain nsp and structural genes in viral virulence and attenuation. Strains with mutations in the nuclear localization signal of the N protein produce lower levels of viremia, a shorter duration of viremia in pigs, higher levels of neutralizing antibodies and reduced viral persistence in tonsils of pigs compared to pigs given the wild-type virus (Lee et al., 2006; Pei et al., 2008). Experiments using reverse genetics indicate that virulence is probably multigenic involving nsp3–8 proteins and GP5 (Kwon et al., 2008; Wang et al., 2008). These latter studies indicated that certain PRRSV chimeras produce attenuated clinical signs in either a respiratory (Wang et al., 2008) or pregnant sow model (Kwon et al., 2008). Finally, the highly virulent strain of PRRSV responsible for the outbreak in China in 2006 contains a unique 30-amino acid deletion in the nsp2 coding region that was presumed to be responsible for the increased virulence of these PRRSV strains. However, a recent study by Zhou et al. (2009) indicates that the nsp2 deletion is not associated with virulence of the high fever disease isolates. As reported in this issue, deletions in nsp2 can attenuate replication and virulence (Faaberg et al., this issue).

Another area of pathogenesis is the interaction between PRRSV and the fetus. At about 90 days gestation, PRRSV can cross the placenta and infect fetuses. In this issue Rowland (this issue) describes several novel aspects of fetal infection, including the thymus as the principal sight of virus replication and the induction of antiviral cytokines by the infected fetus, but not the dam.

2.2. Heterogeneity

Early studies showed that the Lelystad and VR-2332 strain of PRRSV differ by approximately 40% at the genomic level and are also antigenically distinct (Meulenberg et al., 1993; Nelson et al., 1993; Nielson et al., 1999). PRRSV strains are now divided into two genotypes, European genotype (Type 1) and North-American (Type 2), both genotypes circulate globally (Kimman et al., 2009; Zimmerman et al., 2006). Strains within each genotype also vary considerably with sequence differences as high as 20% (Han et al., 2007; Meng, 2000; Nielson et al., 1999). The most variable structural protein is GP5 with 50–55% amino acid homology between North American and European isolates (Mardassi et al., 1996; Meng et al., 1995; Nielson et al., 1999). In this issue Shi et al. (this issue) focus on the evolution of type 1 and 2 PRRSV sequence from a phylogenetic perspective; they assess the potential impact of recombination on the virus epidemiology. Murtaugh et al. (this issue) explore hypotheses accounting for rapid expansion and diversification of PRRSV, including mechanisms specific to PRRSV and other arteriviruses, cellular modification processes, and immunological selection. Variability within GP5 may explain the inefficiency of current vaccines to cross-protect against all strains of PRRSV. At present,

there are no serological assays that link to variability within a genotype.

2.3. Immunology

The quest to achieve a reliable vaccine to prevent clinical disease and re-infection is frustrated by our lack of understanding of virus heterogeneity and what constitutes a protective immune response in the host. PRRSV complicates the ability of the host to respond to infection through several immune evasion capacities (see reviews by Kimman et al., 2009, and in this issue, Diaz et al., this issue). PRRSV infection is characterized by a delayed appearance of neutralizing antibodies (often not appearing for 3–4 months post-infection) and a slow development of virus specific interferon responses. PRRSV is able to downregulate the production of inflammatory cytokines such as type 1 interferons (IFN-α, IFN-β), TNF-α, and interleukin-1 (IL-1) (Van Reeth et al., 1999; Thanawongnuwech et al., 2001). Pigs which clear PRRSV infection have coordinated early expression of IL-1β, IL-8 and IFN-g (Lunney et al., 2010).

Five of the 13 nsps were found to inhibit IFN-β promoter activation (Beura et al., 2010). The strongest inhibitor of IFN-β was nsp1β, which translocates to the nucleus and inhibits TLR3-mediated signaling pathways (Beura et al., 2010) as well as TNF-α promoter activity (Subramaniam et al., 2009). Certain regions in nsp2 that are non-essential for PRRSV replication also downregulate IL-1β and TNF-α (Chen et al., 2010). The inhibition of this early cytokine production in macrophages and dendritic cells contributes to the weak innate immune response, delayed neutralizing antibody, slow IFN-γ response and a depressed cytotoxic T cell response (Costers et al., 2009).

Details related to acquired immunity in pigs following PRRSV infection have been reviewed (Kimman et al., 2009; Mateu and Diaz, 2008; Zimmerman et al., 2006; and in this issue, Diaz et al., this issue; Yoo et al., this issue). Shortly after infection, most antibodies are non-neutralizing and are principally targeted to N and nsp2 proteins. Neutralizing antibodies appear as early as 2–4 weeks after infection but usually do not peak until several weeks to months later (Lopez and Osorio, 2004). The role of neutralizing antibodies has been debated principally because viremia persists in the host in the presence of neutralizing activity. The postulated role of antibody evasion strategies, such as immunodominant "decoy" epitopes that produce non-protective immune responses and "glycan shielding" have been recently reviewed and discussed (Kimman et al., 2009). In this issue Costers et al. (this issue) describe the role of GP4 as sight for neutralization as well as the appearance of neutralizing escape mutants.

The T-cell responses to PRRSV are induced 2–8 weeks post-infection (Bautista and Molitor, 1997; Xiao et al., 2004) and are detected against all structural proteins encoded by ORFs 2–7 (Bautista et al., 1999). Overall, the abundance of T-cells in either acute or persistent infection is highly variable and does not correlate with the level of virus replication in lymphoid tissues (Xiao et al., 2004). Additional studies have shown that the number of PRRSV specific IFN-γ producing T-cells following infection are low in PRRSV infected pigs compared to pigs vaccinated with a pseudorabies vaccine (Meir et al., 2003). In summary, T-cell responses to PRRSV are generally classified as weak, transient and highly variable. One lingering question is whether there are conserved T-cell epitopes that may provide cross-protection against PRRSV strains (Kimman et al., 2009).

In this issue Lunney and Chen (this issue) focus on genomic approaches to identify biomarkers that define genes and pathways that are correlated with swine resistance to PRRSV infection. Besides helping to identify PRRS resistant pigs, these studies may reveal alternate PRRSV control mechanisms that can be exploited for novel drugs, biotherapeutics and vaccine designs.

Table 1
Gaps in our understanding of PRRS and PRRSV vaccines.

What are the determinants of protective immunity?
What is the role of innate and acquired immunity in PRRS?
Which viral proteins bear B- and T-cell epitopes capable of inducing a protective response?
What are the main methods for immune evasion of this virus?
What is the definition of "heterologous protection" and what viral proteins are involved in the heterogeneity?
What are the conserved regions of the virus genome that may function to induce a protective response?
Can conserved viral protein epitopes be exploited to increase the breath of immune protection?

2.4. Vaccines

The first modified-live vaccine for PRRSV, RespPRRS® (later RespPRRS® Repro and now Ingelvac® PRRS MLV), was released in November 1994 by Boerhringer Ingelheim Animal Health. This modified-live vaccine was prepared by sequential passage of a PRRSV in a monkey kidney cell line CL-2621. Since the release of this vaccine a number of other modified-live and killed-virus vaccines have been developed in the U.S. and Europe (Mengeling, 2005).

In 2007, a "Colloquium on Prospects for Development of an Effective PRRS Virus Vaccine" was convened with academic, biologic industry representatives and swine practitioners at the University of Illinois (Rock, 2007). Three general questions were posed to the colloquium participants: (1) what is the efficacy of current PRRS vaccines; (2) what knowledge gaps need to be filled to improve the efficiency of current vaccines and to develop more efficacious PRRS vaccines; and (3) what is the probability that a successful PRRS vaccine can be developed. As to the efficacy it is clear that modified-live PRRS vaccines offer protection against reinfection with homologous and in some cases heterologous viruses. Killed-virus vaccines were viewed as ineffective or of limited efficacy at best against homologous protection. The key question is how to deal with the heterogeneity of PRRSV field isolates and provide cross-protection among heterologous strains of virus. The concept of "heterologous" in terms of PRRSV vaccines remains undefined. Properties of an improved vaccine should include rapid induction of immunity (innate immunity), no adverse outcomes to swine health and the ability to differentiate vaccinated from infected animals (DIVA vaccine) (deLima et al., 2008; Fang et al., 2008). Considerations for the development of vaccines, adjuvants (Charerntantanakul, 2009), and novel designs are reviewed and discussed in this issue by Thanawongnuwech and Suradhat (this issue), Huang and Meng (this issue) and Cruz et al. (this issue). Table 1 highlights continuing issues that must be addressed for effective PRRS vaccines.

2.5. Epidemiology and methods to control PRRSV

Several studies have established the role of infected pigs and semen, and the role of equipment and objects that move between swine farms, as important for herd to herd transmission (see Zimmerman et al., 2006). Others have proposed area spread from neighboring farms as the major form of herd to herd transmission (Mortensen et al., 2002). Proximity to infected herds is recognized as a risk factor with the risk increasing with the density of PRRSV-positive neighboring herds, but decreasing as the distance from positive herds increases (Zhuang et al., 2002). Could PRRSV be transmitted between herds over distances via aerosols? Airborne

transmission could explain area spread in the absence of other sources such as movement of infected pigs, semen, fomites, and breakdowns in biosecurity protocols (Dee et al., 2006; Pitkin et al., 2009).

The PRRSV replicates in alveolar macrophages in the lung and in the tonsil of infected pigs. Thus, the potential for aerosol spread through respiratory and oropharyngeal excretions within swine confinement units is a distinct possibility. In this issue, Kittawornrat et al. (this issue) describe the use of oral fluids a convenient means to track infections in the field. Early laboratory experiments to prove aerosol transmission were disappointing and the conclusion was that aerosol transmission was not common and difficult to achieve over short distances of 1.0–2.5 m (Lager and Mengeling, 2000; Otake et al., 2002; Wills et al., 1997a). In contrast, observations in the field were that extensive efforts to eliminate and eradicate PRRSV from swine herds, while initially successful, always seemed to result in re-infection of these herds and the only explanation appeared to be area spread through aerosols. In this issue, Dee et al. (this issue) describe the use of air filtration systems in the field as an effective means to reduce transmission.

3. Conclusions

Since the discovery of a virus as the etiologic agent of PRRS in the early 1990s, PRRSV continues to be a major economic issue for the swine industry worldwide. Through the efforts of many scientists throughout the world a better understanding of the virology, pathogenesis, immunology, genetic resistance, epidemiology and vaccinology has been gained. Within three years after the discovery of the PRRSV, a modified-live vaccine became available and was quickly used throughout swine producing areas in the world. The question of what drives the evolution of this virus in the field and how to provide cross-protection versus heterologous field strains remains an important and unresolved question.

Despite our lack of understanding of the innate and acquired immune response to PRRSV, it should be remembered that the host eventually eliminates the virus. Experimental evidence indicates that PRRSV is eventually eliminated from the host and persistent infection rarely last more than 200 days (Murtaugh, 2009). Veterinarians have used this information to develop the concept of "herd closure" as a means to eliminate PRRSV from herds. In herd closure all animals are exposed to PRRSV by vaccination or inoculation with virulent virus and then the herd is closed to all animal entry for 200 days. When naïve gilts are introduced into these herds they remain negative. If there are re-breaks, it is almost always with a virus of a different genotype thus indicating a new outbreak (Murtaugh, 2009). This protocol is being used in "regional elimination programs" throughout the U.S. in an effort to eliminate PRRSV from less dense swine areas. The combination of vaccines, herd closure, biosecurity protocols and filtration units on swine housing to prevent area spread may well become the protocol for elimination of this virus in regional areas, as reviewed in this issue by Morrison et al. (this issue).

One of the important lessons learned with PRRS is that understanding this virus and the associated syndrome requires an integrated effort of researchers in various disciplines in collaboration with swine veterinarians. Such interactions would not have been possible without the support of funding programs such as the USDA PRRS Coordinated Agricultural Project (CAP) grants (http://www.prrs.org/), the National Pork Board PRRSV Initiative (http://www.pork.org/Research/), and the yearly updates presented at the International PRRSV Symposium (http://www.prrssymposium.org/).

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References

Allende, R., Laegreid, W.W., Kutish, G.F., Galeota, J.A., Wills, R.W., Osorio, F.A., 2000. Porcine reproductive and respiratory syndrome virus: description of persistence in individual pigs upon experimental infection. J. Virol. 7, 10834–10837.

An, T.-Q., Tian, Z.-J., Xiao, Y., Li, R., Peng, J.-M., Wei, T.-C., Zhang, Y., Zhou, Y.-J., Tong, G.-Z., 2010. Origin of highly pathogenic porcine reproductive and respiratory syndrome virus, China. Emerg. Infect. Dis. 16, 365–367.

Balasuriya, U.B., MacLachlan, N.J., 2004. The immune response to equine arteritis virus: potential lessons for other arteriviruses. Vet. Immunol. Immunopathol. 102, 107–129.

Benfield, D.A., Nelson, E., Collins, J.E., Harris, L., Goyal, S.M., Robison, D., Christianson, W.T., Morrison, B.B., Goryca, D., Chladek, D., 1992. Characteristics of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). J. Vet. Diagn. Invest. 4, 127–133.

Benfield, D., Nelson, E., Rossow, K., Nelson, J., Steffen, M., Rowland, R., 2000. Diagnosis of persistent or prolonged porcine reproductive and respiratory syndrome virus infections. Vet. Res. 31, 71.

Bautista, E.M., Molitor, T.W., 1997. Cell-mediated immunity to porcine reproductive and respiratory syndrome virus in swine. Viral Immunol. 10, 83–94.

Bautista, E.M., Suarex, P., Molitor, T.W., 1999. T-cell responses to the structural polypeptides of porcine reproductive and respiratory syndrome virus. Arch. Virol. 144 (1), 117–134.

Beura, L.K., Sarkar, S.N., Kwon, B., Subramaniam, S., Jones, C., Pattnaik, A.K., Osorio, F.A., 2010. Porcine reproductive and respiratory syndrome virus nonstructural protein 1β modulates host innate immune response by antagonizing IRF3 activation. J. Virol. 84, 1574–1584.

Bush, E.J., Corso, B., Zimmerman, J., Swenson, S., Pyburn, D., Burkgren, T., 1999. Update on the acute PRRS investigative study. Swine Health Prod. 7, 170–180.

Cavananagh, D., 1997. Nidovirales: a new order comprising Coronaviridae and Arteriviridae. Arch. Virol. 142, 629–633.

Charerntantanakul, W., 2009. Adjuvants for porcine reproductive and respiratory syndrome virus vaccines. Vet. Immunol. Immunopathol. 129, 1–13.

Chen, Z., Zhou, X., Lunney, J.K., Lawson, S., Sun, Z., Brown, E., Christopher-Hennings, J., Knudsen, D., Nelson, E.A., Fang, Y., 2010. Immunodominant epitopes in nsp2 of porcine reproductive and respiratory syndrome virus are dispensable for replication, but play an important role in modulation of the host immune response. J. Gen. Virol. 91, 1047–1057.

Collins, J.E., Benfield, D.A., Christianson, W.T., Harris, L., Hennings, J.S., Shaw, D.P., Goyal, S.M., McCullough, S., Morrison, R.B., Joo, H.S., Gorcyca, D., Chladek, D., 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.

Costers, S., Lefebvre, D.J., Goddeeris, B., Delputte, P.L., Nauwynck, H.J., 2009. Functional impairment of PRRSV-specific peripheral CD3+CD8 high cells. Vet. Res. 40, 46.

Costers, S., Vanhee, M., Van Breedam, W., Van Doorsselaere, J., Geldhof, M.F., Nauwynck, H.J., this issue. GP4-specific neutralizing antibodies might be a driving force in PRRSV evolution. Virus Res.

Cruz, J.L.G., Zuñiga, S., Becares, M., Sola, I., Ceriani, J.E., Juanola, S., Plana, J., Enjuanes, L., this issue. Vectored vaccines to protect against PRRSV. Virus Res.

Dea, S.A., Bilodeau, R., Athanassious, R., Sauvageau, R.A., Martineau, G.P., 1992. Swine reproductive and respiratory syndrome in Quebec: isolation of an enveloped virus serologically-related to Lelystad virus. Can. Vet. J. 33, 801–808.

Dea, S., Gagnon, C.A., Mardassi, H., Pirzadeh, B., Rogan, D., 2000. Current knowledge on the structural proteins of porcine reproductive and respiratory syndrome (PRRS) virus: comparison of the North American and European isolates. Arch. Virol. 145, 659–688.

Dee, S., Deen, J., Cano, J.P., Batista, L., Pijoan, C., 2006. Further evaluation of alternative air-filtration systems for reducing the transmission of porcine reproductive and respiratory syndrome virus by aerosol. Can. J. Vet. Res. 69, 64–70.

Dee, S., Otake, S., Deen, J., this issue. Use of a production region model to assess the efficacy of various air filtration systems for preventing airborne transmission of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae*: results from a 2 year study. Virus Res.

deLima, M., Kwon, B., Ansari, I.H., Pattnaik, A.K., Flores, E.F., Osorio, F.A., 2008. Development of a porcine reproductive and respiratory syndrome virus differentiable (DIVA) strain through deletion of specific immunodominant epitopes. Vaccine 26, 3594–3600.

Diaz, I., Darwich, L., Mateu, E.M., this issue. Certainties, doubts and hypotheses in porcine reproductive and respiratory syndrome virus immunobiology. Virus Res.

Dokland, T., this issue. The structural biology of PRRSV. Virus Res.

Duan, X., Nauwynck, H.J., Pensaert, M.B., 1997. Effects of origin and state of differentiation and activation of monocytes/macrophages on their susceptibility to porcine reproductive and respiratory syndrome virus. Arch. Virol. 142, 2483–2497.

Epperson, B., Holler, L., 1997. An abortion storm and sow mortality syndrome. Proc. Ann. Meet. Am. Assoc. Swine Pract., 479–484.

Faaberg, K.S., Kehrli Jr., M.E., Lager, K.M., Guo, B., Han, J., this issue. In vivo growth of porcine reproductive and respiratory syndrome virus engineered Nsp2 deletion mutants. Virus Res.
Fang, Y., Christopher-Hennings, J., Brown, E., Liu, H., Chen, Z., Lawson, S.R., Breen, R., Clement, T., Gao, G., Bao, B., Knudsen, D., Daly, R., Nelson, E., 2008. Development of genetic markers in the non-structural protein 2 region of a US type 1 porcine reproductive and respiratory syndrome virus: implications for future recombinant marker vaccine development. J. Gen. Virol. 89, 3086–3096.
Fang, Y., Snijder, E.J., this issue. The PRRSV replicase: exploring the multifunctionality of an intriguing set of nonstructural proteins. Virus Res.
Halbur, P., Bush, E., 1997. Update on abortion storms and sow mortality. Swine Health Prod. 5, 73.
Han, J., Liu, G., Wang, Y., Faaberg, K.S., 2007. Identification of non essential regions of the nsp2 replicase protein of porcine reproductive and respiratory syndrome virus strain VR-2332 for replication in cell culture. J. Virol. 81, 9878–9890.
Hill, H., 1990. Overview and history of Mystery Swine Disease (swine infertility/respiratory syndrome). In: Proceedings of the Mystery Swine Disease Committee Meeting, Livestock Conversation Institute, Denver, CO, pp. 29–31.
Horter, D.C., Pogranichniy, R.C., Chang, C.C., Evans, R.B., Yoon, K.J., Zimmerman, J.J., 2002. Characterization of the carrier state in porcine reproductive and respiratory syndrome virus infection. Vet. Microbiol. 86 (2), 213–218.
Huang, Y.W., Meng, X.J., this issue. Novel strategies and approaches to develop the next generation of vaccines against porcine reproductive and respiratory syndrome virus (PRRSV). Virus Res.
Keffaber, K.K., 1989. Reproductive failure of unknown etiology. AASP 1, 2–10.
Kimman, T.G., Cornelissen, L.A., Moorman, R.J., Rebel, J.M.J., Stockhofe-Zurwieden, N., 2009. Challenges for porcine reproductive and respiratory syndrome virus (PRRSV) vaccinology. Vaccine 27, 3704–3718.
Kittawornrat, A., Prickett, J.R., Chittick, W.A., Wang, C., Engle, M., Johnson, J.F., Patnayak, D., Schwartz, T., Whitney, D.J., Olsen, C.W., Schwartz, K.J., this issue. Porcine reproductive and respiratory syndrome virus (PRRSV) in serum and oral fluid samples from individual boars: Will oral fluid replace serum for PRRSV surveillance? Virus Res.
Kwon, B., Ansari, I.H., Pattnaik, A.K., Osorio, F.A., 2008. Identification of virulence determinants of porcine reproductive and respiratory syndrome virus through construction of chimeric clones. Virology 380, 371–378.
Lager, K.M., Mengeling, W.L., 2000. Experimental aerosol transmission of pseudorabies virus and porcine reproductive and respiratory syndrome virus. Proc. Ann. Meet. Am. Assoc. Swine Pract., 409–410.
Lawson, S.R., Rossow, K.D., Collins, J.E., Benfield, D.A., Rowland, R.R., 1997. Porcine reproductive and respiratory syndrome virus infection of gnotobiotic pigs: sites of virus replication and co-localization with MAC-387 staining. Virus Res. 51, 105–113.
Lee, C., Yoo, D., 2006. The small envelope protein of porcine reproductive and respiratory syndrome virus possesses ion channel protein-like properties. Virology 355, 30–43.
Lee, C., Hodgins, D., Calvert, J.S., Welch, S.K., Jolie, R., Yoo, D., 2006. Mutations within the nuclear localization signal of the porcine reproductive and respiratory syndrome virus nucleocapsid protein attenuate virus replication. Virology 346, 238–250.
Li, L., Zhao, Q., Ge, X., Teng, K., Kuang, Y., Guo, X., Yang, H., this issue. Porcine reproductive and respiratory syndrome in China. Virus Res.
Lopez, O.J., Osorio, F.A., 2004. Role of neutralizing antibodies in PRRSV protective immunity. Vet. Immunol. Immunopathol. 102, 155–163.
Loula, T., 1991. Mystery pig disease. Agri-practice 12, 23–34.
Lunney, J., Chen, H., this issue. Genetic control of host resistance to porcine reproductive and respiratory syndrome virus (PRRSV) infection. Virus Res.
Lunney, J.K., Fritz, E.R., Reecy, J.M., Kuhar, D., Prucnal, E., Molina, R., Christopher-Hennings, J., Zimmerman, J., Rowland, R.R.R., 2010. Interleukin-8, interleukin-1beta, and interferon-gamma levels are linked to PRRS virus clearance. Viral Immunol. 23, 127–134.
Mardassi, H., Massie, B., Dea, S., 1996. Intracellular synthesis, processing, and transport of proteins encoded by ORFs 5 to 7 of porcine reproductive and respiratory syndrome virus. Virology 221, 98–112.
Mateu, E., Diaz, I., 2008. The challenge of PRRS immunology. Vet. J. 177, 345–351.
Meir, W.S., Galeota, U., Osorio, F.A., Hussman, R.J., Schnitzlein, W.M., Zuckermann, F.A., 2003. development of the interferon-γ response of swine to porcine reproductive and respiratory syndrome virus infection or vaccination. Virology 309, 18–31.
Meng, X.J., 2000. Heterogeneity of porcine reproductive and respiratory syndrome virus: implications for current vaccine efficacy and future vaccine development. Vet. Microbiol. 74, 309–329.
Meng, X.J., Paul, P.S., Halbur, P.G, Morozov, J., 1995. Sequence comparison of opening reading frames 2 to 5 of low and high virulence United States isolates of porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 76, 3181–3188.
Mengeling, W.L., 2005. The porcine reproductive and respiratory syndrome quandary. Part II. Vaccines and vaccination strategy. J. Swine Health Prod. 13, 152–156.
Meulenberg, J.J., Julst, M.M., de Meijer, E.J., Moonen, P.I., den Besten, A., de Khuyver, E.P., Wensvoort, R., Moormann, R.J., 1993. Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARS), is related to LDV and EAV. Virology 192, 62–72.
Morrison, R.B., Davies, P., Dee, S., Torremorell, M., Corzo, C.A., this issue. Control and elimination of porcine reproductive and respiratory syndrome virus. Virus Res.
Mortensen, S., Stryhn, H., Sogaard, R., Borklund, A., Stark, K.D., Christensen, J., 2002. Risk factors for infection of sow herds with porcine reproductive and respiratory syndrome virus (PRRSV). Prev. Vet. Med. 53, 83–101.
Murtaugh, M., 2009. Update on PRRSV immunology and viral genetics: from hopeless to hopeful. In: Proceedings of the 40th Annual Meeting of the American Association of Swine Veterinary, Dallas, TX, pp. 459–462.
Murtaugh, M.P., Stadejek, T., Abrahante, J.E., Lam, T.T., Leung, F.C., this issue. The ever-expanding diversity of porcine reproductive and respiratory syndrome virus. Virus Res.
Nielson, C.J., Murtaugh, M.P., Faaberg, K.S., 1999. Porcine reproductive and respiratory syndrome virus comparison: divergent evolution on two continents. J. Virol. 7, 270–280.
Nelson, E.A., Christopher-Hennings, J., Drew, T., Wensvoort, G., Collins, J.E., Benfield, D.A., 1993. Differentiation of United States and European isolates of porcine reproductive and respiratory syndrome virus by monoclonal antibodies. J. Clin. Microbiol. 31, 3184–3189.
Neumann, E., Kiebenstein, J.B., Johnson, C.D., Mabry, J.W., Bush, E.J., Seitzinger, A.H., Zimmerman, J.J., 2005. Assessment of the economic impact of porcine reproductive and 2 respiratory syndrome von swine production in the United States. J. Am. Vet. Med. Assoc. 227, 385–392.
Otake, S., Dee, S.A., Jacobson, L., Torremorell, M., Pijoan, C., 2002. Evaluation of aerosol transmission of porcine reproductive and respiratory syndrome virus under controlled field conditions. Vet. Rec. 150, 804–808.
Pei, Y., Hodges, D.C., Lee, C., Calvert, J.G., Welch, S.K., Jolie, R., Keith, M., Yoo, D., 2008. Functional mapping of the porcine reproductive and respiratory syndrome virus capsid protein nuclear localization signal and its pathogenic association. Virus Res. 135, 107–114.
Pitkin, A., Deen, J., Dee, S., 2009. Use of a production region model to assess the airborne spread of porcine reproductive and respiratory syndrome virus. Vet. Microbiol. 136, 1–7.
Rock, D.L., 2007. Report: colloquium on prospects for development of an effective PRRS vaccine. AASV Newsletter August 13, 2007, http://www.aasp.org/news/story,phpfid=2527.
Rossow, K., 1998. Porcine reproductive and respiratory syndrome. Vet. Pathol. 35, 1–20.
Rowland, R.R.R., this issue. The interaction between PRRSV and the late gestation pig fetus. Virus Res.
Rowland, R.R.R., Steffen, M., Ackerman, T., Benfield, D.A., 1999. The evolution of porcine reproductive and respiratory syndrome virus: quasispecies and emergence of a virus subpopulation during infection of pigs with VR-2332. Virology 259, 262–266.
Rowland, R., Lawson, S., Rossow, K., Benfield, D., 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.
Shi, M., Lam, T.T., Hon C.-C., Hui, R.H., Faaberg, K.S., Wennblom, T.; Murtaugh, M.P., Stadejek, T., Leung, F.C., this issue. Molecular Epidemiology of PRRSV: A Phylogenetic Perspective. Virus Res.
Snijder, E.J., Meulenberg, J.J., 1998. Molecular biology of arteriviruses. J. Gen. Virol. 79, 961–979.
Snijder, E.J., Dobbe, J.C., Spaan, W.J., 2003. Heterodimerization of the two major envelope proteins is essential for arterivirus infectivity. J. Virol. 77, 97–104.
Subramaniam, S., Kwon, B., Beura, L.K., Pattnaik, A.K., Osorio, F., 2009. Elucidation of mechanisms of TNFα suppression by PRRSV. In: Proceedings of the 90th Meeting of the Conference of Research Workers in Animal Disease, Chicago, IL (Abstract 176).
Sun, Z., Liu, C., Tan, F., Gao, F., Liu, P., Qin, A., Yuan, S., this issue. Characterization of 3′ UTR cis-acting signals for replication of porcine reproductive and respiratory syndrome virus. Virus Res.
Terpstra, C., Wensvoorst, G., 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.
Thanawongnuwech, R., Halbur, P.C., Thacker, E.L., 2000. The role of pulmonary intravascular macrophages in porcine reproductive and respiratory syndrome virus infection. Anim. Health Res. Rev. 1, 95–102.
Thanawongnuwech, R., Suradhat, S., this issue. Taming PRRSV: revisiting the control strategies and vaccine design. Virus Res.
Thanawongnuwech, R., Young, T.F., Thacker, B.J., Thacker, E.L., 2001. Differential production of proinflammatory cytokines: in vivo PRRSV and *Mycoplasma hyopneumoniae* co-infection model. Vet. Immunol. Immunopathol. 79, 115–127.
Tong, G.Z., Zhou, Y.J., Hao, X.F., Tian, Z.J., An, T.Q., Qiu, H.J., 2007. Highly pathogenic porcine reproductive and respiratory syndrome, China. Emerg. Infect. Dis. 13, 1434–1436.
Van Reeth, K., Labarque, G., Nauwynck, H., 1999. Differential production of proinflammatory cytokines in the pig lung during different respiratory virus infections: correlations with pathogenicity. Res. Vet. Sci. 67, 47–52.
Wang, C., Lee, F., Huang, T.S., Pan, D.H., Jong, M.H., Chao, P.H., 2008. Genetic variation in open reading frame 5 gene of porcine reproductive and respiratory syndrome virus in Taiwan. Vet. Microbiol. 131, 339–347.
Welch, S.K., Calvert, J.G., this issue. A brief review of CD163 and its role in PRRSV infection. Virus Res.
Wensvoort, G., Terpstra, C., Pol, J.M.A., Lask, E.A., Bloemraad, M., de Kluyver, E.P., Kragten, C., van Butten, L., den Besten, A., Wagenaar, F., Broekhuijsen, J.M., Moonen, P.J.M., Zetstra, T., de Boer, E.A., Tibben, Ah.J., de Jong, M.F., van'r Veld, P., Groenland, G.J.R., van Gennep, J.A., Voets, M.Th., Verheijden, J.H.M., Braamkamp,

J., 1991. Mystery swine disease in the Netherlands: the isolation of Lelystad virus. Vet. Q. 13, 121–130.
Wills, R.W., Zimmerman, J.J., Swenson, S.L., Yoon, K.J., Hill, H.T., Burady, B.S., McGinley, M.J., 1997a. Transmission of porcine reproductive and respiratory syndrome virus by direct close or indirect contact. Swine Health Prod. 5, 213–218.
Wills, R.W., Zimmerman, J.J., Yoon, K.J., Swenson, S.L., McGinley, M.J., Hill, H.T., 1997b. Porcine reproductive and respiratory syndrome virus: a persistent infection. Vet. Microbiol. 55, 231–240.
Wissink, E.H.J., Kroese, M.V., Van Wijk, H.A.R., Rijsewijk, F.A.M., Meulenberg, J.J.M., Rottier, P.J.M., 2005. Envelope protein requirements for the assembly of infectious virions of porcine reproductive and respiratory syndrome virus. J. Virol. 79, 12495–12506.
Wu, W.H., Fang, Y., Farwell, R., Steffen-Bien, M., Rowland, R.R., Christopher-Hennings, J., Nelson, E.A., 2001. A 10-kDa structural protein of porcine reproductive and respiratory syndrome virus encoded by ORF 2b. Virology 287, 183–191.
Xiao, Z., Batista, L., Dee, S., Halbur, P., Murtaugh, M.P., 2004. The level of virus-specific T-cell and macrophage recruitment in porcine reproductive and respiratory syndrome virus infection in pigs is independent of virus load. J. Virol. 78, 5923–5933.
Yoo, D., Song, C., Sun, Y., Du, Y., Kim, O., Liu, H.-C., this issue. Modulation of host cell responses and evasion strategies for porcine reproductive and respiratory syndrome virus. Virus Res.
Zhou, L., Zhang, J., Zeng, J., Yin, S., Li, Y., Zheng, L., Guo, X., Ge, K., Yang, H., 2009. The 30-amino acid deletion in the nsp2 of highly pathogenic porcine reproductive and respiratory syndrome virus emerging in China is not related to virulence. J. Virol. 83, 5156–5167.
Zhuang, Q., Barfod, K., Wachmann, H., Mortensen, S., Willeberg, P., 2002. Serological surveillance for PRRS in Danish genetic pig herds and risk factors for PRRS infection. Proc. Congr. Int. Pig Vet. Soc. 2, 231.
Zimmerman, J., Sanderson, T., Eernisse, K.A., Hill, H.T., Frey, M.L., 1992. Transmission of SIRS virus from convalescent animals to commingled penmates under experimental conditions. Am. Assoc. Swine Practitioners Newslett. 4 (4), 25.
Zimmerman, J., Benfield, D.A., Murtaugh, M.P., Osorio, F., Stevenson, G., Torremorell, M., 2006. Porcine reproductive and respiratory syndrome virus (porcine arterivirus). In: Straw, R.E., Zimmerman, J.J., D'Allaire, S., Taylor, D.J. (Eds.), Chapter 24, Diseases of Swine, 9th edition. Blackwell Publishing Professional, Ames, IA, pp. 387–417.
Zimmerman, J.J., Yoon, K.-T., Wills, R.W., Swenson, S.L., 1997. General overview of PRRSV: a perspective from the United States. Vet. Microbiol. 55, 187–196.

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