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Polymicrobial respiratory disease in pigs

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Abstract

Respiratory disease in pigs is common in modern pork production worldwide and is often referred to as porcine respiratory disease complex (PRDC). PRDC is polymicrobial in nature, and results from infection with various combinations of primary and secondary respiratory pathogens. As a true multifactorial disease, environmental conditions, population size, management strategies and pig-specific factors such as age and genetics also play critical roles in the outcome of PRDC. While non-infectious factors are important in the initiation and outcome of cases of PRDC, the focus of this review is on infectious factors only. There are a variety of viral and bacterial pathogens commonly associated with PRDC including porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), porcine circovirus type 2 (PCV2), *Mycoplasma hyopneumoniae* (MHYO) and *Pasteurella multocida* (PMULT). The pathogenesis of viral respiratory disease is typically associated with destruction of the mucocilliary apparatus and with interference and decrease of the function of pulmonary alveolar and intravascular macrophages. Bacterial pathogens often contribute to PRDC by activation of inflammation via enhanced cytokine responses. With recent advancements in pathogen detection methods, the importance of polymicrobial disease has become more evident, and identification of interactions of pathogens and their mechanisms of disease potentiation has become a topic of great interest. For example, combined infection of pigs with typically low pathogenic organisms like PCV2 and MHYO results in severe respiratory disease. Although the body of knowledge has advanced substantially in the last 15 years, much more needs to be learned about the pathogenesis and best practices for control of swine respiratory disease outbreaks caused by concurrent infection of two or more pathogens. This review discusses the latest findings on polymicrobial respiratory disease in pigs.

Keywords: porcine, co-infection, respiratory disease, polymicrobial, review

Introduction

The presence of respiratory disease in pigs is commonly associated with significant production losses and is considered one of the main obstacles for the swine industry. If a thorough diagnostic investigation is conducted, results indicate that clinical respiratory disease in pigs is often polymicrobial, resulting from a combined infection with one or more viruses and bacteria. Respiratory pathogens can be further divided into primary pathogens, capable of inducing severe lesions in respiratory tissues as a result of their own virulence, and secondary or opportunistic infectious pathogens that typically need help from other co-infecting pathogens or cofactors to induce substantial lesions in the respiratory system. Moreover, based on their relevance to the swine industry in a particular region or population, respiratory pathogens in pigs can perhaps also be divided into pathogens of major and minor importance or relevance (Table 1). Compared to other domestic animals, pigs are unique because they are typically housed in large groups in relatively small spaces, thereby providing ideal conditions to maintain pathogens for extended time periods. Pathogens involved in respiratory disease in pigs vary significantly among farms, production sites, regions and

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Table 1. Commonly recognized pathogens of major or minor relevance to the pig industry based on their ability to induce respiratory disease and lesions by themselves (major relevance) or under circumstances such as a concurrent infection with other pathogens (minor relevance)

	Pathogen	Abbreviation	Major relevance	Minor relevance
Viruses	Paramyxovirus	PMV		×
	Porcine circovirus type 2	PCV2		×
	Porcine cytomegalovirus	PCMV		×
	Porcine reproductive and respiratory syndrome virus	PRRSV	×	
	Porcine respiratory coronavirus	PRCV		×
	Pseudorabies virus	PRV	×	
	Swine influenza virus	SIV	×	
	Torque teno sus virus	TTSuV		×
Bacteria	*Actinobacillus pleuropneumoniae*	APP	×	
	Actinobacillus suis	ASUIS	×	
	Arcanobacterium pyogenes	APYO		×
	Bordetella bronchiseptica	BORD	×	
	Haemophilus parasuis	HPS		×
	Mycoplasma hyopneumoniae	MHYO	×	
	Mycoplasma hyorhinis	MHYOR		×
	Pasteurella multocida	PMULT		×
	Salmonella spp.	SALM	×	
	Streptococcus suis	SSUIS		×

countries, making generalizations about porcine respiratory disease complex (PRDC) treatment and control difficult (Gutiérrez-Martín *et al.*, 2000; Harms *et al.*, 2002). In some regions of the world, establishment of an accurate diagnosis is often lacking due to limited availability of the most appropriate diagnostic tools which compromises implementation of the most appropriate treatment and control regimens.

The interactions that occur on the cellular and molecular levels during concurrent infection of pigs with two or more respiratory pathogens are multifaceted and complex. The standard paradigm of an initial primary viral infection followed by opportunistic bacterial infection certainly occurs, but the interactions can be much more complex involving multiple viruses and bacteria and different sequences of infection. The modes of action of pathogens can, when combined, be additive or synergistic. Because of the complicated nature of mixed infections, studying their interactions can be a laborious task. Under experimental conditions, it is often difficult to reliably reproduce clinical respiratory disease involving a single pathogen; it is even more difficult to design experimental studies that examine the interactions among two or more pathogens.

PRDC

The term 'porcine respiratory disease complex (PRDC)' is now regularly used to describe mixed respiratory infections of multiple etiologies (Halbur, 1998). The clinical picture of PRDC is often characterized by decreased feed intake, fever, cough and dyspnea ultimately resulting in decreased feed efficiency and decreased growth rate. Morbidity rates associated with PRDC may range from 30 to 70% and mortality rates between 4 and 6% or even higher in affected farms (Halbur, 1998). Macroscopic lesions highly depend on the pathogen involved and can vary from no visible lesions to nearly 100% multifocal tan mottling or consolidation of the lung. Frequently, lesions are located in the cranioventral parts of the lung, where consolidation, discoloration and failure of the lung tissue to collapse may be observed (Harms *et al.*, 2002; Martínez *et al.*, 2009; Fraile *et al.*, 2010). Similarly, microscopic lesions may vary according to the pathogens involved, but bronchopneumonia, sometimes in combination with interstitial pneumonia is often reported (Harms *et al.*, 2002; Kim *et al.*, 2003).

Factors involved in PRDC

The severity of PRDC manifestation in pigs depends upon the interactions and synergy of infectious factors (both viral and bacterial pathogens), environmental factors, type of production system and management, and pig-specific factors (genetics, age and immunological status).

Infectious factors

Many respiratory pathogens are ubiquitous; thus, it is not uncommon for multiple pathogens to circulate in a herd at any given time. New pathogens commonly enter a herd through aerosol, fomites, vectors and in many cases through the introduction of infected animals. The pathogens frequently associated with PRDC and abbreviations used in this review are summarized in Table 1.

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Table 2. Trends in pathogen detection over time in swine respiratory case submissions to the Veterinary Diagnostic Laboratory at Iowa State University

	Pathogen	2003	2004	2005	2006	2007	2008	2009	2010
Viral	PRRSV	1088	928	1094	2191	2113	1679	1104	1217
	SIV	600	535	730	1225	1182	965	824	1000
	PCV2	403	344	378	920	696	248	84	68
Bacterial	PMULT	663	550	634	1060	858	720	388	401
	MHYO	360	279	321	627	497	412	345	469
	BORD	198	151	91	139	216	248	140	92
	APP	163	112	110	97	95	90	91	59
	Bacteria associated with septicemia[1]	748	676	882	1351	1226	1178	642	631
	Total numbers of porcine tissue submissions	2240	2374	3450	5876	6628	5019	3824	4402

[1]APYO, HPS, SALM, SSUIS and ASUIS.

Viral pathogens

Porcine reproductive and respiratory syndrome virus (PRRSV) replicates in macrophages and dendritic cells located in the tonsil, upper respiratory tract and lungs, resulting in viremia as early as 12 h post infection which can persist for several weeks or months (Wills *et al.*, 2003). It has been shown that PRRSV induces cell lysis (Thanawongnuwech *et al.*, 1997), apoptosis (Labarque *et al.*, 2003), changes in function of infected pulmonary alveolar and intravascular macrophages (Thanawongnuwech *et al.*, 1997, 1998b), damage to the mucociliary apparatus (Thanawongnuwech *et al.*, 1998a) and changes in T-cell subpopulations (Shimizu *et al.*, 1996), ultimately resulting in increased susceptibility to opportunistic bacterial pneumonia and septicemia. Based on data from case submissions (Table 2) to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL), PRRSV is the number 1 pathogen detected in swine respiratory disease in the USA. However, the overall trend for PRRSV-induced pneumonia based on percentage of total number of porcine tissue cases received for the last 8 years indicates that PRRSV-associated respiratory disease is slowly declining (Fig. 1). This may be due to recent PRRSV elimination efforts by individual pork producers and systems, and regional PRRS control and eradication projects. Alternatively, this may be due to overall higher numbers of growing pigs that are being vaccinated against PRRSV in the USA, which is mainly due to increased efforts to vaccinate pigs from negative farms before or shortly after they have been sent to finishing units located in pig dense areas (Loula, 2011). As with all prevalence estimates based on submissions to veterinary diagnostic labs the data presented in this review should be evaluated with caution due to potential influence of factors unrelated to pig health. For instance, it is well known that the increased media attention and public awareness during the 2009 flu pandemic negatively impacted submission rates of pigs and pig tissues to veterinary diagnostic labs which may be incorrectly interpreted as an improvement in the overall health of pigs in the Midwest.

Similar to PRRSV, **swine influenza virus (SIV)** also has a tropism for the respiratory system where it attaches

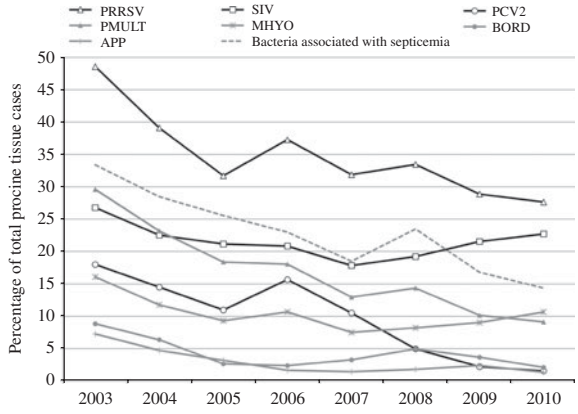


Fig. 1. Trends in pathogens associated with respiratory disease in case submissions to the Veterinary Diagnostic Laboratory at Iowa State University. Data presented as percentage of total number of porcine tissue cases received.

to and replicates in epithelial cells of the upper respiratory tract (Brown *et al.*, 1994). Infection then spreads to bronchi and bronchioles resulting in loss of cilia, extrusion of mucus, exudation of neutrophils and macrophages, and necrosis and metaplasia of airway epithelium (Brown *et al.*, 1994). It has been demonstrated that levels of certain cytokines, tumor necrosis factor α (TNFα), interferon α (IFNα), and interleukin-1 (IL-1), are all elevated 18–24 h after inoculation with SIV and correlate with neutrophil infiltration as well as onset and degree of clinical signs (Karasin *et al.*, 2000; Charley *et al.*, 2006). SIV-induced damage to the respiratory tract reduces the ability to clear the infection and also decreases macrophage function (Dea *et al.*, 1992; Rekik *et al.*, 1994; Thacker, 2001). SIV is the second most commonly identified viral respiratory pathogen based on data from case submissions to the ISU-VDL, and the overall 8-year-trend indicates that the incidence of SIV-induced pneumonia cases remains relatively steady (Fig. 1).

Pseudorabies virus (PRV) replicates in alveolar macrophages and monocytes, and causes necrotic rhinitis and tracheitis with disrupted ciliary activity in the respiratory mucosa. PRV-induced damage to the respiratory tract mucosa may contribute to decreased clearance

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and increased adherence of other pathogens (Narita *et al.*, 1994). Macrophage functions such as phagocytosis, killing of phagocytized bacteria, antibody-dependent and -independent cytotoxicity, IFNα production, phagosome–lysosome fusion and oxygen production after stimulation with opsonized zymosan were reduced in PRV-infected macrophages (Fuentes and Pijoan, 1986; Iglesias *et al.*, 1989, 1992). Moreover, direct destruction of monocytes and macrophages, which are involved in both innate and active immunity, may also contribute to the degree of immunosuppression (Fuentes and Pijoan, 1986; Iglesias *et al.*, 1989, 1992). In the USA, PRV has been eradicated from the domestic pig population; however, since it is still circulating in the wild pig population in certain regions, PRV-associated respiratory disease has to remain on the respiratory disease complex differential diagnosis list.

Porcine circovirus type 2 (PCV2) is now widely accepted to be an important pathogen in PRDC. PCV2-infection has been associated with mild interstitial pneumonia and may induce attenuation and ulceration of the airway epithelium and cause prominent peribronchiolar and peribronchial fibroplasia (Kim *et al.*, 2003). PCV2 antigen has been demonstrated in the cytoplasm of pulmonary macrophages, the bronchiolar epithelium and endothelial cells, which is suggestive that PCV2 may be contributing to the severity and duration of PRDC (Harms *et al.*, 2002). PCV2-associated respiratory disease in the USA reached its peak during 2006 and 2007 (Table 2). In support of the important role of PCV2 in PRDC, implementation of PCV2 vaccination in PRDC affected herds substantially lowers the incidence of respiratory disease and pulmonary co-infections (Fachinger *et al.*, 2008). The efficacy of PCV2 vaccination is also clearly seen in the overall trend for PCV2-induced pneumonia at the ISU-VDL, which indicates a dramatic decline of PCV2-associated pneumonia from 2008 to 2010 (Fig. 1). To better mimic what is going on under field conditions and to have an improved model to test the efficacy of different treatment strategies, poly-viral experimental inoculations have gained importance in recent years in PCV2 research. Researchers have experimentally inoculated pigs concurrently with PCV2 and PRRSV (Opriessnig *et al.*, 2008, Sinha *et al.*, 2010), PCV2, PRRSV and SIV (Opriessnig *et al.*, 2009a), or PCV2, PRRSV and porcine parvovirus (PPV) (Shen *et al.*, 2010; Opriessnig *et al.*, 2011b) to better mimic the field situation and evaluate vaccine efficacy.

Porcine respiratory coronavirus (PRCV) replicates primarily in the epithelium of the lower respiratory tract, in type 2 pneumocytes, and to a lesser extent in bronchi and bronchiolar epithelium (Atanasova *et al.*, 2008). PRCV induces disruption of the mucociliary apparatus and decreases pulmonary alveolar macrophage function, which reduces the ability of macrophages to clear bacteria (Charley *et al.*, 2006).

Porcine cytomegalovirus (PCMV) replicates in and destroys cells of the nasal mucosa and tubuloalveolar glands where it is associated with large intranuclear inclusion bodies (Narita *et al.*, 1987). PCMV-induced damage to the upper respiratory tract epithelium and mucociliary apparatus likely makes pigs more susceptible to bacterial pathogens (Edington *et al.*, 1977; Narita *et al.*, 1987).

Paramyxoviruses (PMV) such as porcine rubulavirus and Nipha virus play a role in respiratory disease in certain geographic regions where they are associated with economically relevant disease outbreaks in affected pig populations (Hooper *et al.*, 2001; Rodríguez-Ropón *et al.*, 2003). A PMV different from porcine rubulavirus and Nipha virus was associated with interstitial pneumonia in pigs in the USA (Janke *et al.*, 2001) indicating that this may be another contributor to PRDC. However, since it is identified only on rare occasions, it is likely of minor relevance.

Torque teno sus virus (TTSuV) was only recently associated with respiratory disease in pigs. When gnotobiotic pigs were infected with TTSuV genogroup 1 (TTSuV-1), the pigs developed mild interstitial pneumonia (Krakowka and Ellis, 2008) implying a potential role for this virus in PRDC. In support of this, a high prevalence of TTSuV infection (54–82%) was detected in Japanese pigs which were 60 days and older, and suffered from PRDC or porcine circovirus-associated disease (Taira *et al.*, 2009). When serum samples from 253 pigs from 83 farms were tested for TTSuV-1 and TTSuV-2 DNA, there was a correlation between the diagnosis of PRDC and the detection of TTSuV (Lang *et al.*, 2011). Moreover, under experimental conditions using gnotobiotic pigs, combined PCV2 and TTSuV-1 infection resulted in systemic clinical disease (Ellis *et al.*, 2008) and combined PRRSV and TTSuV-1 infection resulted in clinical disease resembling porcine dermatitis and nephropathy syndrome (Krakowka *et al.*, 2008). The relevance of these findings is unknown as TTSuV is widely distributed in swine populations. There is only limited data available from field and experimental studies and much more needs to be learned about this virus and its importance in PRDC.

Bacterial pathogens

***Actinobacillus pleuropneumoniae* (APP)** resides in tonsils, necrotic lung tissue and the nasal cavity (Willson *et al.*, 1987). There is a marked difference in virulence between serotypes and strains within serotypes. Virulence factors include three exotoxins (ApxI, ApxII and ApxIII), which play a role in inducing the characteristic necrosis and hemorrhage (Kamp *et al.*, 1997). The capsule of APP is also capable of inhibiting phagocytosis (Sakano *et al.*, 1993; Kamp *et al.*, 1997). Based on diagnostic case submissions to the ISU-VDL, APP-associated respiratory disease is of minor importance compared to other bacterial infections (Table 2) and remains consistently at

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a low level based on overall percentages of cases diagnosed as APP (Fig. 1).

***Mycoplasma hyopneumoniae* (MHYO)** attaches to the cilia and surface of epithelium by adhesion proteins, resulting in clumping, damage and loss of cilia, epithelial cell death and reduced function of the mucociliary apparatus (DeBey and Ross, 1994; Young *et al.*, 2000). The loss of cilia is thought to be important in the increased incidence of secondary bacterial infections associated with MHYO. Infection with MHYO is commonly associated with immunostimulation and immunosuppression (Kishima and Ross, 1985; Kishima *et al.*, 1985; Maes *et al.*, 1996), non-specific mitogenic effects on swine lymphocytes (Messier and Ross, 1991), or cytokines secreted by activated macrophages (Messier and Ross, 1991; Asai *et al.*, 1993; Thacker *et al.*, 2000a; Thanawongnuwech *et al.*, 2001). Among bacterial causes of respiratory disease in pigs in the USA, MHYO is the second most common following *Pasteurella multocida* (PMULT) (Table 2) and despite widespread vaccination, mycoplasmal pneumonia remains a major concern for pig producers. Recent studies indicate that *Mycoplasma hyorhinis* (MHYOR) may also be important in PRDC (Lin *et al.*, 2006; Hansen *et al.*, 2010), but more research in this area is needed.

***Bordetella bronchiseptica* (BORD)** is known to cause attenuation of the respiratory epithelium and loss of cilia (Sakano *et al.*, 1984). The dermonecrotic toxin of BORD causes turbinate atrophy and pneumonic lesions characterized by necrosis, hemorrhage, neutrophil accumulation and fibrosis (Sakano *et al.*, 1992; Horiguchi, 2009). Thus, BORD infection can result in delayed clearance mechanisms, increased accumulation of mucus and exposure of submucosal areas. BORD is also cytotoxic for alveolar macrophages *in vitro* (Brockmeier *et al.*, 2001; Brockmeier and Register, 2007). In the USA, BORD is most commonly seen in younger pigs and has a relatively low prevalence in diagnostic submissions to the ISU-VDL (Table 2 and Fig. 1).

***Pasteurella multocida* (PMULT)** is generally considered to be an opportunistic invader that is rapidly cleared from the lungs of normal pigs. PMULT has a polysaccharide capsule that aids in resisting phagocytosis and the Tox-A protein, which is an essential virulence factor for progressive atrophic rhinitis (Lax and Grigoriadis, 2001; Horiguchi, 2009; Wilson and Ho, 2010). The detection of Tox-A is frequently used for confirmation of pathogenicity of PMULT isolates (Lichtensteiger *et al.*, 1996; Takada-Iwao *et al.*, 2007). PMULT remains the most common respiratory bacterium identified in cases of PRDC at the ISU-VDL (Table 2 and Fig. 1).

***Haemophilus parasuis* (HPS), *Streptococcus suis*, *Actinobacillus suis*, *Arcanobacterium pyogenes* and *Salmonella enterica* serovars Choleraesuis and Typhimurium** may induce lung damage either directly (rare) or in association with septicemia. HPS is commonly isolated from nasal secretions and tracheal swabs of healthy pigs (Bertschinger and Nicod, 1970). The septicemic form of HPS infection is better known as Glässer's disease. *Streptococcus suis* (SSUIS) invades tonsils and reaches the lymph nodes via lymphatics (Cloutier *et al.*, 2003). Infected monocytes may distribute the organism throughout the body including the brain (Zheng *et al.*, 2009). The capsule of SSUIS inhibits phagocytosis and antigens associated with virulence include muramidase-release protein (MRP), capsid and extracellular factor (EF) (Smith *et al.*, 1999; Tanabe *et al.*, 2010). Similar to APP, *Actinobacillus suis* (ASUIS) can be carried in tonsils and nostrils of healthy pigs (Ross *et al.*, 1972) and infection likely occurs via the upper respiratory tract. *Arcanobacterium pyogenes* (APYO) is another common pathogen on the membranes of the upper respiratory tract and is frequently associated with suppurative inflammation and abscess formation. Finally, *Salmonella enterica* (SALM), most commonly serovars Choleraesuis and Typhimurium, can also be associated with respiratory disease in pigs.

Parasites

Disease conditions such as parasitism can also negatively impact on the pig's ability to respond to respiratory pathogens (Tjørnehøj *et al.*, 1992). Especially for pigs raised outdoors, lungworms (*Metastrongylus* spp.) are commonly encountered with respiratory disease. Adult lungworms, ranging from 14 to 55 mm and localized within the terminal bronchioles, elicit bronchitis, bronchoalveolitis, alveolar emphysema and atelecatsis (Stewart and Hoyt, 2006). Ascarid larvae also migrate through lungs and when exposure is high in naïve pigs ascarids can cause severe respiratory disease and lesions. *Ascaris suum* has also been shown to negatively impact MHYO vaccination in pigs (Steenhard *et al.*, 2009).

Non-infectious factors

Various non-infectious factors are significant contributors to respiratory disease by increasing transmission and spread of the pathogens, by creating unfavorable conditions that result in increased stress on the animal, or by directly damaging the respiratory tract (Sorensen *et al.*, 2006; Martínez *et al.*, 2009). The non-infectious factors contributing to the disease process can by divided broadly into three groups including environment, management (system, herd size and nutrition) and pig factors (age, genetics, breed and immune status), which have been summarized previously (White, 2011). Commonly, severe environmental stressors such as, but not limited to, extreme temperature changes, elevated humidity, aerial pollutants and excessive ammonia are known to predispose pigs to development of PRDC (Done, 1991). Feed quality is also important as dust in

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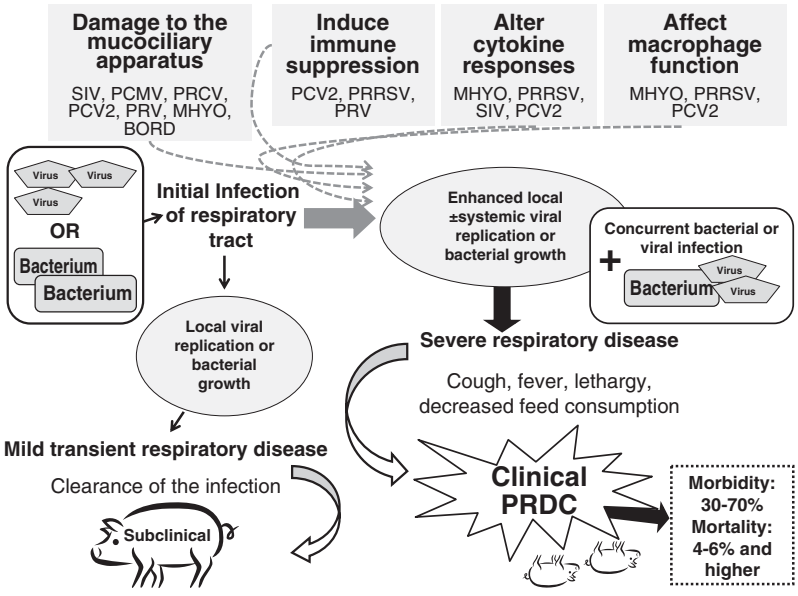


Fig. 2. Summary of possible mechanisms and outcomes of viral and bacterial infections of the respiratory tract.

feed can interfere with the pig's respiratory tract defense. In addition, molds or mycotoxins in feed can either directly damage lungs or compromise immunity. Nutritional deficiencies can also affect the immune system.

The age and breed of the pig at the time of infection can also be of importance. PRDC is predominantly seen in grow–finish pigs, typically around 12–24 weeks of age, and may be associated with waning of passively acquired antibodies (Thanawongnuwech *et al.*, 1998b; Cho *et al.*, 2006; Pomorska-Mól *et al.*, 2011). To the authors' knowledge, to date no specific resistance factors to respiratory pathogens have been identified in pigs. However, evidence of increased susceptibility of certain breeds, or lines within breeds, to disease induced by certain viruses (PRRSV and PCV2) has been reported (Halbur *et al.*, 1998; Vincent *et al.*, 2005; Opriessnig *et al.*, 2006, 2009b; Vincent *et al.*, 2006). Finally, when pigs from multiple sites and with different immune status are mixed, disease spread is enhanced. In problem herds, vaccination can be used as a means to bring the immune status of individuals in the population to a similar level.

Interaction of pathogens in PRDC

The interactions between different pathogens play an important role in the development and outcome of PRDC. Although different pathogens are capable of inducing disease independently, simultaneous infection with two or more viral pathogens commonly results in more severe clinical signs and lesions (Allan *et al.*, 2000; Harms *et al.*, 2001). Some known mechanisms by which pig pathogens interact include damaging of the mucociliary apparatus in respiratory tissues to allow bacterial colonization (Pol *et al.*, 1997; Loving *et al.*, 2010), inducing immune suppression (Renukaradhya *et al.*, 2010), altering cytokine responses (Thanawongnuwech *et al.*, 2004), or affecting macrophage function (Chiou *et al.*, 2000) (Fig. 2). In addition, the sequence of infection is likely also of great importance and should be considered when evaluating outcomes of experimental studies (Table 3). The following paragraphs summarize some of the experimental co-infection models using different combinations of bacteria and viruses or mixed viral–bacterial infections.

Bacterial–bacterial interactions

A summary of selected study outcomes and potential mechanisms in poly-bacterial respiratory disease is provided in Table 3(A). MHYO and PMULT represent the most commonly identified respiratory bacteria based on case submissions to the ISU-VDL in 2010 (Table 2 and Fig. 1). The combination of these pathogens is frequently identified in the same pig indicating a potential interaction. It is well known and experimentally confirmed that inoculation with MHYO alone causes only mild disease and lung lesions (Thacker *et al.*, 1999, Opriessnig *et al.*, 2004). Similarly, inoculation with PMULT alone typically causes no disease and the organism is difficult to recover from experimentally infected pigs (Ciprián *et al.*, 1988; Amass *et al.*, 1994). However, dual infection with MHYO and PMULT induces disease characterized by elevated rectal temperatures, a severe cough, and more extensive lung lesions from which both organisms can be isolated (Ciprián *et al.*, 1988; Amass *et al.*, 1994). In these experiments, if pigs were MHYO vaccinated before MHYO–PMULT challenge or if the pigs were allowed to recover from MHYO challenge, they were not susceptible to

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Table 3. Selected examples of interactions and up-regulating mechanisms in polymicrobial respiratory diseases in pigs. (A) Bacterial–bacterial interactions. (B) Viral–viral interactions. (C) Bacterial–viral interactions. SPF, specific-pathogen-free; CD, colostrum-deprived; CDCD, cesarean-derived, colostrum-deprived pigs

Pathogens	Pig type	Interval between infections	Outcomes and potential up-regulating mechanisms	Reference
(A) Bacterial–bacterial				
MHYO–PMULT	Conventional	23 days	PMULT increases the severity of pneumonia initiated by MHYO.	Ciprián *et al.* (1988)
MHYO–APP	Conventional	28 days	MHYO-induced damage in the respiratory tract enhances APP.	Marois *et al.* (2009)
	Conventional	14 or 28 days	Alveolar macrophages from co-infected pigs have decreased phagocytic abilities.	Caruso and Ross (1990)
BORD–PMULT	CDCD	4 days	BORD predisposes to PMULT colonization. Dermonecrotic toxin is not necessary. Increased adhesion of PMULT.	Brockmeier and Register (2007)
BORD–HPS	Conventional	7 days	BORD predisposes to HPS colonization.	Brockmeier (2004)
(B) Viral–viral				
PRRSV–PRCV	Conventional	3 days	Enhanced lung pathology but replication of PRCV is not affected by prior infection with PRRSV.	Van Reeth *et al.* (1996)
	Gnotobiotic	3 or 7 days	Milder disease compared to conventional pigs. The combination of IFNα and IL-1 could contribute to the systemic signs of the dual PRRSV–PRCV infection.	Van Reeth and Nauwynck (2000)
	SPF	10 days	Enhanced disease due to interactive effects via early innate and adaptive immune responses.	Jung *et al.* (2009)
	Conventional	10 days	Enhanced disease likely due to the fact that each virus modulates the host innate and adaptive immune responses, leading to inflammatory reactions and subsequent lesions.	Renukaradhya *et al.* (2010)
PRRSV–SIV	Conventional	3 days	Enhanced lung pathology but replication of SIV is slightly affected by prior infection with PRRSV.	Van Reeth *et al.* (1996)
PRCV–SIV	Conventional	2–3 days	Increased disease severity.	Van Reeth and Pensaert (1994)
PRRSV–PRV	SPF	7 days	Dual infection with PRRSV and PRV increases and prolongs clinical signs and pneumonic lesions. PRV induced immune-suppression may alter the function and survival of lymphocytes.	Shibata *et al.* (2003)
PRRSV–PCV2	CD	Same day	Disease up-regulation possible due to the fact that monocyte/macrophage cell types are common targets of these viruses.	Allan *et al.* (2000)
	CDCD	Same day	Disease up-regulation and increased severity of PRRSV induced lung lesions.	Harms *et al.* (2001)
	Conventional	7 days	PRRSV up-regulates PCV2 and could act as a systemic immunostimulator or by interfering with PCV2 clearance.	Rovira *et al.* (2002)
	Conventional	Same day	PCV2-induced IFNα reduces PRRSV infection and PRRSV-associated cytopathic effect.	Chang *et al.* (2005)
PCV2–PPV	Conventional	Same day	Porcine alveolar macrophages display reduced phagocytosis and lower IFNα1 and IFNγ expression levels.	Liu *et al.* (2011)
(C) Bacterial–viral				
PRRSV–SSUIS	Conventional	7 days	More severe disease in co-infected groups may be due to PRRSV-induced suppression of pulmonary intravascular macrophage function which may explain PRRSV-associated increased susceptibility to SSUIS infection.	Thanawongnuwech *et al.* (2000)
	Conventional	7 days	PRRSV infection increased the susceptibility to SSUIS.	Xu *et al.* (2010)

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Table 3 (Continued)

Pathogens	Pig type	Interval between infections	Outcomes and potential up-regulating mechanisms	Reference
PRRSV–BORD	Conventional	Same day	Exacerbated disease in co-infected pigs.	Brockmeier *et al.* (2000)
	Conventional	Same day	Co-infection predisposed to infection with PMULT. PRRSV and BORD may interact to adversely affect respiratory tract defense mechanisms.	Brockmeier *et al.* (2001)
PRRSV–PMULT	Conventional	5 days	No clear interaction between PRRSV and PMULT.	Carvalho *et al.* (1997)
MHYO–PRRSV	Conventional	21 days	Higher PRRSV viral load in co-infected pigs.	Cho *et al.* (2006)
	Conventional	21 days	MHYO potentiated PRRSV infection and lesions.	Thacker *et al.* (1999)
	Conventional	5 h	Increased production of pro-inflammatory cytokines IL-1α, IL-1β, IL-8 and TNF in the MHYO–PRRSV group suggests that cytokine-induced inflammation may play an important role in the severe, chronic pneumonia.	Thanawongnuwech *et al.* (2001)
	Conventional	5 h	Enhanced disease in co-infected pigs associated with increased levels of IL-10 which may be a possible mechanism that PRRSV–MHYO co-infection uses to exacerbate the severity and duration of pneumonia.	Thanawongnuwech *et al.* (2004)
PRRSV–APP	SPF	7 days	Enhancement of lesions was seen in only one of three studies suggesting that previous PRRSV infection is not necessary to enhance bacterial infections.	Pol *et al.* (1997)
PRV–PMULT	Conventional	Same day	Severe pneumonia in co-infected pigs.	Fuentes and Pijoan (1987)
PRV–MHYO	CDCD	7 days	PRV infection appears to have effect on the severity of experimentally induced acute MHYO pneumonia in young pigs.	Shibata *et al.* (1998)
PRCV–BORD	Conventional	Same day	Enhanced disease is evident in co-infected pigs which is associated with transcription of pro-inflammatory cytokines, especially IL-6 and Monocyte chemotactic protein-1 (MCP-1).	Brockmeier *et al.* (2008)
MHYO–PCV2	SPF	14 days	Severe PRDC was observed in co-infected pigs mainly due to increase in PCV2.	Opriessnig *et al.* (2004)
	SPF	14 days	Co-infection resulted in enhanced disease.	Opriessnig *et al.* (2011a)
MHYO–SIV	Conventional	21 days	More severe disease in co-infected animals; however, less disease potentiation compared to MHYO–PRRSV co-infection.	Thacker *et al.* (2001)
PCMV–BORD	Gnotobiotic	Same day	Some degree of enhancement of disease due to co-infection; however, no increase in degree of bone atrophy in turbinates.	Edington *et al.* (1976)
SIV–BORD	Conventional	Same day	SIV increased BORD colonization and enhanced production of pro-inflammatory mediators that likely contribute to exacerbated pulmonary lesions.	Loving *et al.* (2010)

subsequent PMULT challenge (Amass *et al.*, 1994). It is thought that the compromised respiratory defense mechanism in this model results from the MHYO-associated ciliostasis that decreases clearance of micro-organisms and mucus.

Similar to what was observed with MHYO and PMULT, lesions in pigs co-infected with MHYO and APP were more severe than with single infection or what would be additive of individual infections (Marois *et al.*, 2009). In fact, pigs infected with the APP isolate used in that study remained healthy and lung lesions were only seen with concurrent MHYO infection (Marois *et al.*, 2009). Alveolar macrophages collected from pigs infected with either MHYO or APP had increased phagocytosis, whereas alveolar macrophages collected from pigs co-infected with both MHYO and APP had decreased phagocytic abilities (Caruso and Ross, 1990). The data suggested that the suppression was due to MHYO rather than APP and that decreased macrophage functions in a pig infected with MHYO may predispose the pig to secondary infections (Caruso and Ross, 1990). Combined challenge of pigs with PMULT and the cytotoxins of APP caused more severe lung lesions than did inoculation of either factor alone (Chung *et al.*, 1994), further confirming a relationship between these pathogens.

BORD is another pathogen that by itself may only cause mild disease, but its interaction with other bacteria, especially toxigenic PMULT, has been well documented (Sakano *et al.*, 1992, 1997). It has been shown that BORD predisposes the upper respiratory tract to colonization with non-toxigenic PMULT type A (Chanter *et al.*, 1989; Brockmeier and Register, 2007). In addition, pretreatment of tracheal rings with BORD has been shown to increase adhesion of PMULT (Dugal *et al.*, 1990, 1992; Brockmeier and Register, 2007). Interestingly, the expression of the dermonecrotic toxin by BORD appears unnecessary for predisposing to PMULT infection (Brockmeier and Register, 2007). BORD has also been shown to predispose the nasal cavity to increasing colonization with HPS (Brockmeier, 2004). Possible mechanisms by which BORD predisposes to infection with other bacteria include use of adhesins secreted by BORD (Nicholson *et al.*, 2009), toxin-induced turbinate atrophy (Brockmeier *et al.*, 2002), damage to ciliated epithelium (Nakai *et al.*, 1988) and others.

Viral–viral interactions

A summary of selected study outcomes and potential mechanisms in poly-viral respiratory disease is provided in Table 3(B). In many pig-producing regions of the world, including the USA (Table 2), PRRSV is the most important viral pathogen in the pig population causing high economic losses (Neumann *et al.*, 2005). When dual infections with PRRSV and PRCV or PRRSV and SIV were examined in conventional pigs, disease was more pronounced in dually infected animals (Van Reeth *et al.*, 1996). When these experiments were repeated with gnotobiotic pigs, the clinical outcome was different. In gnotobiotic pigs, the clinical signs were milder with decreased morbidity and the effect of the co-infection appeared additive rather than synergistic (Van Reeth and Nauwynck, 2000). More recent studies demonstrate that PRRSV modifies the innate immunity, induces immuno-suppression and alters disease outcome in pigs subsequently infected with PRCV, thereby exacerbating the inflammatory response to PRCV in pigs (Jung *et al.*, 2009; Renukaradhya *et al.*, 2010). Exposure to PRCV decreased replication of SIV in the respiratory tract of feeder pigs and disease appeared more severe in co-infected animals than in pigs infected with SIV alone (Van Reeth and Pensaert, 1994). It was speculated that the high IFNα levels induced by PRCV were responsible for inhibition of SIV replication (Van Reeth and Pensaert, 1994). Dual infection of specific pathogen-free pigs with PRRSV and PRV resulted in increased clinical signs and pneumonic lesions in pigs infected with both viruses, as compared to pigs infected with PRRSV or PRV only (Shibata *et al.*, 2003). However, initial infection of 3-week-old pigs with PRRSV followed by SIV 1 week later did not influence clinical disease outcome associated with SIV (Pol *et al.*, 1997).

Until there was widespread implementation of vaccine protocols, the different disease manifestations of PCV2 in pigs including respiratory disease were associated with high morbidity and mortality rates in pigs (Opriessnig *et al.*, 2007). It has been determined that PCV2 by itself is rarely, and only under certain circumstances, associated with disease in conventional pigs. However, it is well known and accepted that PCV2 is up-regulated by a variety of infectious and non-infectious factors including other viruses (Opriessnig and Halbur, 2011). Infection with a combination of PCV2 and PRRSV has been shown to potentiate replication and distribution of PCV2, and results in a more severe clinical outcome than with either virus by itself (Allan *et al.*, 2000; Harms *et al.*, 2001; Rovira *et al.*, 2002; Chang *et al.*, 2005). In this context, it has been speculated that the activation of macrophages in lymphoid tissues may lead to potentiation of PCV2 replication and development of clinical disease (Allan *et al.*, 2000). Additionally, PCV2-infection increased the severity of PRRSV-induced interstitial pneumonia in cesarean-derived, colostrum-deprived pigs (Harms *et al.*, 2001). A longer duration of PRRSV viremia and a higher proportion of PCV2 viremic pigs were identified in the co-infected pigs compared to singularly PRRSV-infected pigs (Rovira *et al.*, 2002). In an *in vitro* study using swine alveolar macrophages, it was determined that PCV2-induced IFNα reduces PRRS infection and the PRRSV-associated cytopathic effect (Chang *et al.*, 2005). There is experimental evidence that synergism occurs between PCV2 and PPV, and inoculation of pigs with these viruses commonly results in systemic disease (Allan *et al.*, 1999;

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Krakowka *et al.*, 2000). In a recent study, the effect of PPV- and PCV2-co-infection on phagocytosis and interferon mRNA expression level of porcine alveolar macrophages was investigated and concurrent PCV2 and PPV infection strongly weakened phagocytosis and constantly lowered IFNα1 and IFNγ mRNA expression levels (Liu *et al.*, 2011). The pathogenesis of PCV2 and SIV co-infection remains unclear. Recently, it was determined that co-infection with SIV did not increase the number of PCV2 genomic copies in serum or target tissues or the severity of microscopic lesions associated with PCV2 in lung or lymph node and the humoral response to PCV2 did not differ between PCV2-SIV- and PCV2-infected groups (Wei *et al.*, 2010).

Bacterial–viral interactions

A summary of selected study outcomes and potential mechanisms in respiratory disease due to combined bacterial–viral infection is provided in Table 3(C). Experimental studies have confirmed that PRRSV-infected pigs have increased pulmonary infections associated with SSUIS (Galina *et al.*, 1994; Thanawongnuwech *et al.*, 2000; Xu *et al.*, 2010) or BORD (Brockmeier *et al.*, 2000, 2001). Moreover, primary infection of pigs with both PRRSV and BORD predisposes pigs to secondary pulmonary infection with PMULT (Brockmeier *et al.*, 2001). It is speculated that the mode of action seen with PRRSV- and BORD-co-infection is based on an interaction of these pathogens that adversely affects respiratory tract defense mechanisms leaving pigs especially vulnerable to infection with secondary agents such as PMULT (Brockmeier *et al.*, 2001). On the other hand, infection of pigs with PRRSV and PRV or PRRSV alone followed by subsequent inoculation with PMULT resulted only in mild disease leading to the conclusion that there is no direct interaction between PRRSV and PMULT (Carvalho *et al.*, 1997), further supporting the findings with concurrent PRRSV–BORD infection.

It has been demonstrated that pulmonary alveolar macrophages from pigs infected with PRRSV have a marked decrease in their ability to kill bacteria (Solano *et al.*, 1998). In contrast, others have demonstrated that PRRSV induces activation of pulmonary alveolar macrophages (Segalés *et al.*, 1998, 1999). Co-infection of pigs with PRRSV and MHYO results in a synergistic effect increasing the severity and duration of respiratory disease and lesions (Thacker *et al.*, 1999; Thanawongnuwech *et al.*, 2001, 2004; Cho *et al.*, 2006). The relatively severe potentiation and prolongation of PRRSV-induced pneumonia by MHYO may be due to the inflammatory responses elicited in the course of both mycoplasmal and PRRSV pneumonia (Thanawongnuwech *et al.*, 2001). It is possible that the MHYO-induced infiltration of macrophages and lymphocytes into the lung parenchyma provides a steady supply of susceptible cells for PRRSV to infect, thus potentiating and prolonging the viral pneumonia. In addition, the inflammatory response induced by both infections may combine to further modulate the local immune response so that clearance of PRRSV is reduced (Thanawongnuwech *et al.*, 2001). Interestingly, PRRSV vaccination in combination with MHYO-infection reduces PRRSV vaccine efficacy and the amount of pneumonia induced by PRRSV is increased (Thacker *et al.*, 2000b). When the influence of PRRSV on APP-infection in the acute stage (PRRSV-infection 2 days after APP) or the more chronic stage (PRRSV-infection 2 weeks after APP) was investigated, PRRSV enhanced APP-associated disease in the acute stage of APP infection, but this finding was not reproducible in subsequent trials (Pol *et al.*, 1997). The authors concluded that previous PRRSV infection does not necessarily enhance the severity of secondary infections of the respiratory tract.

An alternative, reproducible model to study the pathogenesis of PRRSV-induced multi-factorial respiratory disease may be based on bacterial endotoxins (Van Gucht *et al.*, 2004). It has been demonstrated that pigs infected with PRRSV and exposed to bacterial lipopolysaccharide (LPS) had more severe respiratory disease compared to pigs given either PRRSV or LPS alone (Labarque *et al.*, 2002). A synergistic effect between PRRSV and LPS was further confirmed using Chinese PRRSV strains with increased cell surface CD14 expression, resulting in increased cytokine secretion in co-treated pigs (Qiao *et al.*, 2011).

In order to determine whether single or combined infections in 6- to 7-week-old pigs results in pneumonia, pigs were inoculated with different combinations of PRV and PMULT (Fuentes and Pijoan, 1987). Co-infected pigs developed severe pneumonia with decreased average daily gain, whereas pigs given PRV only did not develop pneumonia and use of a modified-live PRV vaccination had no effect on the study outcome (Fuentes and Pijoan, 1987). In another study using cesarean-derived, colostrum-deprived pigs co-infected with PRV and MHYO, PRV infection enhanced the severity of experimentally induced acute mycoplasmal pneumonia (Shibata *et al.*, 1998).

The effect of PRCV on bacterial diseases was investigated in 2008 using BORD (Brockmeier *et al.*, 2008). A synergistic effect between both pathogens with regard to pro-inflammatory cytokine transcription was observed and gross and histopathological lung lesions were found to be more severe in the co-infected group as compared to the groups infected with BORD or PRCV alone (Brockmeier *et al.*, 2008). Furthermore, experimental exposure of pigs to bacterial LPS following PRCV-infection led to more severe respiratory disease and increased TNFα and IL-1 levels than exposure to either LPS or PRCV alone (Van Reeth *et al.*, 2000). Thus, infection with PRCV and Gram-negative bacteria may result in more severe disease. Recently it was determined that blocking of TNFα did not reduce disease in the PRCV–LPS model using cesarean-derived, colostrum-deprived pigs (Atanasova *et al.*, 2010).

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When the effect of MHYO- and PCV2-co-infection was investigated in growing pigs, it was demonstrated that MHYO potentiated the severity of PCV2-associated lung and lymphoid lesions, increased the amount and prolonged the presence of PCV2 antigen, and increased the incidence of respiratory disease in pigs (Opriessnig *et al*., 2004). Similar results were also observed in mature boars (Opriessnig *et al*., 2011a). The results of a subsequent study, which focused on the role of cytokines in the MHYO-PCV2 model, indicated that MHYO potentiated PCV2 infection by increasing IFNγ and IL-10 mRNA expression levels (Zhang *et al*., 2011). The increase of IFNγ and chemokines, and decrease of IFNα in pigs singularly infected with PCV2 or dual-infected was correlated with increased severity of lymphoid lesions and presence of PCV2 antigen. This implies that increased severity of lesions in dual-infected pigs is associated with the presence of PCV2 antigen and alterations of cytokine mRNA expression profiles (Zhang *et al*., 2011).

Additionally, the interaction between MHYO and SIV in the induction of pneumonia in susceptible swine was investigated in the pig model (Thacker *et al*., 2001). While pigs infected with both agents exhibited more severe clinical disease, the relationship between the two pathogens lacked the profound potentiation observed with dual infection with MHYO and PRRSV (Thacker *et al*., 1999) or dual infection with MHYO and PCV2 (Opriessnig *et al*., 2004). In contrast, the relationship between MHYO and SIV appeared independent of each other, additive and transitory in nature, with the pneumonia induced by each pathogen following a normal course over time and with minimal interaction between the two pathogens (Thacker *et al*., 2001). Differences between PRRSV, PCV2 and SIV include differences in cell tropism and differences in duration of infection and induced lesions. SIV has a tropism for airway epithelial cells and SIV infection and the lesions are relatively transitory, suggesting that an effective immune response is mounted quickly. These findings demonstrate that the effect of mycoplasmas on viral infections may vary with the viral agent. MHYO decreases or disrupts the function of the mucociliary apparatus (DeBey and Ross, 1994) and infection with SIV has similar consequences which would potentially lead to increased secondary infections from opportunistic organisms under field conditions. Further characterization of the differences in the interaction between mycoplasmas and viruses will ultimately improve our understanding of the mechanisms that result in the severe pneumonia observed in PRDC cases.

The relationship of PCMV and BORD was investigated, and both PCMV and BORD produced rhinitis and pneumonia when inoculated intranasally into young gnotobiotic pigs (Edington *et al*., 1976). Some exacerbation of the lesions in the nasal mucosa of pigs given both agents was observed, but the degree of bone atrophy was not increased. It is thought that PCMV may predispose pigs to colonization by BORD (Edington *et al*., 1976).

A recent study showed that concurrent infection of pigs with SIV and BORD enhanced bacterial colonization and host responses exacerbating pulmonary lesions (Loving *et al*., 2010). When pigs were inoculated with SIV alone, BORD alone, or both agents, SIV clearance was not altered by BORD infection, but BORD colonization was enhanced. Pulmonary lesions in the co-infected pigs were more severe when compared to singly infected groups, which was associated with an elevated cytokine response leading to the conclusion that SIV-infection increased BORD colonization and enhanced production of pro-inflammatory mediators that likely contribute to exacerbated pulmonary lesions (Loving *et al*., 2010).

Recently, the effect of lipoteichoic acid (LTA) produced by *Staphylococcus aureus* to enhance respiratory disease in pigs infected with PRCV was investigated by inoculating pigs with PRCV followed by *S. aureus* producing LTA 24 h later (Atanasova *et al*., 2011). Specifically, levels of IL-6, IL-12/IL-23 and IFNγ were higher in co-infected groups compared to pigs infected with PRCV alone (Atanasova *et al*., 2011).

Summary and conclusions

Polymicrobial respiratory disease continues to be a major economic threat to the global swine industry. Substantial advancements have been made in the quality, timeliness and breadth of diagnostic and analytic tools available to better understand which pathogens are present over time in different farms and production systems as well as which tissue responses are attributable to specific pathogens individually and in co-infections. Experimental models continue to be developed and analyzed, and field trials conducted to establish best practices for controlling this complex disease syndrome.

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