

Viral co-infections of the porcine respiratory tract: Insight into the local cytokine response*

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Summary

Infections of the porcine respiratory tract are frequently multifactorial, with more than one pathogen involved. They have a significant impact on the efficiency of pig production. One example of such a mixed infection is the porcine respiratory disease complex (PRDC). PRDC can be caused by various viral or bacterial agents. The main viral agents associated with PRDC and considered the primary pathogens are porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), and porcine circovirus type 2 (PCV2). PRRSV, SIV, and PCV2 are known as inducers of inflammatory cytokines. Cytokines play an important role in all aspects of immune responses, but their uncontrolled release in virus-induced diseases may aggravate the course of the disease and the severity of pathological lesions. Although data regarding the kinetics of the local cytokine response in porcine lungs during mono-infection with these pathogens are abundant, their impact on each other during simultaneous infection in different combinations is not thoroughly understood. This paper aims to present the available data on interactions between SIV, PRRSV, and PCV2 in mixed infections of the porcine respiratory tract and the influence of co-infections on local cytokine profiles in the lungs.

Keywords: porcine, co-infection, respiratory tract, cytokines, PRRSV, SIV, PCV2

The respiratory tract, constantly exposed to the environment, is one of the most common sites for pathogen entry. Multiple infections are frequent in pigs, and numerous reports provide evidence for complex interactions between various pathogens (40, 48, 56, 57, 74). Mixed infections in pigs with different viral and/or bacterial pathogens are defined as the porcine respiratory disease complex (PRDC) (9). PRDC is one of the most significant causes of losses in pig husbandry, associated with the necessity of treatment, limited growth performance, as well as increased morbidity (10-40%) and mortality (2-20%) (18, 56). Non-infectious factors contributing to PRDC development include environmental stressors, management (i.a. production system, herd size, and nutrition) and aspects depending on animals (i.a., age, genetics, breed, and immune status) (40). This review aims to identify studies investigating viral co-infections in the pig respiratory tract and presents interactions between viruses and, when possible,

a detailed review of the local inflammatory response and lesions in the lungs during multi-viral infections. Data on the pathophysiology of porcine viral mono-infections, such as those caused by PRRSV (porcine reproductive and respiratory syndrome virus), SIV (swine influenza virus), and PCV-2 (porcine circovirus type 2), are abundant. The complicated nature of mixed infections and the difficulty of experimental reproduction of respiratory diseases with simultaneous dual or mixed infections render the study of co-infections in the respiratory tract challenging and labour-intensive. Nevertheless, understanding the interaction between swine respiratory viruses and their influence on the host inflammatory response, together with the basics of the immune response induced by several combinations of pathogens, can improve and optimize diagnostic methods and herd health surveillance. This knowledge can also facilitate the identification of infectious and non-infectious parameters responsible for the exacerbation of respiratory diseases, thus contributing to the development of more effective methods of eradicating viral diseases.

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Pathogens involved in porcine viral respiratory co-infections

Pathogens involved in swine polymicrobial infections can be divided into primary pathogens, which can induce gross lesions in the respiratory tract because of their virulence, and secondary pathogens, which induce pathological changes on the basis of lesions caused by primary pathogens, co-infecting microorganisms, or other cofactors. A textbook assumption is the primary contribution of viral infection followed by an opportunistic bacterial infection. Due to the numerous pathogens and the diversity of their possible combinations, interactions between respiratory pathogens in the pig respiratory tract in the field are more complex and involve either additive or synergistic effects (40). Viruses (PRRSV, SIV, and PCV-2) are often considered the primary factors of PRDC or other polymicrobial respiratory swine diseases (40). PCV2 and PRRSV are not strictly related to the respiratory system. Nevertheless, they frequently infect tissues in the respiratory tract (57). Other viral pathogens relevant to PRDC, namely Suid herpesvirus 1 (formerly known as Aujeszky's Disease Virus – ADV) (58, 73) and porcine respiratory coronavirus (PRCV) (50), are less frequent. Secondary infection is often related to bacterial agents, namely *Streptococcus suis* (39), *Actinobacillus pleuropneumoniae*, *Glaesserella parasuis*, and *Pasteurella multocida* (57). The clinical outcome of PRDC and other complex respiratory infections may differ depending on the pathogens. The differences between clinical signs produced by different viruses in the respiratory system might be subtle, although the viral disease can often be overshadowed by the effects of concurrent/secondary bacterial infections (58). The primary signs associated with pig respiratory diseases are rhinitis, sneezing, oculonasal catarrhal to purulent discharge, pneumonia, and pleuritis, which may occur due to co-infection or not (58, 72).

SIV is an enveloped RNA virus belonging to the group of influenza A viruses, family of *Orthomyxoviridae*. It is the causative agent of swine influenza (SI), an acute infectious and contagious swine respiratory disease caused by the influenza A virus (IAV) (32). Infection with SIV usually manifests as an acute respiratory disease characterized by fever, apathy, coughing, sneezing, dyspnea, and loss of appetite (1). SI generates losses in the pig industry mainly due to the reduced weight gain of infected pigs. The clinical course may be more severe in the case of co-infection with other viral (PRRSV, PCV2) or bacterial pathogens, which significantly increases mortality (32, 59). Mortality in SIV-infected pigs is usually low, but reaches up to 10-15% in naïve pigs, while morbidity may reach 100% (32). Sarli et al. (58) describe lung lesions in pneumonia caused by SIV as a tan consolidation of the cranial lobe associated with multifocal lobular consolidation of the caudal lobes.

PRRSV is a small enveloped single-stranded positive-sense RNA virus belonging to the genus *Betaarterivirus*, family *Arteriviridae* (55). Two different genotypes of the virus are known: European or type 1 genotype (PRRSV-1) and American or type 2 genotype (PRRSV-2) (55). Due to advances in molecular epidemiology, two viral species of PRRSV are currently distinguished: *Betaarterivirus suis*-1, subgenus *Eurpobarterivirus*, and *Betaarterivirus suis*-2, subgenus *Ampobarterivirus*, for PRRSV-1 and PRRSV-2, respectively (<https://talk.ictvonline.org/ICTV/proposals/2017.001S.012-017S.R.Nidovirales.zip>). Porcine reproductive and respiratory syndrome (PRRS) is characterized by reproductive failure in sows and respiratory problems in growing pigs. Due to its immunosuppressive effect, PRRSV significantly reduces the efficiency of the immune system of pigs and predisposes them to infection with other pathogens, including conditionally pathogenic microorganisms (30, 34). PRRSV infection may lead to a range of consequences, such as secondary infection, co-infection, and persistent infection in the field (74). Simultaneous infections with PRRSV and other viruses, such as SIV, PCV2, PRCV, classical swine fever virus (CSFV), hepatitis E virus (HEV), pseudorabies virus (PRV), porcine parvovirus (PPV), porcine group A rotavirus (PARV), and porcine epidemic diarrhea virus (PEDV), are described across the literature (28, 75). Gross lung lesions caused by PRRSV strains include severe diffuse interstitial pneumonia, usually accompanied by pleurisy, suppurative bronchopneumonia, and a hemorrhagic spot on the lung tissue (45). Highly virulent PRRSV strains are known to form foci of consolidation, mainly in the cranioventral portions of the lungs, together with fibrinous pleuropneumonia (4, 26, 52, 55).

Another virus commonly isolated from pigs with PRDC is PCV2 (41, 67), which belongs to the *Circoviridae* family, *Circovirus* genus (54). Circoviruses are small non-enveloped single-stranded DNA viruses with circular symmetry (33). In addition to PCV2, three other circoviruses (PCV1, PCV3, and PCV4) have been described in pigs (43, 64, 73). Phylogenetic investigations have led to further division of PCV2 into 8 genotypes (from a to h), three of which, a, b, and d, currently dominate in the world (14, 70). At present, the clinical manifestation of PCV2 infection in pigs is referred to as porcine circovirus associated diseases (PCVAD), and five clinical forms have been distinguished: PCV2 subclinical infection (PCV2-SI), PCV2 systemic disease (PCV2-SD), PCV2 lung disease (PCV2-LD), PCV2 enteric disease (PCV2-ED), and PCV2 reproductive disease (PCV2-RD) (60). Clinical signs in the respiratory system are related to PCV2-SD and PCV2-LD (60). Infection with PCV2 in pigs causes lymphoid depletion and immunosuppression, making pigs more susceptible to secondary pathogens (6, 35). Lesions in the porcine lungs are found in the course of PCV2-LD and especially often

during PCV2-SD (58). Macroscopically, tan-mottled lungs are found that fail to collapse with interstitial oedema, which may be moderate to severe (61).

Local cytokine response in porcine lungs after viral challenge: Background

Cytokines play a pivotal role in orchestrating the inflammatory response to self- or non-self danger molecules. They are small secreted proteins (< 40 kDa) produced by nearly every cell to regulate and influence the immune response (2). Chemokines, such as interleukin-8 (IL-8; CXCL8 chemokine), are also crucial in leukocyte movement and interact with other cytokines to manage tissue infiltration and inflammation (31). Cytokines and chemokines contribute to the “cytokine storm” caused by excessive immune activation (17). Previously, the term was applied to a pathological reaction to organ transplantation. Today, however, it is commonly used to describe the uncontrolled release of cytokines in virus-induced diseases, such as influenza, Middle East respiratory syndrome, or COVID-19 (17, 62). However, cytokines and chemokines can have a synergistic effect and potentially trigger signaling cascades, which can make even the smallest amounts of protein tremendously harmful to the lung tissue (20).

SIV and PRRSV are considered key viral pathogens involved in PRDC (40). Both viruses are thought to be strong inducers of inflammatory mediators in the pig respiratory system (10, 44, 47).

During the acute phase of SI, the production of inflammatory cytokines, such as tumour necrosis factor- α (TNF- α), interferon- α (IFN- α), interferon- γ (IFN- γ), interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), and interleukin-12 (IL-12), has been thoroughly documented as a critical determinant of disease severity (3, 23). In acutely SIV-infected pigs, a significant correlation was found between lung lesions and lung concentrations of IL-1 β , IL-8, and TNF- α (44). The highest concentrations of cytokines were significantly correlated with the severity of lung lesions observed in infected animals.

PRRSV has been shown to reduce or suppress IFN- α production during infection (5). In a study by García-Nicolás et al. (15), PRRSV caused an impaired expression of IFN- α and TNF- α in the lungs, leading to a weak and delayed adaptive immune response due to inefficient expression of IL-12 and IFN- γ . However, IFN- α , IL-1 β , and IL-6 were significantly induced in bronchoalveolar lavage fluid (BALF) from infected pigs at 3 day post-inoculation (DPI) in a study by Nazki et al. (37). In addition, the authors reported that the clearance of PRRSV corresponded with a peaked secretion of anti-viral and proinflammatory cytokines locally in BALF from PRRSV-infected animals. Hence, maintained levels of IFN- α in BALF, even at peak viremia, were possibly associated with local viral clearance (37). IFN- α transcript levels were also slightly increased in BALF cells from pigs infected

with PRRSV alone in a study by Czyżewska-Dors et al. (10), but only at 2 DPI (11). Anti-inflammatory interleukin-10 (IL-10) was significantly induced at 3 and 10 DPI in BALF from PRRSV-infected pigs (37), and an upregulated expression of the IL-10 gene was detected in the lungs of PRRSV-inoculated pigs at 10 and 21 DPI (15).

Chae et al. (6) examined cytokine expression profiles (IFN- γ , interleukin-1- α (IL-1 α), IL-8, and IL-10) in the lungs of pigs with clinical signs of PCV2-LD. The expressions of IL-1 α mRNA and IL-8 mRNA were markedly increased, while the expression of IFN- γ mRNA was weak. The expression of IL-10 mRNA in samples from diseased and healthy animals was not detected. The expressions of IL-1 α and IL-8 were significantly up-regulated in the lungs of animals with inflammatory infiltrates in alveolar spaces (6). Another study noted a significant increase in IL-8 concentration with IL-8 mRNA expression in swine alveolar macrophages (AM) inoculated *in vitro* with PCV2 (7). Moreover, TNF- α concentration was also elevated (7). Over-expression of these cytokines may result in increased severity of lung lesions. Furthermore, IL-8 leads to excessive infiltration of inflammatory cells into the affected area and exacerbates host defense reaction, which results in destructive responses (7, 54). In general, Chae et al. (6) indicate that prolonged proinflammatory cytokine expression is related to the pulmonary inflammatory response through exacerbation of inflammatory reactions and recruitment of additional mononuclear phagocytic cells to the lung tissue of pigs infected with PCV2.

PRRSV and SIV co-infection

Czyżewska-Dors et al. (10) evaluated local innate immune response patterns in BALF cells from pigs with PRRSV mono-infection or co-infected with SIV. The study was performed on 26 seven-week-old pigs divided into three groups: PRRSV-infected, PRRSV and SIV-infected, and controls. Pigs from the first group were inoculated intranasally (IN) with 10^5 TCID₅₀ of PRRSV. The second group was co-inoculated IN with 10^5 TCID₅₀ of PRRSV and 10^7 TCID₅₀ SwH1N1. BALF was collected post mortem at 2, 4 and 21 DPI. Gross lung lesions were most severe in the co-infected group at 21 DPI. Therefore, it can be assumed that these two viruses act synergistically with regard to lung lesions. The mean concentration of the PRRSV load in BALF was lower in pigs infected simultaneously with both pathogens than in pigs infected solely with PRRSV, indicating that SIV probably limited PRRSV replication (10).

BALF cells were investigated in terms of expression of IFN- α , IFN- γ , IL-1 β , IL-6, IL-8, and IL-10 mRNA (10). The authors observed a significant downregulation of IFN- γ mRNA at 2 DPI and IFN- α mRNA at 4 DPI in the co-inoculated pigs. On the other hand, the IFN- γ mRNA expression level was later (21 DPI)

higher in the PRRSV-infected and co-infected groups than it was in the control pigs. A significant downregulation of IL-1 β mRNA was observed in the PRRSV-infected and co-infected pigs compared to the control pigs (10). The results showed that co-infection with PRRSV and SIV had additive effects on the mRNA expression of IL-6 and IL-10, and these cytokines were upregulated during the first 4 DPI in co-infected pigs compared to singly infected animals. The IL-6 mRNA level remained high at 4 DPI in animals infected with both viruses. It was in line with a notably lower PRRSV load in BALF, suggesting that IL-6 contributes to virus clearance during PRRSV and SIV infection. The IL-10 transcript level was significantly upregulated at 4 DPI compared to that in pigs infected with PRRSV alone and at 21 DPI compared to that in control animals. In a study by Kowalczyk et al. (24), who investigated SIV H1N1 co-infection with *Bordetella bronchiseptica* (*B. bronchiseptica*), the mRNA expression of IL-10 in SIV-infected pigs remained unchanged. Hence, positive synergistic interaction between PRRSV and SIV in terms of IL-10 mRNA expression in simultaneous infections is possible. The expression of IL-8 was not significantly different. However, Loving et al. (29) showed that SIV co-infection with *B. bronchiseptica* caused an upregulated expression of IL-8 compared to its expression in mono-infection with virus or bacteria. Czyżewska-Dors et al. (10) assumed that PRRSV could diminish SIV-induced proinflammatory IL-8 production.

Turlewicz-Podbielska et al. (68) performed a study aimed at assessing the course of single and dual infection in pigs with PRRSV and SIV, considering the mutual influence of these pathogens on the local cytokine profile and pathological changes in the lung tissue. The cytokine profile included the following cytokines: IFN- α , IFN- γ , IL-1 β , IL-6, IL-8, IL-10, and TNF- α . In that study, 56 healthy seven-week-old piglets were divided into four groups. Three groups were inoculated IN with SwH1N1 10⁷ TCID₅₀ or PRRSV 10⁵ TCID₅₀ in the groups with mono-infection and with SwH1N1 10⁵ TCID₅₀ and PRRSV 10⁷ TCID₅₀ in the group with dual infection. The control group consisted of clinically healthy animals inoculated IN with a placebo. Then, the pigs were euthanised at 4, 10, and 21 DPI, pathological lesions in the lungs were assessed, and samples were taken to evaluate cytokine concentrations in the lungs (68).

At 4 DPI, the concentration of IFN- α was significantly higher in all groups, including the co-infected group, compared to the control animals. No differences were observed between the infected groups (68). At 10 DPI, a significant increase in IFN- α was observed in the group infected with the two pathogens. From 10 DPI onwards, a significant increase in IFN- γ levels was noted only in the PRRSV-infected or co-infected pigs (68). Contrary to the results observed in BALF by Czyżewska-Dors et al. (10), a significant increase

in IL-1 β concentration was found mainly in pigs infected with the two viruses at 4, 10, and 21 DPI. The mean concentration of IL-6 was also higher in the SIV and SIV + PRRSV groups. At 10 DPI, a higher concentration of IL-6 was observed in the PRRSV and SIV + PRRSV groups. At 10 DPI, the concentration of IL-10 was significantly higher in the co-infected group, although it was higher in all infected groups than in the controls. At 21 DPI, the mean concentration of IL-10 was significantly higher only in the SIV + PRRSV group compared to the controls. Significant changes in TNF- α concentration were observed at 10 DPI in the PRRSV and SIV + PRRSV groups and at 21 DPI in the co-inoculated group. IL-8 was elevated in all infected groups, but the local concentration of this cytokine at 2, 4, 10, and 21 DPI was significantly higher in the PRRSV-infected and co-infected groups (68).

Interestingly, the analysis of changes in cytokine concentrations in all groups indicated individual deviations, and no significant differences were observed in the local cytokine response in pigs co-infected with PRRSV and SIV compared to the mono-infected animals (68). Synergistic effects between SIV and PRRSV in terms of the impact on the local cytokine profile were not observed, probably because of the different pathogenesis of the two infections and different timing of lung lesions. Lung lesions typical of IAV were observed mostly up to 10 DPI, whereas lesions characteristic of PRRSV were found later, from 10 DPI onwards. In the group infected only with SIV, the highest levels of cytokines were also observed early (2-4 DPI), which correlated with the period of the most severe lesions in the lungs. Similarly, in the group infected only with PRRSV, the most extensive changes in cytokine levels coincided with the peak of pathological lesions (from 10 to 21 DPI). In addition, a strong positive correlation was observed between the local concentrations of TNF- α and IL-1 and lung lesions, which suggests that these cytokines are involved in inducing lung lesions during PRRS and SI. Moreover, the strong relationships between local TNF- α , IFN γ , IL-8 and SwH1N1 levels in the lungs, as well as TNF- α , IL-8, and the PRRSV genome copy number in the lungs, suggest that the local replication of both viruses also influences the local cytokine response during infection. Otherwise, co-infection did not significantly affect the severity of changes observed in the lungs compared to mono-infection with PRRSV or SIV (68).

Lately, the same research team evaluated the impact on the clinical outcome and the local replication of viruses in the porcine lungs during simultaneous infection with SIV and PRRSV (45). Co-infection resulted in a longer duration of fever (up to 10 DPI) compared to that in single-inoculated groups. In co-inoculated pigs, 2 peaks of fever were observed (at 3 and 7-10 DPI), whereas in the SIV-infected group, fever peaked once at 2 DPI. In the solely PRRSV-infected group, 2 periods with the rectal temperature over 40°C were

also noted. However, mean clinical scores in the inoculated groups did not differ significantly during the study. Mean SIV TCID₅₀ titers in the lungs were not significantly different between co-inoculated and SIV mono-inoculated pigs. In contrast, a significantly higher mean copy number of PRRSV were detected in the lungs of PRRSV mono-inoculated pigs only at 2 DPI. No significant differences in the PRRSV load were identified later until the end of the study (21 DPI). Analysis of the gross lung lesions pattern (lung scores) in the co-infected group showed additive dynamics of SIV mono- and PRRSV mono-infected groups (45).

The above studies demonstrate that co-infection with SIV and PRRSV does not significantly change the kinetics of the local cytokine response and that differences in gross lung lesions between mono-infected and co-infected groups were related to the dynamics of infection with SIV or PRRSV and not to interactions between them. The slight effect observed in co-infection with SIV and PRRSV in studies by Pomorska-Mól (45) and Turlewicz-Podbielska (68) with regard to the local cytokine response or clinical outcome could be related to the different target cells of these two viruses. The primary target cells of PRRSV are macrophages, mainly pulmonary ones (61), whereas SIV infects primarily airway epithelial cells, e.g. bronchioles or alveoli (49). The study was also performed under strictly controlled conditions, where numerous variables, such as bacterial infections, were forcibly limited, and so were additional impacts on the infection and the course of the disease, which could be revealed under field conditions. Differences in cytokine concentrations in lung tissue and BALF may result from the matrix used to assess the concentrations. Detection of cytokines in BALF is difficult because of low to undetectable levels of specific important cytokines (50).

PRRSV and PRCV co-infection

Renukaradhya et al. (50) investigated simultaneous infection with PRRSV and PRCV. Pigs in the co-infected group were inoculated IN with 3×10^4 TCID₅₀, and intramuscularly with 2×10^4 TCID₅₀ of the PRRSV SD23983 strain. Ten days later, the animals were inoculated IN with 4×10^6 plaque-forming units (PFU) and intratracheally with 6×10^6 PFU of the PRCV ISU-1 strain. Clinical signs were more severe in the co-infected group than they were with either single-virus infection. A significantly higher incidence of fever and decreased body weight gains were observed in dually infected pigs compared to pigs infected with only PRRSV or PRCV (50). Similar results regarding the clinical outcome in pigs co-infected with PRCV and PRRSV were observed in a study by Jung et al. (21). Moreover, inoculation with H1N1 or PRRSV before PRCV exacerbated the clinical picture of the disease in weaned pigs compared with PRCV mono-infection (48). Van Reeth et al. (46) reported that the effects of separate inoculation with PRCV and LPS from *E. coli*

0111 : B4 were subclinical, although the co-infection resulted in significant respiratory obstruction, dementia, and loss of appetite in the first 10-12 hours after LPS. In another study, dual infection with PRCV and SIV (H1N1 or H3N2) did not affect clinical responses, gross respiratory lesions, or growth performance compared with single infection in eight-week-old specific pathogen-free (SPF) pigs and did not enhance the pathogenicity of these viruses (25). In addition, PRCV or SIV was isolated more frequently from tissues and nasal swabs from mono-infected animals, suggesting a possible interference between replication of PRCV and SIV (25).

In a study by Jung et al. (21), a subsequent PRCV infection led to increased PRRSV replication in the lungs from 4 to 21 DPI and magnified apoptotic lung lesions, whereas PRCV replication in the lungs decreased compared with PRCV mono-infection. In the group co-infected with PRCV and PRRSV, PRRSV-specific antibody titres in serum were higher compared with PRRSV mono-infection and coincided with increased PRRSV replication in the lungs and severe apoptotic lung lesions in the co-infected group. More severe gross and microscopic lung lesions were also noted during co-infection. In co-infected pigs, more severe bronchointerstitial pneumonia was reported (21). Nevertheless, in animals co-infected with PRCV and bacteria, no synergistic effect of PRCV and LPS on lung lesions was found (46).

In the lungs of pigs co-infected with PRCV and PRRSV, substantially higher levels of IL-12 were detected only during the middle period of infection (8 days post PRRSV inoculation/18 days post PRCV inoculation and 10 days post PRRSV inoculation/20 days post PRCV inoculation – 1/18 and 10-20 DPI), compared to the controls and the PRCV mono-infected groups (50). Higher levels of IL-10 and TGF- β were noted in this group, compared with the other groups, from 2/12 to 8/18 DPI and at 4/14 and 8/18 DPI, respectively (50). Moreover, during the middle period of infection (4/14 and 8/18 DPI), the authors observed increased levels of IL-6 in the lungs of co-infected pigs, compared to mono-infected groups. Contrastingly, higher levels of IL-6 during the early stage of infection (2 and 4 DPI) in both the lungs and serum of pigs infected with PRCV alone, compared with the other three groups, suggest that PRRSV infection may impair IL-6 production in the lungs. In addition, higher levels of IL-6 in the serum and lungs were associated with high fever, reduced body weight gain, and more severe lung pathology (51). Viral-bacterial co-infection (PRCV + LPS from *E. coli*) also potentiated the cytokine response to LPS in BALF of IL-6 compared to single inoculations (46). Moreover, the authors found TNF- α and IL-1 potentiation during dual inoculation with PRCV and LPS (46).

In general, simultaneous infection with PRRSV and PRCV seems to aggravate clinical signs and gross lung

lesions, is likely to promote PRRSV replication in the lungs, and alters the cytokine profile in the lungs compared to mono-infection.

PRRSV and PCV2 co-infection

In most cases, infection with PCV2 has a subclinical course (57), but co-infection with other pathogens may exacerbate clinical signs (57). Pigs co-infected with PCV2 and PRRSV developed more severe clinical signs than those mono-infected with PCV2 (2, 11, 19, 69). Both PCV2 and PRRSV can target host immune cells and impair host defense, resulting in immunosuppression, impaired growth performance, and increased morbidity and mortality (38). In a study by Harms et al. (19), mortality reached 91% ($n = 11$) in co-infected pigs, but was only 26% ($n = 19$) in PCV2-inoculated animals and 0% ($n = 13$) and those inoculated with PRRSV. Lung lesions were similar to, but more severe than those observed in PRRSV pigs (19).

In a study by Tu et al. (66), pigs inoculated simultaneously with PCV2 and PRRSV showed a significant increase in IL-1 α mRNA expression, but the investigated expressions were measured in peripheral blood mononuclear cells (PBMC). An increase in the expression of TLR (toll-like receptor) 2 mRNA, TLR4 mRNA, and TLR8 in PBMC of pigs inoculated with PCV2 and PRRSV triggered a significantly increased secretion of IL-1 α , which could exacerbate microscopic lesions (66). TLR receptors are expressed in leukocytes, and their stimulation induces the production of proinflammatory cytokines and chemokines (16). The authors also reported a significant decrease in the expression of TLR3 mRNA, TLR7 mRNA, and TLR9 mRNA in co-infected pigs, which could drive a reduction in IFN- α secretion, also observed in this group. A decrease in TNF- α mRNA expression and an insignificant increase in the level of IL-10 mRNA were also observed in the co-infected group. Limited INF- α and TNF- α expression and a slight increase in IL-10 expression could result in an insufficient immune response in pigs undergoing co-infection, which could be related to lesions observed in this group: mild interstitial pneumonia and lymphocytic depletion were significantly more severe in pigs co-infected with PCV2 and PRRSV than in those infected solely with PCV2 (66). Significant increases in IL-10 production were also observed in monocyte-derived dendritic cells in pigs inoculated with both PCV2 and PRRSV (NADC-20 or VR-2385 strains) (51). In pigs inoculated with highly pathogenic PRRSV and then inoculated with PCV2 one week later, the earlier PRRSV infection primed the expression of TNF- α and IL-10 in serum, which was then enhanced by the PCV2 infection. Due to the immunosuppression of IL-10, the expression of other cytokines was inhibited, similar to the insufficient immune response reported by Tu et al. (66). Therefore, observations of Tu et al. (66) regarding limited INF- α and TNF- α expression are not consistent with increased

IFN- α mRNA and TNF- α mRNA expression reported by Tsai et al. (65) in porcine alveolar macrophages (PAMs) infected with PCV2 and PRRSV. Pulmonary PAMs are major target cells for PRRSV and PCV2 in the lungs (7, 8, 65). Moreover, Tsai et al. (65) reported that increased expression of the mRNA of IL-8, IFN- α , and TNF- α may contribute to pneumonia and bronchiolar epithelial damage in the lungs of pigs co-infected with PCV2 and PRRSV (65).

The above-mentioned studies thoroughly investigated cytokine concentrations in PBMC, sera, and PAMs, but did not provide data concerning the kinetics of local cytokine profiles in the lung tissue of pigs co-infected with PRRSV and PCV2. Hence, further studies using lung homogenates are required to assess the impact of co-infection with these pathogens on the local cytokine profiles.

PCV2 and SIV co-infection

Co-infections with PCV2 and SIV in pigs seem to occur frequently in the field (42). However, the available data regarding the local inflammatory response associated with this combination of pathogens are scarce.

In a study by Wei et al. (71), 24 pigs were divided into 3 groups. Pigs from groups 1 and 2 were inoculated twice with PCV2b (strain ADDLPP 10069, 1.6×10^5 TCID₅₀/mL). During the second inoculation, pigs from group 1 were also inoculated with SIV H1N1 (A/Swine/A07-7967/IN, 2×10^7 PFU/mL) (71). The course of the disease in pigs from group 2 was mild and short (without coughing). On the other hand, the dually infected animals exhibited an increased respiratory rate and occasional coughing. Clinical signs in this group lasted 4 times as long as in the PCV2b mono-infected group. Respiratory scores were significantly higher for the co-infected pigs than for the PCV2 group from 9 to 23 DPI. Pooled lung homogenates were subjected to real-time PCR, and results revealed that co-infection with PCV2 and SIV did not increase the number of PCV2 genome copies in serum or lung tissue or the severity of microscopic lesions associated with PCV2 in the lungs or lymph nodes. Although the clinical course in co-inoculated pigs was more severe than in pigs inoculated with PCV2 only, the scoring of lesions and the titer of PCV2 in the tissues did not differ significantly between groups 1 and 2 (71). Co-infection in this study did not increase PCV2 replication, and only limited synergism between the two viruses was observed. Gross lung lesions were found during necropsy of pigs inoculated with PCV2 and SIV. No similar lung lesions were found in pigs inoculated with PCV2 alone. The limited synergism between these two viruses may have resulted from the low pathogenicity of the SIV strain used in that study (71). Hence, the insufficient level of SIV replication may have failed to induce adequate levels of proinflammatory cytokines (IFN- α , IL-6, TNF- α) or monocyte-attracting chemokines to enhance PCV2 replication in the lungs. The insufficient

SIV level probably limited the number of pulmonary cell deaths, consequently limiting their regeneration and production of enzymes required for PCV2 DNA synthesis.

However, in the latest research on SIV and PCV2 interaction, mice were used to investigate whether SIV infection could induce excessive inflammation and drive immunosuppression together with attenuating protective immune responses to PCV2 vaccines (63). SIV significantly decreased the TNF- α mRNA

level and enhanced the IL-10 mRNA level in the lungs of mice from a group inoculated with subunit PCV2-vaccine followed by a PCV2 challenge. Further studies are required to investigate the local effects of co-infection with SIV and PCV2 on the respiratory tract, especially in pigs.

In pigs, mixed respiratory infections are a common problem leading to significant economic losses and pig welfare aggravation. Understanding the underlying mechanisms of the local immune response in the lungs during viral co-infections in pigs can contribute to the development of more effective preventive and therapeutic methods and eradication strategies, thus reducing economic losses associated with swine complex respiratory infections. Cytokine profiles in co-infected pigs (Tab. 1) were altered and dependent on the combination of viruses or the matrix used to measure cytokine concentration/expression. Simultaneous infections with SIV and PRRSV did not significantly influence the lung cytokine profile compared to mono-infections. The differences observed in gross lung lesions probably resulted from infection dynamics rather than interaction between viruses. Compared with mono-infection, co-infection with PRCV and PRRSV resulted in more acute clinical signs, more severe lung lesions, and more significant changes in lung cytokine profiles. Data regarding the local inflammatory response during other co-infections (PRRSV + PCV2 or PCV2 + SIV) in the porcine respiratory tract are scarce.

It should be emphasized that, given the current state of research, it seems that analysis of cytokine concentrations is insufficient to determine the nature of the infection (viral, bacterial, or mixed). Nevertheless, currently available data indicate that in some infections serum or BALF cytokine levels may serve as markers of disease or lung lesion severity. The knowledge of the impact of single and mixed infections on the profile of the inflammatory response in the lungs may be used to develop an appropriate therapy, including recommen-

Tab. 1. Cytokine concentration/expression in lungs during porcine respiratory tract mono- and co-infections

Pathogen	Cytokine concentration/expression		Reference
	Increased	Decreased	
PRRSV	IFN- α , IL-1 β , IL-6 (BALF) IL-10 (BALF, lung)	IFN- α , TNF- α , IFN- γ , IL-12 (lung)	15, 37
SIV	IL-1 (BALF) IL-1 β , IL-8, IL-10 (lung) IL-6, IL-12, TNF- α , IFN- α , IFN- γ (lung, BALF)	IL-10 (lung)	3, 23, 44, 68
PCV2	IL-1 α (lung) IL-8 (lung, AM) TNF- α (AM)	–	6, 7
SIV + PRRSV	IFN- α , IL-1 β , IL-8, TNF- α (lung) IFN- γ , IL-6, IL-10 (lung, BALF)	IFN- γ , IFN- α , IL-1 β , IL-8 (BALF)	10, 68
PRRSV + PRCV	IL-6, IL-10, IL-12, TGF- β (lung)	IL-6 (lung)	50
PRRSV + PCV2	IL-8, IFN- α , TNF- α (AM)	–	65

Explanations: BALF – bronchoalveolar lavage fluid; AM – alveolar macrophages; IL – interleukin; TNF – tumor necrosis factor; TGF – transforming growth factor

dations regarding the usage of anti-inflammatory drugs. Further investigations are advisable to evaluate the local immune response during polyetiological infections of the porcine respiratory tract and its usefulness in the diagnostics and/or treatment.

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