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Porcine Respiratory Disease Complex and the involved pathogens

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Összefoglaló

A sertések légzőszervi betegségkomplexe (Porcine Respiratory Disease Complex, PRDC) egy többtényezős betegség, amely jelentős hatással van a globális sertéstenyésztésre, és komoly gazdasági veszteségeket okoz. A betegség különböző fertőző ágenseket – vírusokat, baktériumokat és parazitákat –, valamint nem fertőző tényezőket, például környezeti stresszhatásokat, genetikai tényezőket és állománykezelési gyakorlatokat foglal magában. A dolgozat célja a PRDC elsődleges, vírusos és bakteriális kórokozóinak átfogó bemutatása. A dolgozat keretében a sertés reprodukciós és légzőszervi szindróma vírusa (PRRSV), a sertés circovírus 2-es típusa (PCV2) és az influenza-A vírus (IAV), valamint a *Mycoplasma hyopneumoniae*, az *Actinobacillus pleuropneumoniae*, a *Glaesserella parasuis*, a *Bordetella bronchiseptica*, a *Pasteurella multocida* és a *Streptococcus suis* bemutatását kíséreltük meg. A dolgozat részletes elemzést nyújt e kórokozók taxonómiájáról, genomjuk felépítéséről, terjedési mechanizmusairól, klinikai tüneteiről, diagnosztikájáról, a betegség kezeléséről és megelőzéséről, átfogó képet adva azok szerepéről a PRDC kialakulásában.

Ezeken felül a dolgozat a társfertőzések előfordulását is vizsgálja, amelyek bonyolítják a betegség klinikai megjelenését és kezelését, növelve a halálozási arányt, a növekedési visszamaradottságot és az antibiotikumhasználatot. Rámutat a PRDC diagnosztizálásának nehézségeire, amelyeket a fertőző és nem fertőző tényezők összetett kölcsönhatása okoz, és hangsúlyozza a kórokozókimutatás fontosságát a társfertőzések és másodlagos fertőzések mintázatainak azonosításában. A dolgozat elemzi a PRDC gazdasági hatásait is, különös tekintettel a légzőszervi betegségek által okozott termeléscsökkenésre, beleértve a súlygyarapodás csökkenését, a magasabb halálozást és a gyengébb takarmányhasznosulást, valamint az antimikrobiális rezisztencia növekvő problémáját. A dolgozat célja, hogy hozzájáruljon a PRDC jobb megértéséhez, és betekintést nyújtson a sertéstenyésztésben alkalmazható hatékony megelőzési és ellenőrzési stratégiákba.

Abstract

Porcine respiratory disease complex (PRDC) is a multifactorial disease that significantly impacts global swine production, causing substantial economic losses. It involves a variety of infectious agents, including viruses, bacteria, and parasites, along with non-infectious factors such as environmental stressors, genetics, and herd management practices. This thesis provides an in-depth exploration of the primary pathogens responsible for PRDC, focusing on viral agents such as porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus 2 (PCV2), and influenza-A virus (IAV), as well as bacterial agents including *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Glaesserella parasuis*, *Bordetella bronchiseptica*, *Pasteurella multocida*, and *Streptococcus suis*. Detailed analyses of these pathogens' taxonomy, genome organization, transmission mechanisms, clinical signs, diagnosis, treatment, and control are presented, offering a comprehensive overview of their role in PRDC.

Additionally, the thesis investigates the occurrence of co-infections, which complicate the clinical presentation and management of the disease, leading to increased mortality, growth retardation, and antibiotic use. It highlights the challenges of diagnosing PRDC due to the complex interactions between infectious and non-infectious factors and emphasizes the importance of pathogen detection in identifying patterns of co-infections and secondary infections. The economic impact of PRDC is also analysed, with a focus on the production losses associated with respiratory diseases, including reduced weight gain, higher mortality, and diminished feed efficiency, alongside the increasing concern of antimicrobial resistance. This work aims to contribute to our understanding of PRDC and provides insights into effective strategies for its prevention and control in the swine industry.

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List of Abbreviations

A. pleuropneumoniae – Actinobacillus pleuropneumoniae

ADV – Adenovirus

ADWG – Average daily weight gain

ALP – Alkaline phosphatase

Apx – Actinobacillus pleuropneumoniae toxin

B. bronchiseptica – Bordetella bronchiseptica

BALF – Bronchoalveolar lavage fluid

Cp – Capsid protein

CPS – Capsular polysaccharide

DNA – Deoxyribonucleic acid

DNT – Dermonecrotic toxin

ELISA – Enzyme-linked immunosorbent assay

EP – Enzootic pneumonia

G. parasuis – Glaesserella parasuis

HA – Hemagglutinin

HP-PRRSV – Highly pathogenic PRRSV

IFNs – Interferons

IHA – Indirect hemagglutination

IHC – Immunohistochemistry

IL-1, IL-1β, IL-1α, IL-6, IL-8 – Interleukin-1/1β/1α/8

ISH – *In situ* hybridization

kb – kilobases

LAIV – Live attenuated influenza vaccines

LAMPs – Lipid-associated membrane proteins

LPS – Lipopolysaccharides

MAPK – Mitogen-activated protein kinase

MCC – Mucociliary clearance

MLVs – Modified live vaccines

mPCR – Multiplex polymerase chain reaction

NA – Neuraminidase

NAD – Nicotinamide adenine dinucleotide

NF-κB – Nuclear factor kappa B

Nsps – nonstructural proteins

ORFs – Open reading frames

Ori – Origin of replication

P. multocida – *Pasteurella multocida*

PAM – Porcine alveolar macrophage

PCR – Polymerase chain reaction

PCV – Porcine circovirus

PCVD – Porcine circovirus disease

PDNS – Porcine dermatitis and nephropathy syndrome

PMT – *P. multocida* toxin

PMWS – Postweaning multi-systemic wasting syndrome

PNP – Proliferative and necrotizing pneumonia

PRCoV – Porcine respiratory coronavirus

PRDC – Porcine respiratory disease complex

PRRS – Porcine reproductive and respiratory syndrome

PRRSV – Porcine reproductive and respiratory syndrome virus

RdRp – RNA-dependent RNA polymerase

Rep – Replication-associated protein

RT-PCR – Real-time polymerase chain reaction

S. suis – *Streptococcus suis*

SIV – Swine influenza virus

ssDNA - Single-stranded deoxyribonucleic acid

TCT – Tracheal cytotoxin

TNF-α – Tumor necrosis factor-α

VI – Virus isolation

vtaA – Virulence-associated trimeric autotransporter

1 Introduction

Porcine respiratory disease complex (PRDC) represents a significant challenge to the global swine industry due to its multifactorial nature, involving a wide range of infectious agents, including viruses, bacteria, and parasites, in addition to non-infectious factors such as genetics, herd management, and environmental conditions. This complex disease often results from the interplay between primary and secondary pathogens, opportunistic infections, and stressors like overcrowding, poor ventilation, and fluctuating environmental conditions [1]. The presence of co-infections is common in PRDC, complicating diagnosis and treatment, and necessitating the detection of multiple pathogens in the swine respiratory tract. Understanding the patterns of these co-infections is essential for determining the full scope of disease impact and effectively managing it in affected populations [2].

PRDC primarily manifests as pneumonia with diverse presentations, including both aerogenous and hematogenous forms, each caused by different etiological agents [2; 3]. Pathological examination of the lungs, including macroscopic and histological findings, plays a crucial role in diagnosing PRDC [4]. However, these findings must be interpreted in conjunction with clinical history, epidemiological data, and other diagnostic methods to understand the underlying causes fully [5]. While infectious factors are the primary contributors, non-infectious factors such as environmental stressors significantly influence the severity and spread of the disease, affecting morbidity and mortality rates [6; 7].

The economic impact of PRDC on the swine industry is profound, as respiratory diseases are the leading cause of production losses worldwide. These losses are linked to reduced average daily weight gain (ADWG), increased mortality rates, lower feed efficiency, and heightened antibiotic usage, all of which contribute to increased production costs [8]. Given the complexity of PRDC, comprehensive research into its viral and bacterial pathogens, including co-infections, is crucial for developing more effective management strategies to mitigate its impact on global pig production [1].

2 Viral Infections in PRDC

PRDC involves several viral pathogens that significantly impact swine health, particularly porcine reproductive and respiratory syndrome virus, swine influenza virus, and porcine circovirus 2. These viruses significantly impact the respiratory health of pigs, predisposing them to secondary bacterial infections and complicating disease management. Detailed knowledge of their taxonomy, pathogenesis, clinical signs, diagnostic methods, treatment options, and control measures is crucial for managing PRDC and reducing its impact on swine herds effectively [1].

2.1 Porcine Reproductive and Respiratory Syndrome

Porcine reproductive and respiratory syndrome (PRRS) is an immunosuppressive viral disease, which significantly affects the global pork industry by causing severe reproductive failure in sows and respiratory disease in young pigs [9].

PRRSV belongs to the family *Arteriviridae* and is a member of the genus *Arterivirus*, which comprises of enveloped RNA viruses with a linear, positive-sense genome of approximately 12.7 to 15.7 kilobases (kb). PRRSV is classified into two distinct species: *Betaarterivirus europensis* (PRRSV-1) and *Betaarterivirus americense* (PRRSV-2) [10]. PRRSV-1 originates from Europe (specifically the Lelystad strain), while PRRSV-2 originates from North America (specifically the VR2332 strain) [11].

The PRRSV genome consists of 10 open reading frames (ORFs) that encode its polyproteins and structural proteins. ORF1a and ORF1b encode the replicase polyproteins, which are processed into nonstructural proteins. ORF2a, ORF2b, ORF3, ORF4, ORF5, and ORF6 encode the viral structural proteins GP2, GP3, GP4, GP5, M, and N. Additionally, ORF7 encodes the nucleocapsid protein, essential for virion structure. PRRSV encodes several key proteins essential for its replication and virion structure [9]. The nonstructural proteins (nsps), including nsp1α, nsp2, and nsp3–12, are involved in viral replication, transcription, and immune evasion. Structural proteins include the glycoproteins GP2, GP3, and GP4, which form a complex for viral entry, and GP5, the major glycoprotein crucial for viral attachment. The membrane protein (M) and the nucleocapsid protein (N), which packages the viral RNA, are also integral to the virus's structural integrity and functionality. The RNA-dependent RNA polymerase (RdRp) of PRRSV is encoded by ORF1b, specifically the nsp9 protein. It plays a crucial role in the replication and transcription of the virus, synthesizing

both the genomic RNA and subgenomic RNAs. The RdRp, along with helicase (nsp10), form the core replication machinery. Additionally, the 5′ and 3′ untranslated regions (UTRs) of the PRRSV genome are essential for the virus's replication and transcription, as they interact with both viral and host factors to regulate RNA synthesis [12]. PRRSV is considered one of the most variable RNA viruses due to its high mutation rate, which is primarily driven by its RdRp lacking proofreading activity. This leads to a rapid accumulation of mutations and increases genetic diversity [13].

PRRSV can be transmitted through direct contact with infected pigs and indirectly via fomites. The virus is shed primarily in nasal secretions and saliva, but also in urine, faeces, and mammary gland secretions. It can be transmitted through semen, presenting a significant risk during artificial insemination. Major risks for introducing PRRSV into sow herds are posed by purchasing infected replacement gilts or sows and using semen from infected boars [9].

PRRSV infection in pigs follows a well-defined pathogenesis, beginning with a narrow cell tropism that primarily targets macrophages in tissues such as the lungs, placenta, and lymphoid organs. The virus enters host cells through the scavenger receptor CD163, utilizing clathrin-mediated endocytosis for viral uptake. Once it is inside the cell, the virus replicates and employs mechanisms like viral apoptotic mimicry to facilitate further infection. During infection, PRRSV induces apoptosis both in infected cells and neighbouring cells, contributing to the spread of the virus and its persistence. The infection progresses through three stages: acute, persistent, and extinction [9]. During the acute phase, viral replication occurs predominantly in macrophages, leading to high viremia, which peaks within the first 1-2 weeks post-infection [14]. Clinical signs appear, but viral load decreases after several weeks. Following the acute phase, PRRSV enters a persistent phase where the virus can persist in secondary lymphoid tissues for 3 to 4 months or longer, even after clinical symptoms have resolved. Antibodies against PRRSV appear around 10 days post-infection, but the cellular immune response, including cytotoxic T lymphocytes, is delayed, becoming detectable 4 to 8 weeks post-infection. This immune response primarily occurs in lymphoid tissues [15]. PRRSV can evade adaptive immune responses, contributing to its persistence and continued viral shedding. While most animals clear the virus within 2 to 4 months, it can persist longer in some cases [9; 14].

PRRSV infection can sometimes remain subclinical, with no noticeable clinical signs. However, when signs do appear, they often include respiratory issues such as coughing, sneezing, and dyspnea, particularly in infected piglets and fattening pigs. These signs may lead to growth delays and increased mortality rates. In gilts and sows, respiratory symptoms may also be present, but reproductive failure is more prominent, especially during late gestation. At this stage, PRRSV can cause significant damage to the endometrium and placenta, leading to transplacental infection of foetuses and resulting in reproductive complications such as abortion or stillbirth. The increased susceptibility to transplacental infection in late gestation is likely due to the presence of PRRSV-susceptible cells in the placenta. Infected boars also experience respiratory distress, but more importantly, the infection disrupts male reproductive function, damaging the seminiferous tubules and leading to reduced semen quality, decreased libido, and the potential for transmission via semen during artificial insemination. In cases of highly pathogenic PRRSV (HP-PRRSV) infections, pigs often show signs of high fever, lethargy, anorexia, coughing, dyspnoea, periocular oedema, and occasionally cyanosis and muscle tremors. HP-PRRSV infections are associated with severe clinical disease, high mortality, and significant lung pathology, including interstitial pneumonia and pneumocytic hyperplasia, whereas infections with low-pathogenic strains generally result in milder clinical signs [9; 16; 17].

PRRSV diagnosis in swine involves clinical observations, pathological evaluation, and laboratory tests. Laboratory confirmation is essential for a definitive diagnosis, with common methods including virus isolation (VI), serology, polymerase chain reaction (PCR), and antigen detection. Specimens like serum, tissues (e.g. lung, tonsil, lymph nodes), bronchoalveolar lavage fluid (BALF), and oral fluids are often collected for testing. VI is performed using cell cultures such as porcine alveolar macrophages (PAMs) and African monkey kidney cells, while PCR-based techniques, including real-time PCR (RT-PCR), are used to detect viral RNA. Additionally, immunohistochemistry (IHC) and fluorescent antibody staining are used to identify viral antigens in tissue samples, and sequencing methods provide genetic characterization of the virus. The detection of antibodies through enzyme-linked immunosorbent assay (ELISA) is commonly used, though it may be influenced by maternal antibodies and cannot distinguish between infection and vaccination [18; 19].

PRRSV is a virus that frequently remains clinically silent, complicating detection and early intervention efforts. Effective prevention and eradication require the continuous collection

of population data to establish baseline levels, track changes, and assess the success or failure of control measures. Due to the lack of specific treatments for PRRSV, management during acute outbreaks typically involves the use of anti-inflammatory medications to reduce fever and antibiotics to address secondary bacterial infections [18]. Modified live vaccines (MLVs) for PRRSV have been extensively used to control and prevent PRRS. These vaccines can induce protective immune responses against homologous virus strains, reducing clinical signs and virus shedding in pigs [20]. However, MLVs do not confer sterilizing immunity, failing to protect against various field strains and allowing for potential viral mutation and recombination. Additionally, MLVs replicate within the host, causing viremia and virus shedding, which does not prevent onward transmission of the virus. The safety and efficacy of MLVs are therefore under scrutiny, especially given the high genetic diversity and rapid evolution of PRRSV. Inactivated vaccines are safer since they do not replicate in vaccinated animals, but unfortunately, they could not induce satisfactory protective immunity. Recent advancements in vaccine development, including reverse genetics, novel adjuvants, DNA vaccine platforms, and viral vector expression systems, aim to address these issues, although no new commercial vaccines have emerged yet. The development of more effective and safer vaccines relies on further research into the mechanisms of cross-protection and immune response [21].

The study of the economic impact of PRRSV in Irish pig farms performed by Calderón Díaz et al. (2020) reveals significant losses due to increased weaner mortality, slower growth rates, and higher feed costs. PRRSV-positive farms experienced a delay of one week in reaching target slaughter weight, increasing feed and disposal costs. Moreover, finisher sales were lower, with vaccinated PRRSV-positive farms seeing a 3.9% reduction and unvaccinated farms a 0.8% reduction. When comparing with studies from the United States, the Irish farm's losses per pig were higher: €5.7 per pig in vaccinated farms and €3.7 in unvaccinated farms, versus $2.08 per pig in United States systems [22]. Another study revealed results of the total annual economic losses due to PRRSV in the United States, which were estimated at $66.75 million for breeding herds and $493.57 million for grower pigs [23]. A study found in Germany that farms experienced an average total loss of €74,181 annually, with losses per sow amounting to €255. The financial impact on farm profits was substantial, with an average decline of 19.1%, and in the worst cases, it could reach up to 41% [24]. These differences reflect variations in production systems and cost structures between countries. Interestingly, unvaccinated farms had higher net profits than vaccinated

ones, possibly due to better biosecurity practices and less severe co-infection with other pathogens [22].

2.2 Porcine Circovirus 2

PCV-2 (*Circovirus porcine 2*) is a major pathogen in pigs, causing significant economic losses worldwide and is responsible for porcine circovirus disease (PCVD) [25].

PCV-2 belongs to the genus *Circovirus* of the *Circoviridae* family, which comprises viruses that are the smallest known viral pathogens of animals. PCV-2 is currently classified into four major genotypes: PCV2a, PCV2b, PCV2c, and PCV2d [26].

The genome of *Circovirus* ranges from 1.7 to 2.1 kb in length and contains two major ORFs. The genera are differentiated by the location of the origin of replication (ori) in relation to the coding regions and the length of the intergenic regions. In *Circovirus* genomes, the ori is located on the same strand as the rep ORF. Additionally, *Circovirus* genomes have two intergenic regions between the major ORFs [27]. PCV-2 has a circular, single-stranded DNA genome of 1.76 kb in length. The PCV-2 genome features two major ORFs, each exceeding 600 nucleotides, encoding the replication-associated protein (Rep) and the capsid protein (Cp) [27].

Unlike PCV-1, which is not associated with clinical diseases [25], PCV-3, identified in the USA, have been linked to porcine dermatitis and nephropathy syndrome (PDNS) and reproductive failure [28], while PCV-4 is a newly emerging virus detected in swine herds in China and South Korea [29].

PCV-2 is primarily transmitted through horizontal and vertical routes, with direct contact being a frequent method of transmission. Infected pigs shed the virus through respiratory, digestive, and urinary secretions, facilitating the spread of the virus when susceptible animals are exposed to these secretions. While airborne transmission cannot be completely excluded, it plays a lesser role compared to direct contact. Transmission between farms typically occurs through the introduction of infected animals or animal products, such as semen. PCV-2 can persist within pig populations for extended periods, as modern pig farming practices, including the continuous renewal of susceptible animals and movements between compartments, enabling the virus to remain endemic within farms [30].

PCV-2 infection in swine manifests in both subclinical and clinical forms. Subclinical infections are common worldwide, with PCV-2 being ubiquitous in pig populations. On the other hand, clinical infections of PCV-2 can result in several disease syndromes, with PCVD being the most prominent. PCV-2 infection leads to a variety of pathological lesions affecting multiple organ systems, particularly in cases of PCVD. Key findings include necrotizing lymphadenitis, which often features granulomatous inflammation and thrombosis in lymph nodes. Myocarditis and vasculitis are common, with PCV-2 antigen detected in myocardial cells and endothelial cells of affected organs, suggesting endothelial involvement in disease progression. Neurological lesions, though rare, include cerebellar vasculitis and haemorrhages, while kidney lesions such as tubulointerstitial nephritis and glomerulitis are frequently observed, with PCV-2 present in both renal inflammatory and epithelial cells. Lastly, pulmonary lesions, marked by interstitial oedema and vascular damage, highlight the complex, systemic nature of PCV-2 infection. These pathological findings collectively underscore the widespread impact of PCV-2 on multiple organ systems, often involving vascular and inflammatory changes [25; 31].

Clinical signs of PCV-2 infection may include systemic disease (PCV2-SD) characterized by wasting, pallor, respiratory distress, diarrhoea, and occasionally jaundice [32]. Other associated conditions include proliferative and necrotizing pneumonia (PNP), PDNS, reproductive failures such as abortions and stillbirths, and enteric diseases. These clinical manifestations may vary in severity, with the disease potentially leading to high mortality in affected herds, particularly in cases of systemic disease and PDNS [25].

The diagnosis of PCV-2 infections relies on several methods, with the most common being molecular and immunological assays. IHC and *in situ* hybridization (ISH) are traditional techniques used to detect PCV-2 antigens or nucleic acids within tissue samples, respectively. These methods, however, have limitations in terms of sensitivity, specificity, and cost, prompting a shift towards PCR-based assays. PCR is now widely used due to its higher speed, cost-effectiveness, and ability to detect PCV-2 in a variety of samples, including serum, tissues, oral fluids, and environmental samples. Quantitative PCR can also help assess the viral load, offering valuable information on the correlation between viral presence and clinical disease progression. Additionally, serological assays can detect antibodies against PCV-2, although their utility in diagnosing active disease is limited since most herds are subclinically infected. For accurate diagnosis, a combination of clinical signs, histopathological examination, and detection of PCV-2 in tissues remains crucial [25; 32].

The control of PCV2 in swine is largely achieved through vaccination, with two main types currently used: inactivated vaccines and subunit vaccines. Inactivated vaccines use killed PCV-2 virus to stimulate immune responses. Subunit vaccines utilize the Cap protein of the virus to generate virus-like particles. These vaccines provide robust protection by inducing strong neutralizing antibody and cellular immunity, resulting in reduced viremia, lower viral shedding, and improved growth performance in pigs [33].

A study by Alarcon et al. (2013) estimated the significant economic impact of PCV-2 and postweaning multi-systemic wasting syndrome (PMWS) using farm-level data, with the cost for the English pig industry in 2008 estimated at £52.6 million per year. This increased to £88 million annually during the epidemic period from 2001 to 2004. Subclinical PCV-2 infections were identified as the largest source of economic loss at the farm level. The financial losses from affected pigs were significant, with the average losses per pig being £84.1 for those with PMWS, £82.3 for subclinically infected pigs, and £24.5 for pigs that recovered from PMWS. Vaccination programs and improved management practices that reduce subclinical infections have been shown to improve farm productivity, underlining the importance of addressing PCV2 in reducing economic losses [34].

2.3 Swine influenza A

Swine influenza A virus is a significant pathogen that affects pigs, causing respiratory disease with major economic impacts and zoonotic potential, playing a key role in PRDC [35].

Influenza A viruses (IAV, *Alphainfluenzavirus influenzae*) belong to the genus *Alphainfluenzavirus* of the *Orthomyxoviridae* family. This family encompasses viruses that cause significant diseases in humans, birds, and mammals. The swine influenza virus (SIV) is an antigenic variant of IAV [36].

The virus has a segmented, negative-stranded RNA genome, which consists of 8 segments. Its segmented genome allows for genetic reassortment, which contributes to the generation of new strains and can lead to the emergence of more virulent and novel variants. The virus's primary surface glycoproteins are hemagglutinin (HA) and neuraminidase (NA), based on which subtypes are classified. The diverse subtypes of HA (H1-H16) and NA (N1-N9) contribute to the antigenic variation, with the three major subtypes being H1N1, H1N2, and H3N2 [37-39].

SIV primarily spreads through respiratory droplets when infected pigs cough, sneeze, or exhale, facilitating transmission via direct nose-to-nose contact. When isolating SIV from air samples, Corzo et al. (2013) successfully demonstrated VI in two out of four farms, with the virus detected both inside the barn and at the exhaust point, suggesting short-distance aerosol transmission. These findings highlight the importance of airborne transmission in swine populations and support the risk of human exposure through infectious aerosols generated in confined environments [40].

SIV primarily affects the epithelial cells of the upper and lower respiratory tract, including the nasal mucosa, trachea, and lungs. The virus replicates within these tissues, and virus excretion occurs from the respiratory tract and can be detected in nasal, tonsillar, and oropharyngeal swabs. The infection typically remains localized to the respiratory system, with limited viral presence in other organs. The severity of the infection depends on factors such as viral strain, the route of inoculation, and dose. Cytokine production plays a key role in the severity of disease, with greater viral replication in the lungs leading to more pronounced inflammation [35].

Clinical signs of SIV in pigs primarily include fever, coughing, sneezing, and respiratory distress, often accompanied by lethargy and anorexia. The severity of these symptoms can vary, with coughing and sneezing being more common in infected pigs. Clinical signs can also differ between farms, and factors like concomitant infections may influence the presentation. Respiratory distress is commonly seen with rapid onset and widespread symptoms in affected groups. SIV may occasionally induce abortions and decrease fertility rates in sows. Morbidity is often high; however, mortality solely attributable to SIV is generally regarded as minimal [40].

Lung lesions in pigs infected with SIV are typically mild and consist of viral pneumonia, predominantly affecting the apical and cardiac lobes, with consolidation visible in over 50% of the lung tissue. Microscopically, SIV causes necrosis of lung epithelia, airway obstruction by necrotic cells and neutrophils, and later infiltration of lymphocytes, with these lesions often complicated by bacterial infections in naturally occurring cases [35].

Diagnosis of SIV involves several methods. Upper respiratory specimens such as nasopharyngeal aspirates, throat, and nose swabs are commonly used. Rapid antigen tests can detect influenza A but cannot distinguish between subtypes and have varied sensitivity. RT-PCR assays are highly specific, distinguishing swine-origin H1N1 from other strains

with results available within hours. While viral culture is diagnostic, it is often too slow for clinical management. Immunofluorescent antibody testing can identify influenza A but may yield false negatives. Despite limitations, RT-PCR remains the gold standard for its speed and accuracy. Future developments may include rapid, sensitive biosensors for field use, offering a low-cost, efficient diagnostic tool [41].

Antimicrobial treatment should mitigate SIV-related mortality and enhance clinical symptoms, particularly those linked to subsequent bacterial infections of the respiratory tract [42].

Vaccination is the primary method for preventing SIV infection, with most commercial vaccines being inactivated whole-virus vaccines with adjuvants or autogenous inactivated vaccines. Autogenous vaccines, formulated with farm-specific strains, have gained popularity in the United States in recent years and are restricted for use only within those production systems [37]. Inactivated vaccines typically require two doses 2 to 4 weeks apart, followed by biannual boosters for sows to maintain high and prolonged maternal antibody levels, protecting piglets during the nursery phase. Inactivated vaccines induce serum neutralizing antibodies against the viral HA, and these antibodies are then transferred to the respiratory mucosa, where they act to neutralize the virus [43]. In Europe, trivalent vaccines containing H1N1, H3N2, and H1N2 strains are widely used, while in North America, polyvalent vaccines containing multiple H1 and H3 strains are available. Live attenuated influenza vaccines (LAIV) have also been developed to induce respiratory mucosal antibodies, offering protection against SIV transmission. LAIV vaccines, available in the United States, are less affected by maternal antibody interference and are particularly useful for younger pigs. Although inactivated vaccines provide protection, challenges remain due to the diversity of circulating virus strains, requiring vaccines that closely match field isolates for optimal efficacy. Other vaccine types, such as recombinant protein and RNA vaccines, are under investigation but have shown limited success in the field [35].

Positive herd status for SIV is associated with significant economic losses in the swine industry. Farms exposed to SIV experience increased feed usage during the weaner and finisher stages, resulting in higher feed costs as pigs require more time to reach adequate slaughter weight due to reduced ADWG. This increase in feed usage was quantified as 133.1 tons and 272.6 tons of additional feed for weaners and finishers, respectively, in vaccinated SIV-positive farms compared to SIV-negative farms. The higher mortality rates in SIV-

positive farms further reduce the number of piglets produced weekly, thereby decreasing overall income. Specifically, vaccinated SIV-positive farms reported financial losses of €7.2 per pig, whereas unvaccinated SIV-positive farms reported losses of €2.8 per pig. These economic impacts are compounded by additional costs associated with higher numbers of dead animals for disposal, increased health care costs, and reduced annual sales. Farms with higher biosecurity measures tend to have lower SIV prevalence, highlighting the importance of biosecurity in mitigating economic losses [44].

3 Bacterial Infections in PRDC

PRDC is a multifactorial disease driven by both primary and secondary bacterial pathogens. Primary pathogens, such as *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, and *Bordetella bronchiseptica* [4], initiate respiratory symptoms, while secondary pathogens like *Pasteurella multocida*, *Glaesserella parasuis*, and *Streptococcus suis* often exacerbate the condition [2]. Understanding the role of each pathogen, their pathogenesis, clinical presentation, diagnostic methods, and treatment options is essential for managing PRDC and minimizing its impact on swine herds.

3.1 *Mycoplasma hyopneumoniae*

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is the causative agent of porcine enzootic pneumonia (EP). This infection results in a respiratory tract invasion and leads to a chronic disease of pigs resulting in combination with other bacterial agents. This bacterium is one of the major pathogens in PRDC and its presence is global [45].

M. hyopneumoniae is classified within the family *Metamycoplasmataceae*, and the genus *Mesomycoplasma* [36]. There is a large diversity at genomic and proteomic level of *M. hyopneumoniae* isolates which have different virulence factors [46]. The J-strain, which was isolated in 1973, is still considered as a reference strain referring to the degree of disease or lesion severity [47]. Mycoplasmas have no cell wall and are therefore very sensitive to environmental conditions [48]. The genome of *M. hyopneumoniae* is small, ranging from 0.86 to 0.96 Mb, and contains approximately 600 protein-coding genes. Despite its compact size, many genes have unknown functions. Approximately 20-30% of these genes encode surface proteins, some of which play roles in the pathogen's virulence and pathology. This gene content contributes to the bacterium’s ability to infect and evade the host immune system [49].

M. hyopneumoniae is primarily transmitted through direct pig-to-pig contact, mainly when infected animals are introduced to the herd. Indirect transmission via fomites has been suggested, it can spread over distances, raising concerns for farms in close proximity [45; 48; 50].

In host colonization, *M. hyopneumoniae* adhere to the ciliated epithelium of the respiratory tract of swine, which is influenced by bacterial adhesins interacting with host ligands. This adhesion is facilitated by surface proteins, particularly P97, which plays a crucial role in

colonization. This adhesion induces inflammatory responses, triggering the release of pro-inflammatory cytokines such as interleukin-1 (IL-1), TNF-α, and IL-6. These cytokines contribute to the development of bronchopneumonia. The bacterium produces hydrogen peroxide (H_2O_2) and other virulence factors, which impair the respiratory mucosa and disrupt mucociliary clearance (MCC). This creates a favourable environment for secondary bacterial infections. Moreover, *M. hyopneumoniae* exhibits antigenic variation, which enables it to evade the host immune system, facilitating chronic infection [49].

M. hyopneumoniae infection alters host defences, promoting a Th2 immune response, though some studies suggest a mixed Th1/Th2 response. The bacterium's cytotoxic effects, mediated by lipid-associated membrane proteins (LAMPs), lead to apoptosis of immune cells, weakening immune defences, and aiding in immune evasion [51]. Moreover, during infection, host cells produce high levels of nitric oxide (NO) and superoxide radicals, resulting in oxidative stress and tissue damage. *M. hyopneumoniae* also modulates the immune response through antigenic variation, enabling persistence in the host by evading immune detection. The prolonged inflammatory response, along with tissue damage, weakens the immune defence, leading to increased susceptibility to co-infections [49; 51].

M. hyopneumoniae infects pigs of all ages, but grower and finisher pigs are more susceptible to develop moderate to severe symptoms [45]. The disease can be either subclinical or clinical. In uncomplicated cases, some animals may remain subclinically infected for several weeks without showing coughing or having pulmonary lesions at slaughter [52]. The primary clinical sign of EP is a gradual onset of a chronic, non-productive cough, especially in finishing pigs [50]. When secondary pathogens are involved, clinical signs can include laboured breathing, pyrexia, and potentially death. Lung lesions caused by *M. hyopneumoniae* appear as purple to grey consolidated areas, mainly in the apical and middle lobes, and sometimes the cranial part of the diaphragmatic lobes [52]. These pathological lesions are characterized by catarrhal exudate, uniform lung parenchyma coloration, and a "meaty" texture. Over time, these active lesions may heal, leaving behind reddish-purplish interlobular scar retractions called fissures [45]. Histopathological analysis reveals infiltration of neutrophils and lymphocytes around the airways and alveoli, progressing to broncho-interstitial pneumonia with a neutrophilic exudate [52].

There are several methods for detecting *M. hyopneumoniae* in tissue, such as PCR, ISH, and IHC. PCR testing is frequently employed in standard diagnostic laboratories to identify *M.*

hyopneumoniae. PCR, especially real-time and multiplex variants, is highly sensitive for detecting the bacterium in lung tissue and swabs. ELISAs are used for antibody detection, although they cannot distinguish between infection, vaccination, or maternal antibodies. They are effective for population-level analysis, though antibody levels may diminish in chronic infections, limiting their use for individual diagnosis [50].

Antibiotic treatment, vaccination, and management of housing conditions can effectively control *M. hyopneumoniae* infections [47; 49]. Treatment of *M. hyopneumoniae* often involves macrolides, tetracyclines, and tiamulin, though resistance to macrolides and tiamulin is increasing. Fluoroquinolones and aminoglycosides are also used, showing mycoplasmacidal effects. Due to the absence of a cell wall, *M. hyopneumoniae* is resistant to β-lactam antibiotics, such as penicillins. Strategic antimicrobial use in reproductive herds and weaned pigs can reduce bacterial shedding and control infections, however antimicrobial resistance remains a concern [48; 52].

Vaccination against *M. hyopneumoniae* is widely used to control infections in pigs, with inactivated whole-cell vaccines being the most common. These vaccines improve growth and feed conversion and reduce clinical signs and lung lesions [49]. Subunit vaccines use purified proteins or antigens derived from the bacterium to stimulate an immune response. Although these vaccines are less common than inactivated ones, they offer advantages such as reduced risk of adverse reactions and better specificity. Vaccination strategies vary by herd type and management system, with early vaccination of piglets before 4 weeks of age, while late vaccination is administered to piglets at the age of 4 to 10 weeks. Vaccinating sows to reduce pathogen transmission to the offspring is also practiced [48; 52].

When implementing management practices, biosecurity, and hygiene measures, such as thorough cleaning and disinfection between batches, are essential to reduce exposure. The all-in, all-out system plays a significant role in disease control. Isolating sick animals and managing wild birds and rodents further reduce disease spread. Environmental conditions can be optimized with proper ventilation, minimizing relative humidity, and avoiding temperature fluctuations during cold seasons. Ensuring good air quality is vital to prevent high dust concentrations, ammonia buildup, and air pollution. Ideal housing conditions include appropriate stocking density, bedding, and feeding systems [48; 50].

M. hyopneumoniae significantly impacts swine production, leading to reduced performance, higher mortality, and increased antibiotic use, resulting in substantial economic losses.

Gustavo et al. (2019) highlight that implementing *M. hyopneumoniae* elimination protocols can be economically viable within a few months, with benefits exceeding the costs. For instance, herd closure with medication could yield an annual benefit of $877,375 per farm, with a payback period of just two months. Even short-term benefits from maintaining a one-year negative herd can justify the initial investment. These results support *M. hyopneumoniae* elimination as a cost-effective strategy for swine producers [53].

3.2 *Actinobacillus pleuropneumoniae*

Actinobacillus pleuropneumoniae (*A. pleuropneumoniae*) causes porcine pleuropneumonia, a contagious respiratory disease that affects pigs [54], and is one of the most significant respiratory bacterial pathogens of swine [55].

A. pleuropneumoniae is part of the genus *Actinobacillus* within the family *Pasteurellaceae* [36]. The bacterium is a small, gram-negative, encapsulated, haemolytic coccobacillus. It is a lactose fermenter and grows *in vitro* in aerobic or anaerobic environments at 37°C within 24 to 72 h [54; 55].

A. pleuropneumoniae isolates are categorized according to their nicotinamide adenine dinucleotide (NAD) requirements for *in vitro* growth into biotype 1 (NAD-dependent) and biotype 2 (NAD-independent). Biotype 1 consists of 12 serotypes, and biotype 2 entails 6 serotypes based on the surface polysaccharide antigens [5]. The correlation between serotype and biotype is not certain, as a single serotype may occasionally exhibit features of either biotype 1 or biotype 2. The occurrence of serotypes associated with clinical course varies significantly across different countries. Whereas strains of a specific serotype may exhibit high virulence in one area, strains of the same serotype may have low virulence in a different region [56].

Regarding *A. pleuropneumoniae* infections, lesions are caused by secreted toxins, called *Actinobacillus pleuropneumoniae* toxin (Apx), are produced by serovar and are among the most significant virulence factors contributing to disease progression [1]. Apx1 has strongly haemolytic and cytotoxic properties, Apx2 displays weak haemolytic and moderate cytotoxic effects, while Apx3 is non-haemolytic but possesses substantial strong cytotoxicity. Whereas Apx4 serves as a determinant for confirmation of an *A. pleuropneumoniae* infection [55]. Various serotypes generate one or two of such toxins [54], which then target immune cells and induce lysis of alveolar cells, epithelial cells, red blood

cells, neutrophils, and macrophages [2; 55]. Serotype 2 strains exhibit high virulence in Europe and Asia, secreting Apx2 and Apx3, whereas North American strains secrete just Apx2 and possess lower virulence. Additional virulence factors have been proposed to significantly contribute to the pathogenesis of the infection, including outer membrane proteins, capsular polysaccharide (CPS), proteases, and lipopolysaccharide (LPS) [56].

A. pleuropneumoniae is initially transmitted from the sow to the piglets within the first two weeks of life, via vertical transmission. Direct contact between pigs through the oro-nasal route is the most common way of transmission in the production cycle. By coughing or sneezing, droplet infections spread via aerosols within short distances. Indirect transmission is possible but poses a lower risk. Asymptomatic carriers of the bacterium, whether survivors of acute diseases or subclinical infected animals, may harbor *A. pleuropneumoniae* primarily in tonsillar crypts, serving as a source of infection for naïve populations [54].

The lungs are the most common site for visible pathological lesions, which might change as the disease progresses. Pneumonia may be unilateral or bilateral, multifocal, or diffuse. The most virulent strains can promptly cause fatal necrotizing and fibrino-haemorrhagic pleuropneumonia in pigs of all ages [2; 54]. *A. pleuropneumoniae* is more common in grower and fattening pigs, and is mostly associated with pleuropneumonia [6]. In acute instances, consolidated regions varying from dark red to black may be seen, alongside discrete to pronounced interlobular oedema and mild to severe fibrinous pleuritis [56]. Virulent strains of *A. pleuropneumoniae* can induce significant mortality on a farm, even in absence of risk factors or concurrent infections. In such circumstances, it may be regarded as the primary cause of respiratory diseases [2]. Moreover, less virulent strains may considerably enhance their pathogenic potential when accompanied by concomitant factors, although this is not consistently observed [56].

Bacteriological examination of the lung tissue is one of the methods that can be used for diagnosis of *A. pleuropneumoniae*. Samples suitable for genome detection are oral fluids, BALF, nasal swabs, and tonsillar scrapes, with the tonsillar scrape tested by PCR being the most sensitive method [54]. Although PCR is frequently used for the direct detection of *A. pleuropneumoniae* from nasal swabs, to determine the serovar using PCR, it is essential to first isolate the *A. pleuropneumoniae* strains [56]. Isolation is very challenging, and the direct detection of DNA in these contaminated environments remains difficult. Therefore, serology is the most economical method for identifying such herds. The Apx4-based ELISA

is widely applied for detecting antibodies but does not indicate the virulence or serovar [56]. An alternative is the LPS-based ELISA, which is sensitive and specific to the main serovars found in clinical cases. In herds consistently free of *A. pleuropneumoniae*, regular monitoring can be done with species-specific tests that have high specificity but lower sensitivity, as introducing the infection into a naïve herd is expected to cause a noticeable seroconversion and high seroprevalence [54].

Antibiotics are most effective in the early stages of the disease, as they can help to reduce mortality [57]. For the treatment of *A. pleuropneumoniae*, various antimicrobials can be used, including tetracyclines, fluoroquinolones, phenicols, cephalosporins, penicillins, and trimethoprim-sulphonamides. However, there has been a noted increase in resistance to non-critical antibiotics such as tetracyclines, penicillins, and trimethoprim-sulphonamides [54]. The initial objective should be to minimize mortality by treating affected individuals and often all contact animals within the impacted pen. However, it is important to note that highly effective antibiotics can suppress the immune response, potentially leaving animals susceptible to reinfection. On the other hand, delaying treatment can lead to chronic lesions that impair respiratory function, even after recovery [57].

Multiple vaccines have been developed to prevent *A. pleuropneumoniae* infection, categorized into three groups: killed-whole-cell vaccines (bacterins), subunit toxin-based vaccines, and combined bacterin-toxin vaccines. Bacterin vaccines are serotype-specific and should only be used on farms where the relevant serotype is present. Subunit vaccines contain the main toxins (Apx1, Apx2, and Apx3). It is generally recommended to vaccinate piglets, with the first dose administered after the initial weeks of life to avoid interference from maternal antibodies. Immunizing sows with bacterins may delay or reduce piglet colonization, potentially decreasing clinical symptoms in grower and finisher pigs. However, antibodies, whether naturally acquired or vaccine-induced, do not eliminate the carrier state in the tonsillar stage [54; 58].

In the USA the economic impact of the diagnosis of *A. pleuropneumoniae* in grower and finisher pigs resulted in a total annual loss of approximately 32 million dollars, with a standard deviation of 30 million dollars. Meat production and total number of sold pigs decreased on farms tested positive for respiratory infections [59].

3.3 *Glaesserella parasuis*

Glaesserella parasuis (*G. parasuis*) is widely known as the causative agent of Glässer's disease [60].

G. parasuis, formerly known as *Haemophilus parasuis* [61], belongs to the family *Pasteurellaceae* [36]. *G. parasuis* is a pleomorphic Gram-negative bacterium [60] and is one of the first pathogens to colonize piglets after birth. A single animal often harbors multiple strains of *G. parasuis*, exhibiting variations in antigen (serovar) and virulence features. The strains of *G. parasuis* are diverse and display significant variety in virulence, ranging from non-virulent to highly virulent strains [62; 63].

G. parasuis is a ubiquitous bacterium found in the microbiota of the respiratory tract of swine. It typically colonizes the upper respiratory tract of piglets shortly after birth through contact with the sow. The bacteria can spread between pigs through direct contact, and transmission risk is increased when pigs from different origins and age groups are mixed together [64]. Non-virulent *Glaesserella* strains are often present in the nasal cavities and upper airways as part of the natural microbiota, without inducing any disease. Such strains are vulnerable to PAMs and are efficiently eliminated from the lungs under normal circumstances [62]. As early as two days after birth, *G. parasuis* can be seen colonizing the nasal cavity; however, the peak level of colonization occurs sixty days later [65]. Following the initial invasion of the upper respiratory system, virulent strains may infiltrate the lungs, where they persist owing to their capacity to evade phagocytosis. Virulent strains show a biofilm-like growth in the mucosa of the trachea. After reaching the lungs, contact between virulent *G. parasuis* and PAM is restricted, resulting in delayed macrophage activations [62]. *G. parasuis* modifies its metabolism to endure the pulmonary environment during infection, exhibiting virulence characteristics that overcome host defences. Survival of *G. parasuis* triggers inflammation and neutrophil recruitment, causing suppurative bronchopneumonia. Virulent strains may endure in pneumocytes, resulting in chronic, persistent infections. Systemic invasion, attributable to serum resistance, may lead to significant inflammation and lesions that are typical of Glässer's disease (fibrinous polyserositis) [63].

The clinical presentation of pigs infected with *G. parasuis* typically includes swollen joints and respiratory distress, often accompanied by elevated body temperature and reduced ADWG. A hallmark of Glässer's disease is the presence of fibrinous serositis and arthritis.

In severe cases, systemic disease can lead to serofibrinous to fibrinopurulent lesions on various organs, including the pleura (pleuritis), pericardium (pericarditis), peritoneum (peritonitis), meninges (meningitis), and joints (polyarthritis) [66; 67].

To diagnose *G. parasuis*, sampling must target organs associated with systemic lesions, including the brain, joints, and serous surfaces, where the pathogen is most likely to be present. Samples must be collected aseptically to avoid contamination and should be transported to the laboratory under cool conditions (4-8°C) to maintain bacterial viability. Bacterial isolation and PCR can be used for the diagnosis of *G. parasuis*. To speed up the identification of *G. parasuis* after it has been isolated, molecular techniques such as PCR assays can be used [67].

Indirect hemagglutination (IHA) is the standard method for serotyping *G. parasuis*, allowing the identification of 15 distinct serovars. A multiplex PCR (mPCR) has been developed to improve diagnosis and serovar identification. Additionally, it is a rapid and efficient method with the ability to handle cases of cross-reactivity [60]. Advancements in molecular diagnostics have enhanced the ability to differentiate virulent from non-virulent *G. parasuis* strains. This improvement has led to the development of simple molecular tests to identify higher virulent strains. Several genes have been identified as potential virulence markers for *G. parasuis*. Recent advances in genome analysis have highlighted the leader sequences of virulence-associated trimeric autotransporter (*vtaA*) genes as promising targets for predicting strain virulence through PCR [68]. These PCR-based methods are designed to detect virulent strains and could be applied to nasal samples to identify carriers of virulent strains, allowing for an assessment of the risk of developing Glässer's disease on individual farms [67].

Antimicrobials may be employed for the control of *G. parasuis*. The detrimental impact of antimicrobials on piglets' natural microbiota must be acknowledged, since diminished diversity of nasal microbiota in pigs has been linked to an increased likelihood of Glässer's disease [60]. Antimicrobial treatment of Glässer's disease is recommended during outbreaks, but the choice of antibiotic should be guided by antimicrobial susceptibility testing. The use of antibiotics is closely linked to the development of antimicrobial resistance, posing challenges to effective disease management in animal health. Various antimicrobials, including aminoglycosides, macrolides, tetracyclines, fluoroquinolones, florfenicol, amoxicillin, ceftiofur, and colistin, have shown efficacy against *G. parasuis* [67].

Vaccines against *G. parasuis* are mainly inactivated or serovar-specific, which limits cross-protection because they are only protective against strains of the same serovar [60; 62]. Some vaccines combine multiple serovars to enhance cross-protection, and the choice of adjuvant is important for boosting efficacy. However, multiple doses are required for long-term protection. Autogenous vaccines, made from farm-specific strains, have limitations due to lower antigen concentrations but may be useful if the serovar is different from that targeted by commercial vaccines. Subunit vaccines, targeting specific *G. parasuis* antigens, are under development and may offer broader protection without affecting beneficial non-virulent strains. Sow vaccination can delay colonization and reduce bacterial load in piglets, but for lasting protection, piglets may require additional vaccination post-weaning [67].

3.4 *Bordetella bronchiseptica*

The bacterium *Bordetella bronchiseptica* (*B. bronchiseptica*) is found in a broad variety of animals, including pigs and other mammals. Apart from contributing to atrophic rhinitis [69], it is also one of the pathogenic bacteria that contributes to PRDC [70]. *B. bronchiseptica* has a worldwide distribution in pig farming [2].

B. bronchiseptica is one of nine species of the genus *Bordetella*, within the family *Alcaligenaceae* [36]. The species *B. bronchiseptica*, *B. parapertussis*, and *B. pertussis* are known as the "classical" Bordetella species. Approximately 50% of the core genome is present in all strains, with this genomic diversity being attributed to different hosts or various pathogenic factors [71]. *B. bronchiseptica* is a gram-negative bacterium with a rod or coccobacillus morphology. It grows under aerobic conditions on blood agar as a non-fermenter and shows haemolytic characteristics. Additionally, it is oxidase-, catalase-, urease-, and citrate-positive [72].

B. bronchiseptica is a ubiquitous bacterium in pigs and is frequently isolated in pneumonia cases [73]. It is most commonly detected in the respiratory tract in ciliated cells of the turbinates, trachea, and lungs [2]. Transmission mainly occurs through direct contact between animals via respiratory secretions, fomites, or by aerosols. Indirect transmission has also been reported but occurs rarely [74].

B. bronchiseptica leads to degradation of the respiratory epithelium and ciliary loss [1] including the production of adhesins that facilitate the colonization of the respiratory mucosa by other bacteria [75]. Virulence factors include fimbriae, filamentous HA, pertactin, and

toxins, such as the dermonecrotic toxin (DNT) or the tracheal cytotoxin (TCT) [73]. *B. bronchiseptica* secretes DNT, which induces damage to mucosal surfaces and turbinates, leading to ciliary stasis and diminished clearance of inhaled microorganisms [1; 75]. Toxins like these are cytotoxic to neutrophils and PAMs, disrupting the innate immune defenses [69; 76].

The symptoms of *B. bronchiseptica* infection vary depending on the age and immunological condition of the pigs, as well as the presence of co-infections with other pathogens. *B. bronchiseptica* can affect pigs at any age and, as a primary pathogenic agent, can cause mild to moderate bronchopneumonia and non-progressive atrophic rhinitis [75]. The primary illness caused by *B. bronchiseptica* is often asymptomatic unless accompanied by more complex co-infections, which result in severe bronchopneumonia with symptoms such as dyspnoea and lethargy or progressive atrophic rhinitis [77]. In swine atrophic rhinitis, *B. bronchiseptica* plays two roles: when *B. bronchiseptica* is the sole pathogen, atrophic rhinitis is typically mild to moderately severe, non-progressive, and usually reversible. Microscopically, non-progressive atrophic rhinitis is characterized by ciliary loss, mucosal squamous metaplasia, and neutrophil infiltration in the submucosa of the bronchial and bronchiolar epithelium [2; 75]. Initial clinical signs manifest 2-3 days post-infection and include sneezing, nasal and ocular discharge, and a dry cough. Mortality is usually low, although morbidity can be widespread throughout the herd [75]. When co-infected with the toxigenic strain of *P. multocida*, atrophic rhinitis leads to a more severe and progressive condition [78]. Clinical signs of chronic progressive atrophic rhinitis include epistaxis, brachygnathia, and lateral deformities of the snout [69].

Acute lung infection presents as clearly defined regions of cranio-ventral consolidation exhibiting a red to plum coloration [79]. Microscopically, suppurative bronchopneumonia is characterized by haemorrhage, necrosis, and a high level of neutrophils in the respiratory tract and alveoli [77]. Lesions of chronic bronchopneumonia resulting from *B. bronchiseptica* infection are depicted by firmness, fibrosis, and a grey-to-tan coloration. Over time, fibroplastic replacement of lung parenchyma and sequestration of necrotic areas may occur. Complex infections involving other viruses and/or other bacteria may modify the characteristics of lesions; nonetheless, suppurative bronchopneumonia is typically observed [79].

B. bronchiseptica is commonly identified through bacterial isolation and biochemical identification from nasal or tracheal swabs and washes or lung tissues from pigs. Additionally, molecular analysis using DNA probe hybridization or PCR testing have been developed for diagnosis of *B. bronchiseptica*, both of which are high in sensitivity and specificity [80] compared to isolation and biochemical testing. Immunological analysis, such as ELISA and agglutination assays, has also been used for diagnosis; however, it is not routinely employed [72].

The treatment of *B. bronchiseptica* infections is generally effective with appropriate antimicrobial agents. Tetracyclines, including oxytetracycline and chlortetracycline, are commonly used and are highly effective, showing low MIC values, which indicates good susceptibility. Treatment with sulphonamides, fluoroquinolones, and phenicols is also effective, as resistance is relatively rare and usually manageable. Aminoglycosides, such as gentamicin, are effective against *B. bronchiseptica*, though resistance to streptomycin may occur. The bacterial pathogen exhibits varying degrees of antimicrobial resistance, although overall resistance remains relatively low [81].

Vaccines targeting *B. bronchiseptica* primarily consist of whole-cell bacterins, often combined with *P. multocida* whole-cell bacterins and/or toxoids to control atrophic rhinitis. These vaccines are effective at reducing colonization levels of *B. bronchiseptica*. Attenuated live vaccines, typically administered intranasally within the first few days after birth, aim to prevent colonization by virulent strains through competitive exclusion while stimulating a mucosal immune response [82]. Additionally, vaccinating sows approximately six weeks and again two weeks before farrowing has proven effective in protecting piglets and minimizing pathogen transmission [83].

3.5 *Pasteurella multocida*

Pasteurella multocida (*P. multocida*) belongs to the *Pasteurellaceae* family, which consists of three genera: *Pasteurella*, *Haemophilus*, and *Actinobacillus* [84]. The phylogenetic relationships within the *Pasteurellaceae* taxonomy can be elucidated through the evaluation of their 16S rRNA genes [83]. Knowledge in research on the new genera classification along with the reclassification of primary *Pasteurella genus* is constantly advancing [84]. *P. multocida* is associated with several infectious diseases as pathogenic agent in many mammals, including humans [84]. Considering the multitude of genotypes and subspecies, *P. multocida* can be regarded as a heterogeneous species. Genotypes can be differentiated

into A, B, C, D, E, and F, which all manifest various clinical symptoms [83]. Depending on the subspecies of *P. multocida*, it can cause significant diseases in different animals, such as fowl cholera in poultry, shipping fever in ruminants, haemorrhagic septicaemia in cattle, snuffles in rabbits, as well as progressive atrophic rhinitis and pneumonic pasteurellosis in swine. Furthermore, the bacterium can be grouped into toxigenic and nontoxigenic strains depending on the presence of the toxin-encoding gene [85]. The toxinogenic strain of *P. multocida* contains 14 genes, such as the *tox*A gene, which is the *P. multocida* toxin (PMT) causing atrophic rhinitis [83].

P. multocida is a Gram-negative bacterium with the morphological characteristics of small, pleomorphic, nonmotile coccobacilli without flagella. The bacterium grows in aerobic or facultative anaerobic environments and can be cultured in the laboratory on 5% sheep's blood agar or chocolate agar at 37°C [83]. *P. multocida* strains are catalase- and oxidase-positive; additionally, they can ferment carbohydrates including glucose, fructose, mannose, and galactose [85].

Most *Pasteurella* species are found in the normal microbiota of mouth and pharynx. On the other hand, *P. multocida* can act as a primary or opportunistic pathogen of the respiratory tract [85]. Transmission of toxigenic *P. multocida* strains occurs horizontally via direct contact between the animals, such as nose-to-nose contact, or through indirect routes via contaminated materials. Additionally, it can also be transmitted vertically from sows to piglets [86].

P. multocida is regarded as a major respiratory pathogen in pigs and is the predominant secondary bacterial agent identified in swine pneumonia cases [87]. The clinical manifestation of swine infected with *P. multocida* is characterized by bronchopneumonia, septicaemia, and serositis. The onset of clinical signs is typically linked to stressors and concurrent respiratory infections caused by viruses or bacteria such as *Mycoplasma*. Apart from pneumonia, other clinical symptoms have been described, including fever, dyspnoea, sporadically cough and vomiting [88]. Aside from pneumonic pasteurellosis, there is another very important disease called atrophic rhinitis, a type of osteopenia that primarily manifests as facial distortion due to the breakdown of nasal turbinate bones. This condition is triggered by PMT, specifically from capsular type D and some type A strains, together with *B. bronchiseptica*. The toxin impacts the development and function of bone cells, including osteoblasts and osteoclasts, leading to the disease.

The main clinical signs include severe degeneration of the nasal turbinate bones, resulting in a shortened or twisted snout, and it often leads to growth retardation in young pigs. Specifically, PMT inhibits osteoblastic differentiation of stromal cells, including mesenchymal stem cells and primary osteoblasts, through a signaling pathway involving Gαq/11 proteins, RhoA activation, and the mitogen-activated protein kinase (MAPK) cascade. This results in a reduction of osteoblast markers, such as alkaline phosphatase (ALP) and the late transcription factor SP7, which are essential for bone formation. PMT also stimulates RhoA activity, which plays a critical role in inhibiting osteoblastogenesis. The toxin activates Gαq/11, which in turn stimulates RhoA. This activation leads to MAPK signalling, blocking osteoblast differentiation. The inhibition of osteoblast activity is significant in the clinical manifestation of atrophic rhinitis [89].

The diagnosis of *P. multocida* in pigs typically involves culture methods to isolate the bacteria from affected tissues. Conventional biochemical tests help identify the bacteria, though PCR methods targeting specific genes offer superior specificity and sensitivity [86]. Capsular typing by mPCR has largely replaced serological methods for strain identification, while toxin detection, including PMT-specific ELISA tests, is crucial for diagnosing diseases like progressive atrophic rhinitis [90].

Pasteurella infections may be managed with antibiotics, and immunization against toxigenic *P. multocida* strains is accomplished by vaccinating young piglets [91]. Antimicrobial treatment is widely used for the control of pasteurellosis, whereas broad-spectrum antibiotics are commonly administered. The best choices of antibiotics are cephalosporins like cefazolin and ceftiofur, florfenicol, and fluoroquinolones such as ciprofloxacin. However, other antimicrobials have already developed resistance among the *P. multocida* isolates and therefore had less therapeutic effect [86]. To reduce the risk of *P. multocida* transmission, both vertically from sows to suckling piglets and laterally among recently weaned pigs, it is a common practice to vaccinate sows once or twice before farrowing. As a result, the piglets establish passive immunity through the colostrum from the vaccinated mother [83].

Several vaccines have been developed to protect against *P. multocida* infection. Currently, bacterins with toxoids are extensively used, as well as modern subunit vaccines, which contain various antigen fragments. The subunit vaccine offers a higher safety profile and ensures cross-protection compared to inactivated or live-attenuated vaccines. Nevertheless, the best choice of antigen and suitable adjuvant still need to be determined. Studies have

shown that vaccines containing biological adjuvants can substantially reduce the occurrence of gross lesions, improving their potential in vaccine efficacy [92].

3.6 *Streptococcus suis*

Streptococcus suis (*S. suis*) is the most important streptococcal swine pathogen worldwide and is also an emerging zoonotic agent that has increased in importance [93; 94].

S. suis is a species within the genus *Streptococcus*, which belongs to the family *Streptococcaceae* [36]. In total, 35 serotypes of *S. suis* have been described, categorized by the antigenicity of their CPS. Sequence analysis of the 16S rRNA and cpn60 genes has revealed a high genetic diversity within the *S. suis* species [94]. The most common serotypes found in pigs are serotype 1, 2, 3, 7, 9, and ½, which have also been associated with human infections. Among these, *S. suis* serotype 2, which has multiple genome sequences available, remains the most isolated serotype and the one most associated with disease in pigs. The genome of *S. suis* typically consists of a single circular chromosome, which contains a variety of genes involved in virulence and metabolic functions [95].

S. suis is a Gram-positive bacterium that inhabits the respiratory and digestive systems of swine [93]. The bacterium grows in an aerobic environment, but microaerophilic conditions promote growth. *S. suis* strains are mostly alpha-haemolytic and can be cultured on sheep or bovine blood agar at 37°C [94].

Animals can be contaminated with *S. suis* through vertical transmission, wherein piglets acquire microbes from their mother, or through horizontal transmission by interacting with other herd members. Transmission to humans can occur via aerosols, posing a high risk to people who work in the swine industry or have frequent contact with pigs. However, the predominant route for human infection with *S. suis* is through contaminated pork meat [93].

S. suis infection causes septic disease mostly in 5- to 10-week-old piglets. Affected pigs often show fluctuating fever, poor appetite, depression, and lameness. Neurological signs due to meningitis include incoordination, paddling, convulsions, and nystagmus. Other signs include sudden death, anorexia, arthritis-induced lameness, and respiratory distress [96]. Pathological lesions associated with *S. suis* infections are most significant in the brain, heart, joints, and serous membranes. Common findings include septicaemia, pneumonia, meningitis, arthritis, polyserositis, and endocarditis [93; 94].

The diagnostic methods for *S. suis* in pigs include bacterial isolation from affected tissues, such as the brain, joints, lungs, or spleen, depending on the clinical signs. Serotyping and biochemical identification can also be used. PCR-based techniques, including mPCR and serotyping PCR, are used to detect *S. suis* and differentiate between serotypes, although these are not routinely used in veterinary practice. Serological tests like ELISA may detect antibodies but have limited utility due to cross-reactions and strain diversity [96].

Treatment of *S. suis* infections in pigs typically involves the use of antibiotics. Penicillin is the drug of choice, although it may not always be effective due to the increasing resistance of *S. suis* strains. Other antibiotics used include tetracycline, ampicillin, and florfenicol. Vaccination against *S. suis* holds promise as an alternative to antibiotics in the swine industry. However, due to the pathogen's genetic diversity, developing a universal vaccine has been challenging. Inactivated bacterial vaccines are not very effective, typically only protecting against the strain used. Autogenous vaccines, made from bacteria isolated from infected pigs, have also shown limited success. Current research is focused on subunit vaccines targeting specific virulence factors [93].

4 Discussion of coinfections

In PRDC, viral and bacterial pathogen interactions determine the course and severity of clinical signs [1; 97]. The study of coinfections plays a crucial role in the prevention and control of the disease in the swine industry. Although several studies on coinfections have been conducted, the mechanisms of pathogen interactions have not yet been fully elucidated [3]. Therefore, studies that investigate coinfections or superinfections in the pig respiratory tract and analyzing pathogen interactions, including mechanisms that regulate these interactions when available are important (Figure 1).

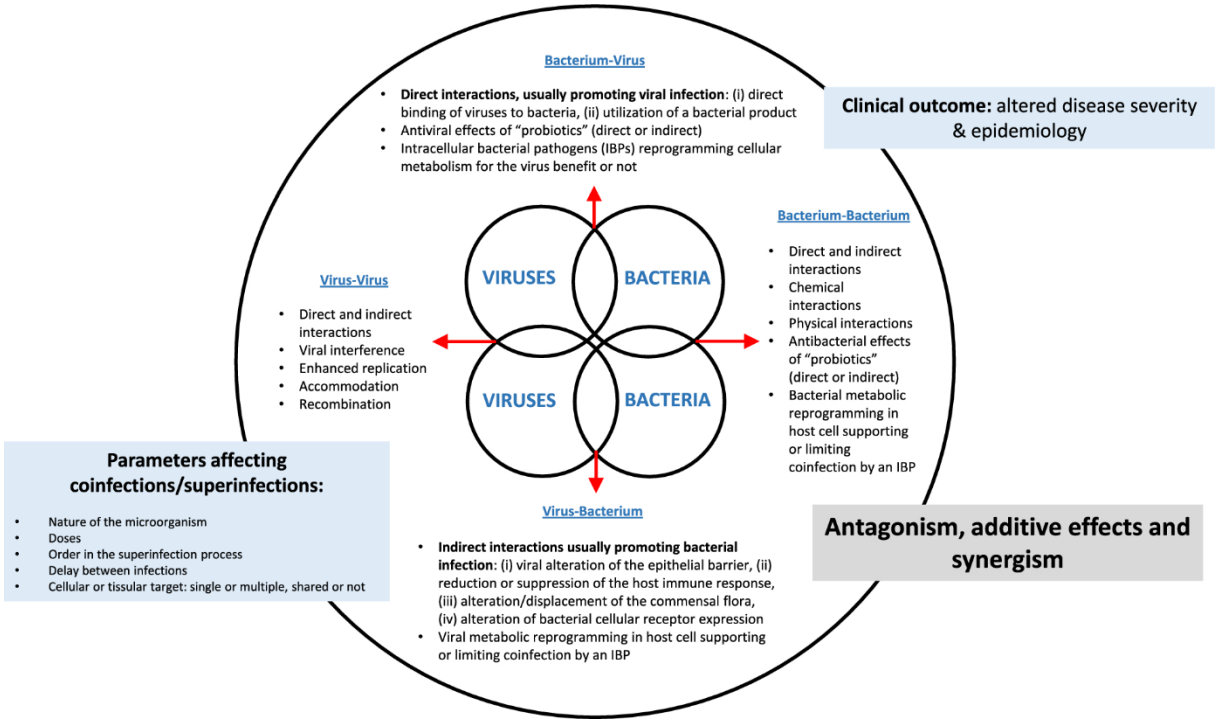

Figure 1 Consequences of the different types of coinfections for the microorganisms and for the host. In the left box some parameters enable to affect coinfections and superinfections are listed. IFN: Interferon, IBP: Intracellular Bacterial Pathogen [2].

4.1 Viral coinfections

Results from diagnostic examinations in pigs show that respiratory infections are typically polymicrobial, involving a combination of one or more viruses and bacteria [1]. Research indicates that viral respiratory coinfections are a major contributor to PRDC, with several studies focusing on the detection of various viral pathogens in pigs displaying respiratory symptoms in endemic areas. The primary viruses are SIV, PRRSV, and PCV-2, with minor

contributions from porcine coronavirus (PRCoV) and adenovirus (ADV). Over the past twenty years, research has increasingly focused on PCV-2, PRRSV, and SIV due to their rapid spread and economic impact [2; 6].

Viral interactions may result in viral interference, where one virus inhibits the replication of another, often through the production of type 1 and type 3 interferons (IFNs), which activate antiviral responses. These IFNs can also influence bacterial growth and, in some cases, increase susceptibility to bacterial infections. Non-interferon-mediated interference, or intrinsic interference, occurs when viruses or bacteria compete for host resources, thereby inducing cellular resistance. This interference can impact various stages of the viral replication cycle. Coinfections may also result in enhanced replication (Figure 1) and virulence of one or both viruses, or in some cases, they may coexist without affecting replication. Additionally, viral coinfections can result in genetic recombination, which influences viral evolution and disease severity [2; 5].

The most important virus among coinfections is PRRSV, which has a significant economic impact, as discussed in Chapter 2.1, where the consequences of PRRSV infection for the swine industry are highlighted [5]. PRRSV and PCV-2 are both significant primary pathogens and therefore a predominant cause of PRDC [98]. Coinfection of these viruses weakens the host's immune system, which increases their susceptibility to additional infections and worsening both disease development and severity in comparison to single infections. *In vitro* models have shown that coinfection enhances the replication of both PRRSV and PCV-2 [1], with notable increases in certain protein levels, indicating activation of the nuclear factor kappa B (NF-κB) pathway and suggesting a synergic interaction between the two viruses. Research also indicates that PRRSV may prolong viremia and shedding of PCV-2 [1; 5], particularly affecting subtypes PCV-2a and PCV-2b. Moreover, mutation rates of specific genes in pigs coinfected with PRRSV and PCV-2b were significantly higher than in those infected with PRRSV alone, suggesting that such coinfections may facilitate the persistence of PRRSV in swine populations [5]. PAMs that were initially infected with PCV-2 and subsequently exposed to PRRSV showed a significant decrease in the PRRSV antigen detection rate, cytopathic effects, and TNF-α expression levels [98]. This finding was further supported by an *in vitro* study by Chang et al. (2005) using PAMs, and it was found that IFN-α induced by PCV-2 reduces PRRSV infection and the associated cytopathic effects [1].

As previously mentioned with PRRSV and PCV-2 coinfection, there are similar effects with PRRSV and SIV interaction, such as more severe disease and reduced growth rates compared to single infections [5]. Regarding the symptoms, increased inflammation in the bronchial walls is one of the clinical signs observed. A study on dual infection of PRRSV and SIV H3N2 found that prior PRRSV infection does not significantly alter the disease outcome during subsequent influenza infection [99]. SIV includes various strains as discussed in Chapter 2.3. Among these, the H1N1 strain of Influenza-A can rapidly become pandemic, but also H1N2 and H3N2 occur frequently in swine herds [5]. Recent research indicates that PRRSV-1 modifies the interaction between SIV and its primary target cells. PRRSV-1 interacts with tracheal epithelial cells, triggering ERK signalling protein phosphorylation and inhibiting the phosphorylation of AKT, AMPK, and JAK2 signalling proteins. This interaction can inhibit SIV H1N2 infection of epithelial cells when PRRSV-1 is inoculated. Consequently, the vaccination programs could potentially influence their effectiveness [100]. The complex nature of these synergistic interactions highlights the need for further research into the interactions between these viruses during coinfection. Outcomes may be affected by factors such as viral strains, the order of infection, and the susceptibility of host cells [5].

However, co-infection with SIV does not appear to significantly affect the severity of PCV-2-related lesions or the replication of the virus, overall, these co-infections demonstrate complex interactions that often lead to more severe disease manifestations in pigs [1].

4.2 Bacterial coinfections

As discussed in chapter 3, the primary pathogens include *M. hyopneumoniae*, *B. bronchiseptica*, and highly virulent strains of *A. pleuropneumoniae*, all of which are of great significance in the case of bacterial co-infections and may be followed by subsequent infectious agents. The secondary pathogens include *G. parasuis, P. multocida,* low-virulent strains of *A. pleuropneumoniae*, *A. suis*, and *S. suis*, which are often responsible for pathological changes and clinical symptoms [2].

Coinfection with multiple respiratory bacteria in pigs represents a similar outcome to those observed in viral coinfections. The bacterial-bacterial coinfections result in a more severe course of the disease with increased pulmonary lesions compared to single infections [1]. Additionally, bacterial coinfections alter the immune response, leading to reduced phagocytic activity [2].

M. hyopneumoniae increases pigs' susceptibility to secondary bacterial infections, which is why secondary pathogens are frequently found during field outbreaks of enzootic pneumonia [52]. *M. hyopneumoniae* induces strong inflammatory responses, characterized by the production of various cytokines, contributing to pulmonary tissue damage [101].

P. multocida type A is usually considered as a secondary pathogen of enzootic pneumonia caused by *M. hyopneumoniae* infection [88]. Coinfections with *M. hyopneumoniae* and *P. multocida* can result in severe respiratory symptoms and extensive lung lesions, which may lead to inflammation of the visceral pleura and subsequent bacterial colonization in thoracic cavity fluids, causing pleuritic lesions. This was demonstrated in a study which utilized macroscopic lung lesions and pleura samples from slaughtered pigs to investigate the causes of pleuritis. This study also found a high prevalence of *M. hyopneumoniae* in lung samples, which may enhance *P. multocida's* adherence to lung epithelial cells [4]. When pigs are infected with both *M. hyopneumoniae* and *P. multocida*, they suffer from elevated temperatures, severe coughing, and extensive lung damage. Similarly, coinfection with *M. hyopneumoniae* and *A. pleuropneumoniae* results in more severe lesions and decreased phagocytic activity of alveolar macrophages, making pigs more susceptible to further infections [1].

The study mentioned above also showed a strong correlation between *M. hyopneumoniae* and *S. suis* in carcasses with high pleurisy scores, suggesting that *M. hyopneumoniae* may facilitate the colonization of *S. suis* by inducing ciliostasis and altering the mucociliary tract [4]. In contrast, a study by Pageaut et al. (2023) revealed that the effect of pre-infection with *M. hyopneumoniae* did not significantly affect the adhesions or invasion of *S. suis* to tracheal epithelial cells, suggesting that *M. hyopneumoniae* does not enhance the ability of *S. suis* to invade these cells [102]. Similar results were previously observed in a co-infection model involving *S. suis* and *G. parasuis* [103]. The study also showed a clear increase in cytotoxicity in cells pre-infected with *M. hyopneumoniae* before being infected with *S. suis*. The co-infection significantly increased cell death, which could potentially facilitate the systemic invasion of *S. suis* [102].

B. bronchiseptica alone causes mild disease, but it facilitates colonization by other bacteria, such as non-toxigenic *P. multocida*, which can cause atrophic rhinitis [1; 89]. Elevated mortality and manifestations of systemic disease, including lameness, lethargy, and

neurological symptoms, may arise when infections with *B. bronchiseptica* predispose individuals to invasive bacterial infections caused by *G. parasuis* or *S. suis* [4].

Although some instances of these complex relationships in bacterial coinfections have been reported, the fundamental processes remain largely unexamined [2]. Due to the large diversity of bacteria, assessing bacterial interactions is both complex and challenging. The current state of scientific research has resulted in limited knowledge regarding the mechanisms of coinfections [2].

4.3 Viral-bacterial coinfections

Co-infections of bacteria and viruses in pigs lead to significant complications in respiratory diseases, primarily due to intricate interactions affecting the immune system and increasing disease severity [1; 2; 5].

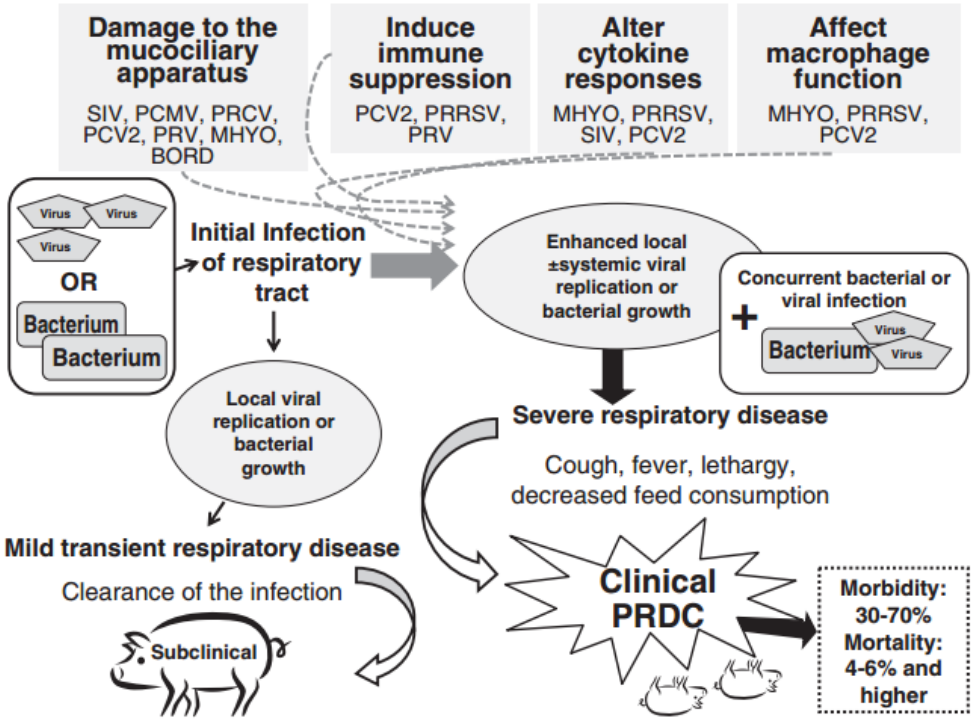

Figure 2 Summary of possible mechanisms and outcomes of viral and bacterial infections of the respiratory tract [1].

Viral and bacterial coinfection of pigs often results in more prominent disease outcomes compared to infections with a single pathogen. One key example is the interaction between PRRSV and various bacterial pathogens. PRRSV weakens the pigs' immune defences, particularly by impairing pulmonary alveolar macrophages (Figure 2), which are crucial for

killing bacteria. This impairment makes pigs more susceptible to secondary bacterial infections, such as *S. suis* and *B. bronchiseptica*. Studies have shown that this viral-bacterial synergy significantly exacerbates respiratory diseases [1; 2].

The mechanisms underlying these co-infections can be either direct or indirect. Direct interactions include viruses exploiting bacterial products to enhance their own infectivity. Indirectly, viruses can modify the host's epithelial barriers (Figure 1), immune responses, and bacterial cellular receptors, creating a favourable environment for bacterial superinfections. This has been observed with viruses like PCV-2 and SIV, which, when combined with bacterial pathogens such as *A. pleuropneumoniae* and *S. suis*, lead to severe respiratory diseases [2].

Specifically, co-infection with PRRSV and *M. hyopneumoniae* results in prolonged and more severe respiratory illnesses. The strong inflammatory response elicited by both pathogens provides a steady supply of cells for PRRSV to infect, intensifying viral pneumonia. This inflammation, characterized by elevated levels of pro-inflammatory cytokines such as TNF-α and IL-1, not only worsens the viral infection but also increases the severity of bacterial diseases [1; 2; 5].

Coinfection with PRRSV and *S. suis* leads to increased morbidity and mortality in swine. PRRSV suppresses the immune function of PAMs as mentioned previously, reducing their ability to clear *S. suis* and allowing it to spread more widely in tissues. Early *S. suis* infection can increase the virulence of PRRSV strains, causing excessive inflammation and tissue damage [5; 104].

The interaction between PRRSV and *A. pleuropneumoniae* has shown inconsistent results. While secondary *A. pleuropneumoniae* infection during the acute phase of PRRSV infection leads to more severe disease, PRRSV pre-infection does not affect *A. pleuropneumoniae* adhesion in vitro. Interestingly, pre-infection with *A. pleuropneumoniae* or its culture supernatant can block PRRSV infection by activating cofilin and causing actin depolymerization, which hinders PRRSV endocytosis. This suggests that *A. pleuropneumoniae* has an inhibitory effect on PRRSV. However, the molecular mechanisms underlying this interaction remain insufficiently researched [5; 54].

PRRSV compromises the immune system by destroying PAMs and inducing nasal mucosa inflammation. This creates a favourable environment for *G. parasuis* invasion. Studies have

shown that co-infection with *G. parasuis* triggers a strong pro-inflammatory response in PAM cells, increasing the expression of cytokines like TNF-α, IL-1β, and IL-8. Additionally, PRRSV promotes *G. parasuis* proliferation in blood and tissues, exacerbating the infection [5].

Moreover, interactions between *M. hyopneumoniae* and PCV-2 amplify lung and lymphoid tissue damage, prolong the presence of PCV-2 antigen, and increase respiratory disease incidence [105]. Similarly, co-infection with SIV and *B. bronchiseptica* leads to increased bacterial colonization and severe lung lesions due to elevated pro-inflammatory cytokines [1].

The complex interactions between viral and bacterial pathogens in pigs emphasize the importance of understanding immune responses and the underlying mechanisms of co-infections for managing PRDC [106].

5 Conclusion

PRDC continues to represent one of the most challenging and economically impactful diseases in the swine industry, characterized by the involvement of multiple pathogens, both viral and bacterial, as well as significant environmental and management factors. This thesis has comprehensively reviewed the role of key viral agents such as PRRSV, PCV-2, and Influenza-A and bacterial pathogens, including *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae, Glaesserella parasuis* and others, in the pathogenesis of PRDC. The detailed examination of these pathogens has underscored the complexity of the disease and the various ways in which these infectious agents interact with the host and each other, often complicating diagnosis and treatment.

The significant challenge in managing PRDC lies not only in the direct effects of these pathogens but also in the role of co-infections and opportunistic infections that arise in a compromised immune environment. The frequent presence of secondary bacterial infections, alongside primary viral infections, can exacerbate the severity of clinical signs, leading to more difficult treatment and management strategies.

Economically, PRDC has far-reaching implications for pork production. Respiratory diseases contribute to considerable losses. Additionally, the increasing prevalence of antimicrobial resistance makes the long-term effectiveness of some treatments uncertain, which points to the need for alternative strategies in managing and controlling these infections.

Future research should focus on improving diagnostic tools, understanding the interactions between pathogens in co-infections, and developing sustainable approaches to disease control, particularly those that minimize the reliance on antibiotics. Effective control of PRDC will require continuous adaptation of both scientific knowledge and management practices to address the evolving nature of the disease and its impact on the swine industry.

6 References

1. Opriessnig T, Giménez-Lirola LG, Halbur PG (2011) Polymicrobial respiratory disease in pigs. Anim Health Res Rev 12(2):133-148. https://doi.org/10.1017/s1466252311000120
2. Saade G, Deblanc C, Bougon J, Marois-Créhan C, Fablet C, Auray G, Belloc C, Leblanc-Maridor M, Gagnon CA, Zhu J, Gottschalk M, Summerfield A, Simon G, Bertho N, Meurens F (2020) Coinfections and their molecular consequences in the porcine respiratory tract. Veterinary Research 51(1):80. https://doi.org/10.1186/s13567-020-00807-8
3. Burrai GP, Hawko S, Dei Giudici S, Polinas M, Angioi PP, Mura L, Alberti A, Hosri C, Hassoun G, Oggiano A, Antuofermo E (2023) The Synergic Role of Emerging and Endemic Swine Virus in the Porcine Respiratory Disease Complex: Pathological and Biomolecular Analysis. Vet Sci 10(10). https://doi.org/10.3390/vetsci10100595
4. Petri FAM, Ferreira GC, Arruda LP, Malcher CS, Storino GY, Almeida HMdS, Sonalio K, Silva DGd, Oliveira LGd (2023) Associations between Pleurisy and the Main Bacterial Pathogens of the Porcine Respiratory Diseases Complex (PRDC). Animals 13(9):1493. https://doi.org/10.3390/animals13091493
5. Zhao D, Yang B, Yuan X, Shen C, Zhang D, Shi X, Zhang T, Cui H, Yang J, Chen X, Hao Y, Zheng H, Zhang K, Liu X (2021) Advanced Research in Porcine Reproductive and Respiratory Syndrome Virus Co-infection With Other Pathogens in Swine. Frontiers in Veterinary Science 8. https://doi.org/10.3389/fvets.2021.699561
6. Ruggeri J, Salogni C, Giovannini S, Vitale N, Boniotti MB, Corradi A, Pozzi P, Pasquali P, Alborali GL (2020) Association Between Infectious Agents and Lesions in Post-Weaned Piglets and Fattening Heavy Pigs With Porcine Respiratory Disease Complex (PRDC). Frontiers in Veterinary Science 7. https://doi.org/10.3389/fvets.2020.00636
7. Renzhammer R, Auer A, Loncaric I, Entenfellner A, Dimmel K, Walk K, Rümenapf T, Spergser J, Ladinig A (2023) Retrospective Analysis of the Detection of Pathogens Associated with the Porcine Respiratory Disease Complex in Routine Diagnostic Samples from Austrian Swine Stocks. Vet Sci 10(10). https://doi.org/10.3390/vetsci10100601
8. Boeters M, Garcia-Morante B, van Schaik G, Segalés J, Rushton J, Steeneveld W (2023) The economic impact of endemic respiratory disease in pigs and related interventions - a systematic review. Porcine Health Manag 9(1):45. https://doi.org/10.1186/s40813-023-00342-w
9. Fiers J, Cay AB, Maes D, Tignon M (2024) A Comprehensive Review on Porcine Reproductive and Respiratory Syndrome Virus with Emphasis on Immunity. Vaccines 12(8):942. https://doi.org/10.3390/vaccines12080942
10. Brinton MA, Gulyaeva AA, Balasuriya UBR, Dunowska M, Faaberg KS, Goldberg T, Leung FCC, Nauwynck HJ, Snijder EJ, Stadejek T, Gorbalenya AE (2021) ICTV Virus Taxonomy Profile: Arteriviridae 2021. Journal of General Virology 102(8). https://doi.org/10.1099/jgv.0.001632
11. Kikuti M, Sanhueza J, Vilalta C, Paploski IAD, VanderWaal K, Corzo CA (2021) Porcine reproductive and respiratory syndrome virus 2 (PRRSV-2) genetic diversity and occurrence of wild type and vaccine-like strains in the United States swine industry. PLoS One 16(11):e0259531. https://doi.org/10.1371/journal.pone.0259531

12. Kappes MA, Faaberg KS (2015) PRRSV structure, replication and recombination: Origin of phenotype and genotype diversity. Virology (New York, NY) 479:475-486. https://doi.org/10.1016/j.virol.2015.02.012
13. Gao X, Bian T, Gao P, Ge X, Zhang Y, Han J, Guo X, Zhou L, Yang H (2024) Fidelity Characterization of Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus and NADC30-like Strain. Viruses 16(5):797. https://doi.org/10.3390/v16050797.
14. Costers S, Lefebvre DJ, Goddeeris B, Delputte PL, Nauwynck HJ (2009) Functional impairment of PRRSV-specific peripheral CD3+CD8high cells. Vet Res 40(5):46. https://doi.org/10.1051/vetres/2009029.
15. Xiao Z, Batista L, Dee S, Halbur P, Murtaugh MP (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(11):5923-5933. https://doi.org/10.1128/jvi.78.11.5923-5933.2004.
16. Weesendorp E, Morgan S, Stockhofe-Zurwieden N, Popma-De Graaf DJ, Graham SP, Rebel JM (2013) Comparative analysis of immune responses following experimental infection of pigs with European porcine reproductive and respiratory syndrome virus strains of differing virulence. Vet Microbiol 163(1-2):1-12. https://doi.org/10.1016/j.vetmic.2012.09.013.
17. Zhang L, Liu J, Bai J, Wang X, Li Y, Jiang P (2013) Comparative expression of Toll-like receptors and inflammatory cytokines in pigs infected with different virulent porcine reproductive and respiratory syndrome virus isolates. Virol J 10:135. https://doi.org/10.1186/1743-422x-10-135.
18. Zimmerman JJ, Dee SA, Holtkamp DJ, Murtaugh MP, Stadejek T, Stevenson GW, Torremorell M, Yang H, Zhang J (2019) Porcine Reproductive and Respiratory Syndrome Viruses (Porcine Arteriviruses). In: Diseases of Swine. pp 685-708. https://doi.org/10.1002/9781119350927.ch41
19. Mengeling W, Lager K (2000) A brief review of procedures and potential problems associated with the diagnosis of porcine reproductive and respiratory syndrome. Veterinary Research 31(1):61-69. https://doi.org/10.1051/vetres:2000057
20. Madapong A, Saeng-Chuto K, Boonsoongnern A, Tantituvanont A, Nilubol D (2020) Cell-mediated immune response and protective efficacy of porcine reproductive and respiratory syndrome virus modified-live vaccines against co-challenge with PRRSV-1 and PRRSV-2. Sci Rep 10(1):1649. https://doi.org/10.1038/s41598-020-58626-y
21. Zhou L, Ge X, Yang H (2021) Porcine Reproductive and Respiratory Syndrome Modified Live Virus Vaccine: A "Leaky" Vaccine with Debatable Efficacy and Safety. Vaccines (Basel) 9(4). https://doi.org/10.3390/vaccines9040362
22. Calderón Díaz JA, Fitzgerald RM, Shalloo L, Rodrigues da Costa M, Niemi J, Leonard FC, Kyriazakis I, García Manzanilla E (2020) Financial Analysis of Herd Status and Vaccination Practices for Porcine Reproductive and Respiratory Syndrome Virus, Swine Influenza Virus, and Mycoplasma hyopneumoniae in Farrow-to-Finish Pig Farms Using a Bio-Economic Simulation Model. Frontiers in Veterinary Science 7. https://doi.org/10.3389/fvets.2020.556674
23. Neumann EJ, Kliebenstein JB, Johnson CD, Mabry JW, Bush EJ, Seitzinger AH, Green AL, Zimmerman JJ (2005) Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. J Am Vet Med Assoc 227(3):385-392. https://doi.org/10.2460/javma.2005.227.385
24. Renken C, Nathues C, Swam H, Fiebig K, Weiss C, Eddicks M, Ritzmann M, Nathues H (2021) Application of an economic calculator to determine the cost of

porcine reproductive and respiratory syndrome at farm-level in 21 pig herds in Germany. Porcine Health Manag 7(1):3. https://doi.org/10.1186/s40813-020-00183-x

25. Segalés J (2012) Porcine circovirus type 2 (PCV2) infections: clinical signs, pathology and laboratory diagnosis. Virus Res 164(1-2):10-19. https://doi.org/10.1016/j.virusres.2011.10.007
26. Franzo G, Cortey M, Olvera A, Novosel D, Castro AM, Biagini P, Segalés J, Drigo M (2015) Revisiting the taxonomical classification of Porcine Circovirus type 2 (PCV2): still a real challenge. Virol J 12:131. https://doi.org/10.1186/s12985-015-0361-x
27. Breitbart M, Delwart E, Rosario K, Segalés J, Varsani A, Consortium IR (2017) ICTV Virus Taxonomy Profile: Circoviridae. Journal of General Virology 98(8):1997-1998. https://doi.org/10.1099/jgv.0.000871
28. Palinski R, Piñeyro P, Shang P, Yuan F, Guo R, Fang Y, Byers E, Hause BM (2017) A Novel Porcine Circovirus Distantly Related to Known Circoviruses Is Associated with Porcine Dermatitis and Nephropathy Syndrome and Reproductive Failure. J Virol 91(1). https://doi.org/10.1128/jvi.01879-16
29. Wang D, Mai J, Yang Y, Xiao C-T, Wang N (2022) Current knowledge on epidemiology and evolution of novel porcine circovirus 4. Veterinary Research 53(1):38. https://doi.org/10.1186/s13567-022-01053-w
30. Rose N, Opriessnig T, Grasland B, Jestin A (2012) Epidemiology and transmission of porcine circovirus type 2 (PCV2). Virus Res 164(1-2):78-89. https://doi.org/10.1016/j.virusres.2011.12.002
31. Opriessnig T, Langohr I (2012) Current State of Knowledge on Porcine Circovirus Type 2-Associated Lesions. Veterinary Pathology 50(1):23-38. https://doi.org/10.1177/0300985812450726
32. Opriessnig T, Langohr I (2013) Current state of knowledge on porcine circovirus type 2-associated lesions. Vet Pathol 50 (1):23-38. doi:10.1177/0300985812450726
33. Guo J, Hou L, Zhou J, Wang D, Cui Y, Feng X, Liu J (2022) Porcine Circovirus Type 2 Vaccines: Commercial Application and Research Advances. Viruses 14(9). https://doi.org/10.1177/0300985812450726
34. Alarcon P, Rushton J, Wieland B (2013) Cost of post-weaning multi-systemic wasting syndrome and porcine circovirus type-2 subclinical infection in England - an economic disease model. Prev Vet Med 110(2):88-102. https://doi.org/10.1016/j.prevetmed.2013.02.010
35. Van Reeth K, Vincent AL (2019) Influenza Viruses. In: Diseases of Swine. pp 576-593. https://doi.org/10.1002/9781119350927.ch36
36. Schoch CL, Ciufo S, Domrachev M, Hotton CL, Kannan S, Khovanskaya R, Leipe D, McVeigh R, O'Neill K, Robbertse B, Sharma S, Soussov V, Sullivan JP, Sun L, Turner S, Karsch-Mizrachi I (2020) NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database (Oxford) 2020. https://doi.org/10.1093/database/baaa062
37. Romagosa A, Allerson M, Gramer M, Joo HS, Deen J, Detmer S, Torremorell M (2011) Vaccination of influenza a virus decreases transmission rates in pigs. Vet Res 42(1):120. https://doi.org/10.1186/1297-9716-42-120
38. Diaz A, Marthaler D, Culhane M, Sreevatsan S, Alkhamis M, Torremorell M (2017) Complete Genome Sequencing of Influenza A Viruses within Swine Farrow-to-Wean Farms Reveals the Emergence, Persistence, and Subsidence of Diverse Viral Genotypes. J Virol 91(18). https://doi.org/10.1128/jvi.00745-17

39. Lefkowitz EJ, Dempsey DM, Hendrickson RC, Orton RJ, Siddell SG, Smith DB (2017) Virus taxonomy: the database of the International Committee on Taxonomy of Viruses (ICTV). Nucleic Acids Research 46 (D1):D708-D717. https://doi.org/10.1093/nar/gkx932
40. Corzo CA, Culhane M, Dee S, Morrison RB, Torremorell M (2013) Airborne detection and quantification of swine influenza a virus in air samples collected inside, outside and downwind from swine barns. PLoS One 8(8):e71444. https://doi.org/10.1371/journal.pone.0071444
41. Chauhan N, Narang J, Pundir S, Singh S, Pundir CS (2013) Laboratory diagnosis of swine flu: a review. Artificial Cells, Nanomedicine, and Biotechnology 41(3):189-195. https://doi.org/10.3109/10731199.2012.716063
42. Rewar S, Mirdha D, Rewar P (2015) Treatment and Prevention of Pandemic H1N1 Influenza. Ann Glob Health 81 (5):645-653. https://doi.org/10.1016/j.aogh.2015.08.014
43. Mancera Gracia JC, Pearce DS, Masic A, Balasch M (2020) Influenza A Virus in Swine: Epidemiology, Challenges and Vaccination Strategies. Frontiers in Veterinary Science 7. https://doi.org/10.3389/fvets.2020.00647
44. Calderón Díaz JA, Fitzgerald RM, Shalloo L, Rodrigues da Costa M, Niemi J, Leonard FC, Kyriazakis I, García Manzanilla E (2020) Financial Analysis of Herd Status and Vaccination Practices for Porcine Reproductive and Respiratory Syndrome Virus, Swine Influenza Virus, and Mycoplasma hyopneumoniae in Farrow-to-Finish Pig Farms Using a Bio-Economic Simulation Model. Front Vet Sci 7:556674. https://doi.org/10.3389/fvets.2020.556674
45. Pieters MG, Maes D (2019) Mycoplasmosis. In: Diseases of Swine. pp 863-883. https://doi.org/10.1002/9781119350927.ch56
46. Betlach AM, Maes D, Garza-Moreno L, Tamiozzo P, Sibila M, Haesebrouck F, Segalés J, Pieters M (2019) Mycoplasma hyopneumoniae variability: Current trends and proposed terminology for genomic classification. Transbound Emerg Dis 66(5):1840-1854. https://doi.org/10.1111/tbed.13233
47. Marchioro S (2013) Immune responses following vaccination of pigs and mice against Mycoplasma hyopneumoniae. Journal of Immunology Research, 45(3), 123-132. https://doi.org/10.1007/jir.2013.12345
48. Maes D, Segales J, Meyns T, Sibila M, Pieters M, Haesebrouck F (2008) Control of Mycoplasma hyopneumoniae infections in pigs. Vet Microbiol 126(4):297-309. https://doi.org/10.1016/j.vetmic.2007.09.008
49. Leal Zimmer FMA, Paes JA, Zaha A, Ferreira HB (2020) Pathogenicity & virulence of Mycoplasma hyopneumoniae. Virulence 11(1):1600-1622. https://doi.org/10.1080/21505594.2020.1842659
50. Sibila M, Pieters M, Molitor T, Maes D, Haesebrouck F, Segalés J (2009) Current perspectives on the diagnosis and epidemiology of Mycoplasma hyopneumoniae infection. Vet J 181(3):221-231. https://doi.org/10.1016/j.tvjl.2008.02.020
51. Wang J, Liang K, Chen L, Su X, Liao D, Yu J, He J (2023) Unveiling the stealthy tactics: mycoplasma's immune evasion strategies. Frontiers in Cellular and Infection Microbiology 13. https://doi.org/10.3389/fcimb.2023.1247182
52. Maes D, Sibila M, Kuhnert P, Segalés J, Haesebrouck F, Pieters M (2018) Update on Mycoplasma hyopneumoniae infections in pigs: Knowledge gaps for improved disease control. Transbound Emerg Dis 65 Suppl 1:110-124. https://doi.org/10.1111/tbed.12677
53. Silva GS, Yeske P, Morrison RB, Linhares DCL (2019) Benefit-cost analysis to estimate the payback time and the economic value of two Mycoplasma

hyopneumoniae elimination methods in breeding herds. Preventive Veterinary Medicine 168:95-102. https://doi.org/10.1016/j.prevetmed.2019.04.008
54. Sassu EL, Bossé JT, Tobias TJ, Gottschalk M, Langford PR, Hennig-Pauka I (2018) Update on Actinobacillus pleuropneumoniae-knowledge, gaps and challenges. Transbound Emerg Dis 65 Suppl 1:72-90. https://doi.org/10.1111/tbed.12739
55. Soto Perezchica MM, Guerrero Barrera AL, Avelar Gonzalez FJ, Quezada Tristan T, Macias Marin O (2023) Actinobacillus pleuropneumoniae, surface proteins and virulence: a review. Front Vet Sci 10:1276712. https://doi.org/10.3389/fvets.2023.1276712
56. Gottschalk M (2015) The challenge of detecting herds sub-clinically infected with Actinobacillus pleuropneumoniae. Vet J 206(1):30-38. https://doi.org/10.1016/j.tvjl.2015.06.016
57. Papatsiros V, Tzika E, Athanasiou L, Tassis P, Chaintoutis S, Christodoulopoulos G (2018) In Vivo Effectiveness of Injectable Antibiotics on the Recovery of Acute Actinobacillus pleuropneumoniae-Infected Pigs. Microbial Drug Resistance 25(4):603-610. https://doi.org/10.1089/mdr.2018.0277
58. Zhang L, Luo W, Xiong R, Li H, Yao Z, Zhuo W, Zou G, Huang Q, Zhou R (2022) A Combinatorial Vaccine Containing Inactivated Bacterin and Subunits Provides Protection Against Actinobacillus pleuropneumoniae Infection in Mice and Pigs. Frontiers in Veterinary Science 9. https://doi.org/10.3389/fvets.2022.902497
59. Losinger WC (2005) Economic impacts of reduced pork production associated with the diagnosis of Actinobacillus pleuropneumoniae on grower/finisher swine operations in the United States. Preventive Veterinary Medicine 68(2):181-193. https://doi.org/10.1016/j.prevetmed.2004.12.004
60. Howell KJ, Peters SE, Wang J, Hernandez-Garcia J, Weinert LA, Luan SL, Chaudhuri RR, Angen Ø, Aragon V, Williamson SM, Parkhill J, Langford PR, Rycroft AN, Wren BW, Maskell DJ, Tucker AW (2015) Development of a Multiplex PCR Assay for Rapid Molecular Serotyping of Haemophilus parasuis. J Clin Microbiol 53(12):3812-3821. https://doi.org/10.1128/jcm.01991-15
61. Dickerman A, Bandara AB, Inzana TJ (2020) Phylogenomic analysis of Haemophilus parasuis and proposed reclassification to Glaesserella parasuis, gen. nov., comb. nov. International Journal of Systematic and Evolutionary Microbiology 70(1):180-186. https://doi.org/10.1099/ijsem.0.003730
62. Macedo N, Rovira A, Torremorell M (2015) Haemophilus parasuis: infection, immunity and enrofloxacin. Vet Res 46:128. https://doi.org/10.1186/s13567-015-0263-3
63. Bello-Orti B, Costa-Hurtado M, Martinez-Moliner V, Segalés J, Aragon V (2014) Time course Haemophilus parasuis infection reveals pathological differences between virulent and non-virulent strains in the respiratory tract. Vet Microbiol 170(3-4):430-437. https://doi.org/10.1016/j.vetmic.2014.01.011
64. Aragon V, Segalés J, Tucker AW (2019) Glässer's Disease. In: Diseases of Swine. pp 844-853. https://doi.org/10.1002/9781119350927.ch54
65. Cerdà-Cuéllar M, Naranjo JF, Verge A, Nofrarías M, Cortey M, Olvera A, Segalés J, Aragon V (2010) Sow vaccination modulates the colonization of piglets by Haemophilus parasuis. Vet Microbiol 145(3-4):315-320. doi:10.1016/j.vetmic.2010.04.002
66. Aragon V, Cerdà-Cuéllar M, Fraile L, Mombarg M, Nofrarías M, Olvera A, Sibila M, Solanes D, Segalés J (2010) Correlation between clinico-pathological outcome and typing of Haemophilus parasuis field strains. Vet Microbiol 142(3-4):387-393. https://doi.org/10.1016/j.vetmic.2009.10.025

67. Costa-Hurtado M, Barba-Vidal E, Maldonado J, Aragon V (2020) Update on Glässer's disease: How to control the disease under restrictive use of antimicrobials. Veterinary Microbiology 242:108595. https://doi.org/10.1016/j.vetmic.2020.108595
68. Galofré-Milà N, Correa-Fiz F, Lacouture S, Gottschalk M, Strutzberg-Minder K, Bensaid A, Pina-Pedrero S, Aragon V (2017) A robust PCR for the differentiation of potential virulent strains of Haemophilus parasuis. BMC Vet Res 13(1):124. https://doi.org/10.1186/s12917-017-1041-4
69. Chanter N, Magyar T, Rutter JM (1989) Interactions between Bordetella bronchiseptica and toxigenic Pasteurella multocida in atrophic rhinitis of pigs. Res Vet Sci 47(1):48-53
70. Loving CL, Brockmeier SL, Vincent AL, Palmer MV, Sacco RE, Nicholson TL (2010) Influenza virus coinfection with Bordetella bronchiseptica enhances bacterial colonization and host responses exacerbating pulmonary lesions. Microb Pathog 49(5):237-245. https://doi.org/10.1016/j.micpath.2010.06.004
71. Park J, Zhang Y, Buboltz AM, Zhang X, Schuster SC, Ahuja U, Liu M, Miller JF, Sebaihia M, Bentley SD, Parkhill J, Harvill ET (2012) Comparative genomics of the classical Bordetella subspecies: the evolution and exchange of virulence-associated diversity amongst closely related pathogens. BMC Genomics 13(1):545. https://doi.org/10.1186/1471-2164-13-545
72. Brockmeier SL, Register KB, Nicholson TL, Loving CL (2019) Bordetellosis. In: Diseases of Swine. pp 767-777. https://doi.org/10.1002/9781119350927.ch49
73. Vötsch D, Willenborg M, Baumgärtner W, Rohde M, Valentin-Weigand P (2021) Bordetella bronchiseptica promotes adherence, colonization, and cytotoxicity of Streptococcus suis in a porcine precision-cut lung slice model. Virulence 12(1):84-95. https://doi.org/10.1080/21505594.2020.1858604
74. Mattoo S, Cherry JD (2005) Molecular Pathogenesis, Epidemiology, and Clinical Manifestations of Respiratory Infections Due to Bordetella pertussis and Other Bordetella Subspecies. Clinical Microbiology Reviews 18(2):326-382. https://doi.org/10.1128/cmr.18.2.326-382.2005
75. Brockmeier SL, Palmer MV, Bolin SR, Rimler RB (2001) Effects of intranasal inoculation with Bordetella bronchiseptica, porcine reproductive and respiratory syndrome virus, or a combination of both organisms on subsequent infection with Pasteurella multocida in pigs. Am J Vet Res 62(4):521-525. https://doi.org/10.2460/ajvr.2001.62.521
76. Brockmeier SL, Palmer MV, Bolin SR (2000) Effects of intranasal inoculation of porcine reproductive and respiratory syndrome virus, Bordetella bronchiseptica, or a combination of both organisms in pigs. Am J Vet Res 61(8):892-899. https://doi.org/10.2460/ajvr.2000.61.892
77. Goodnow RA (1980) Biology of Bordetella bronchiseptica. Microbiol Rev 44 (4):722-738. https://doi.org/10.1128/mr.44.4.722-738.1980
78. Yoshimura M, Honda Y, Yonemitsu E, Kuraoka K, Suenaga K (2024) Method for quantifying the Pasteurella multocida antigen adsorbed on aluminum hydroxide adjuvant in swine atrophic rhinitis vaccine. PLoS One 19(5):e0301688. https://doi.org/10.1371/journal.pone.0301688
79. Rutter JM, Francis LM, Sansom BF (1982) Virulence of Bordetella bronchiseptica from pigs with or without atrophic rhinitis. J Med Microbiol 15(1):105-116. https://doi.org/10.1099/00222615-15-1-105
80. Hozbor D, Fouque F, Guiso N (1999) Detection of Bordetella bronchiseptica by the polymerase chain reaction. Research in Microbiology 150(5):333-341. https://doi.org/10.1016/S0923-2508(99)80059-X

81. Kadlec K, Schwarz S (2018) Antimicrobial Resistance in Bordetella bronchiseptica. Microbiology Spectrum 6 (4):10.1128/microbiolspec.arba-0024-2017. https://doi.org/10.1128/microbiolspec.arba-0024-2017
82. Hsuan S-L, Liao C-M, Huang C, Winton JR, Chen Z-W, Lee W-C, Liao J-W, Chen T-H, Chiou C-J, Yeh K-S, Chien M-S (2009) Efficacy of a novel Pasteurella multocida vaccine against progressive atrophic rhinitis of swine. Vaccine 27(22):2923-2929. https://doi.org/10.1016/j.vaccine.2009.03.005
83. Wilson BA, Ho M (2013) Pasteurella multocida: from Zoonosis to Cellular Microbiology. Clinical Microbiology Reviews 26(3):631-655. https://doi.org/10.1128/cmr.00024-13
84. Christensen H, Bisgaard M (2018) Classification of genera of Pasteurellaceae using conserved predicted protein sequences. Int J Syst Evol Microbiol 68(8):2692-2696. https://doi.org/10.1099/ijsem.0.002860
85. Peng Z, Wang X, Zhou R, Chen H, Wilson BA, Wu B (2019) Pasteurella multocida: Genotypes and Genomics. Microbiol Mol Biol Rev 83(4). https://doi.org/10.1128/mmbr.00014-19
86. Tang X, Zhao Z, Hu J, Wu B, Cai X, He Q, Chen H (2009) Isolation, Antimicrobial Resistance, and Virulence Genes of Pasteurella multocida Strains from Swine in China. Journal of Clinical Microbiology 47(4):951-958. https://doi.org/10.1128/jcm.02029-08
87. Piva MM, Schwertz CI, Bianchi RM, Henker LC, Morés MAZ, Rebelatto R, Kemper RT, Goslar MS, Nagae RY, Pavarini SP (2023) Pasteurella multocida polyserositis in growing-finishing pigs. Journal of Comparative Pathology 202:16-22. https://doi.org/10.1016/j.jcpa.2023.03.003
88. Oliveira Filho JX, Morés MAZ, Rebellato R, Kich JD, Cantão ME, Klein CS, Guedes RMC, Coldebella A, Barcellos D, Morés N (2018) Pathogenic variability among Pasteurella multocida type A isolates from Brazilian pig farms. BMC Vet Res 14(1):244. https://doi.org/10.1186/s12917-018-1565-2
89. Siegert P, Schmidt G, Papatheodorou P, Wieland T, Aktories K, Orth JH (2013) Pasteurella multocida toxin prevents osteoblast differentiation by transactivation of the MAP-kinase cascade via the Gα(q/11)--p63RhoGEF--RhoA axis. PLoS Pathog 9(5):e1003385. https://doi.org/10.1371/journal.ppat.1003385
90. Takada-Iwao A, Uto T, Mukai T, Okada M, Futo S, Shibata I (2007) Evaluation of an Indirect Enzyme-Linked Immunosorbent Assay (ELISA) using Recombinant Toxin for Detection of Antibodies against Pasteurella multocida Toxin. Journal of Veterinary Medical Science 69(6):581-586. https://doi.org/10.1292/jvms.69.581
91. Kubatzky KF (2022) Pasteurella multocida toxin - lessons learned from a mitogenic toxin. Front Immunol 13:1058905. https://doi.org/10.3389/fimmu.2022.1058905
92. Wu M-C, Wu H-C, Lee J-W, Chang W-C, Chu C-Y (2023) A protein-based subunit vaccine with biological adjuvants provides effective protection against Pasteurella multocida in pigs. Veterinary Research 54 (1):17. https://doi.org/10.1186/s13567-023-01150-4
93. Haas B, Grenier D (2018) Understanding the virulence of Streptococcus suis: A veterinary, medical, and economic challenge. Médecine et Maladies Infectieuses 48 (3):159-166. https://doi.org/10.1016/j.medmal.2017.10.001
94. Goyette-Desjardins G, Auger JP, Xu J, Segura M, Gottschalk M (2014) Streptococcus suis, an important pig pathogen and emerging zoonotic agent-an update on the worldwide distribution based on serotyping and sequence typing. Emerg Microbes Infect 3(6):e45. https://doi.org/10.1038/emi.2014.45

95. Zhang A, Yang M, Hu P, Wu J, Chen B, Hua Y, Yu J, Chen H, Xiao J, Jin M (2011) Comparative genomic analysis of Streptococcus suis reveals significant genomic diversity among different serotypes. BMC Genomics 12:523. https://doi.org/10.1186/1471-2164-12-523
96. Gottschalk M, Segura M (2019) Streptococcosis. In: Diseases of Swine. pp 934-950. https://doi.org/10.1002/9781119350927.ch61
97. Zhang J, Wang P, Xie C, Ha Z, Shi N, Zhang H, Li Z, Han J, Xie Y, Qiu X, Tao Y, Jin N, Lu H (2022) Synergistic Pathogenicity by Coinfection and Sequential Infection with NADC30-like PRRSV and PCV2 in Post-Weaned Pigs. Viruses 14(2):193. https://doi.org/10.3390/v14020193
98. Tsai YC, Chang HW, Jeng CR, Lin TL, Lin CM, Wan CH, Pang VF (2012) The effect of infection order of porcine circovirus type 2 and porcine reproductive and respiratory syndrome virus on dually infected swine alveolar macrophages. BMC Vet Res 8:174. https://doi.org/10.1186/1746-6148-8-174
99. Pol JMA, van Leengoed LAMG, Stockhofe N, Kok G, Wensvoort G (1997) Dual infections of PRRSV/influenza or PRRSV/Actinobacillus pleuropneumoniae in the respiratory tract. Veterinary Microbiology 55(1):259-264. https://doi.org/10.1016/S0378-1135(96)01323-5
100. Saade G, Ménard D, Hervet C, Renson P, Hue E, Zhu J, Dubreil L, Paillot R, Pronost S, Bourry O, Simon G, Dupont J, Bertho N, Meurens F (2020) Porcine Reproductive and Respiratory Syndrome Virus Interferes with Swine Influenza A Virus Infection of Epithelial Cells. Vaccines (Basel) 8(3). https://doi.org/10.3390/vaccines8030508
101. Fourour S, Marois-Créhan C, Martelet L, Fablet C, Kempf I, Gottschalk M, Segura M (2019) Intra-Species and Inter-Species Differences in Cytokine Production by Porcine Antigen-Presenting Cells Stimulated by Mycoplasma hyopneumoniae, M. hyorhinis, and M. flocculare. Pathogens 8(1):34. https://doi.org/10.3390/pathogens8010034
102. Pageaut H, Lacouture S, Lehoux M, Marois-Créhan C, Segura M, Gottschalk M (2023) Interactions of Mycoplasma hyopneumoniae and/or Mycoplasma hyorhinis with Streptococcus suis Serotype 2 Using In Vitro Co-Infection Models with Swine Cells. Pathogens 12(7):866. https://doi.org/10.3390/pathogens12070866
103. Mathieu-Denoncourt A, Letendre C, Auger J-P, Segura M, Aragon V, Lacouture S, Gottschalk M (2018) Limited Interactions between Streptococcus Suis and Haemophilus Parasuis in In Vitro Co-Infection Studies. Pathogens 7 (1):7. https://doi.org/10.3390/pathogens7010007
104. Auray G, Lachance C, Wang Y, Gagnon CA, Segura M, Gottschalk M (2016) Transcriptional Analysis of PRRSV-Infected Porcine Dendritic Cell Response to Streptococcus suis Infection Reveals Up-Regulation of Inflammatory-Related Genes Expression. PLOS ONE 11(5):e0156019. https://doi.org/10.1371/journal.pone.0156019
105. Opriessnig T, Thacker EL, Yu S, Fenaux M, Meng XJ, Halbur PG (2004) Experimental reproduction of postweaning multisystemic wasting syndrome in pigs by dual infection with Mycoplasma hyopneumoniae and porcine circovirus type 2. Vet Pathol 41(6):624-640. https://doi.org/10.1354/vp.41-6-624
106. Thacker EL (2001) Immunology of the porcine respiratory disease complex. Vet Clin North Am Food Anim Pract 17(3):551-565. https://doi.org/10.1016/s0749-0720(15)30006-2

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8 Thesis progress report

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Thesis progress report for veterinary students

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Department: Pathology

Thesis title: The Course of Porcine Respiratory Disease Complex and the involved pathogens

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2024	02	11	Thesis topic discussion & Finalisation	
2.	2024	02	23	Exchange of thesis examples	
3.	2024	06	28	Thesis 1 grade & Update	
4.	2024	07	01	Meeting & Update	
5.	2024	07	02	Update on current thesis status	

Grade achieved at the end of the first semester: excellent (5)

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2024	10	27	Thesis Update & Discussion of current issues	
2.	2024	11	04	Discussion of citation, literature	
3.	2024	11	07	Correction of current thesis status	
4.	2024	11	18	Correction of thesis	

1

5.	2024	11	25	Revision & final correction	

Grade achieved at the end of the second semester: ..good (4)..

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

.....
signature of the supervisor

Signature of the student: ..Reinhold Franciska.....

Signature of the secretary of the department: .....

Date of handing the thesis in..... .....