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Epidemiology and pathobiology of novel porcine parvoviruses (PPV2 – PPV8)

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List of abbreviations

PPV - Porcine Parvovirus

SMEDI - Stillbirth, Mummification, Embryonic Death, Infertility

ORF - Open Reading Frames

PRDC - Porcine Respiratory Disease Complex

PCV - Porcine Circovirus

PRRSV - Porcine Reproductive and Respiratory Syndrome Virus

BPV - Bovine Parvovirus

PARV4 – Human Parvovirus 4

BHoV - Bovine Hokovirus

ELISA - Enzyme-Linked Immunosorbent Assay

CRISPR - Clustered Regularly Interspaced Short Palindromic Repeats

PCR - Polymerase Chain Reaction

qPCR - Quantitative Polymerase Chain Reaction

mPCR – Multiplex Polymerase Chain Reaction

HTS - High-Throughput Sequencing

NGS – Next-generation sequencing

LFD - Lateral Flow Dipstick

RHR - Rolling Hairpin Replication

PMWS - Post-Weaning Multisystemic Wasting Syndrome

PCVAD - Porcine Circovirus-Associated Disease

PTTV - Porcine Torque Teno Virus

ERA – Enzyme recombinase amplification

LFD -Lateral flow dipstick

Abstract

Porcine parvoviruses (PPVs) are important pathogens in pig production, affecting the reproductive health of the animals and contributing to complex diseases. Although PPV1 has long been recognized for its role in reproductive failures such as the SMEDI complex, the discovery of seven novel PPVs (PPV2–PPV8) has extended our knowledge of their epidemiological and pathological significance.

This thesis discusses the emergence, global distribution, transmission mechanisms and clinical implications of these emerging viruses. Frequently detected in co-infections with other pathogens, such as porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV), novel PPVs complicate disease dynamics and diagnostic efforts. Recent advances in diagnostic technologies have enhanced the detection and characterization of these viruses. Taken together, these findings, indicate that further research is needed to clearly define their independent pathogenic roles and interaction with other important swine pathogens, as well as the development of targeted preventive and control measures to reduce their impact on swine health and productivity.

Összefoglaló

A *Parvoviridae* családba tartozó sertés parvovírusok (porcine parvoviruses, PPVs) kisméretű DNS-vírusok , amelyek kiemelt fontossággal bíró kórokozók a sertésiparban. Az általuk okozott fertőzések különböző szaporodásbiológia problémák és különböző komplex megbetegedések kialakulásához járulhatnak hozzá. Az első ismert PPV, a PPV1 szerepe már régóta ismert az általa okozott reprodukciós zavarok, pontosabban a SMEDI kórkép kapcsán. Az elmúlt két évtizedben hét új PPV-t (PPV2–PPV8) fedeztek fel, amelyek megjelenése bővítette tudásunkat a sertések parvovírusainak járványtani és patológiai jelentőségéről.

A szakdolgozatom ezen új vírusok megjelenését, globális elterjedtségét, terjedési mechanizmusait és klinikai vonatkozásait tárgyalja. Az újonnan leírt PPV-k igen gyakran fordulnak elő más kórokozókkal társfertőzésben; például sertés circovírus 2-es típusával (PCV2) és sertések reprodukciós zavarokkal és légzőszervi tünetekkel járó szindrómájának vírusával (PRRSV) történő egyidejű fertőzéseik, jelentősen bonyolítják a betegségek lefolyását és a megfelelő diagnosztikát. A molekuláris biológiai technológiák rohamos fejlődésével egyre pontosabb képet tudunk alkotni ezen vírusok elterjedtségéről és genetikai, járványtani jellemzőiről. Összességében az eddigi irodalmi adatok arra utalnak, hogy további kutatás szükséges az új PPV-k pontos patogén szerepének meghatározásra illetve más sertéskórokozókkal való kölcsönhatásainak a feltérképezésére. Célzott megelőzési és ellenőrzési intézkedések kidolgozásá a sertéstelepeken, jelentősen csökkenthetné ezen kórokozók negatív hatását az állatok egészségére és termelési paraméterekre.

1. Introduction

Parvoviridae family comprises a diverse group of viruses capable of infecting a wide range of hosts, including both vertebrates and invertebrates. Within the swine population, eight distinct PPVs (PPV1–8) have been identified, belonging to various subfamilies and genera. Despite their shared family classification, they exhibit significant genomic diversity which may influence their pathogenicity, host immune response and interactions with co-infecting pathogens.

The first identified PPV, the PPV1 was described in the 1960s and is primarily associated with porcine reproductive failure (PRF) in sows, particularly in first-parity gilts and second-parity sows. This virus is the primary causative agent of the SMEDI complex, which results in stillbirths, mummification, embryonic death, and infertility.

Since the discovery of PPV1, seven novel PPVs have been reported, with PPV2 first identified in 2001 and later recognized as a potential primary agent of the porcine respiratory disease complex (PRDC). The ability of the other novel PPVs (PPV3–8) to cause disease independently is still under investigation, along with their clinical and pathological implications. These viruses are often detected with other bacterial and viral pathogens complicating the determination of their clinical significance.

Novel PPVs are widely distributed globally and have been detected in both domestic pigs and in wild boar populations, indicating potential transmission between species. These viruses have been detected in various swine samples, including serum, feces, lungs, heart, spleen, kidney, lymph nodes, tonsils, and aborted fetuses suggesting broad tissue tropism and diverse routes of transmission. Advances in diagnostic techniques, especially new-generation sequencing (NGS) technologies, have been employed to study these emerging pathogens. Understanding the effects of PPVs on swine health, the dynamics of viral evolution within this family, and the implications for cross-species transmission requires ongoing research, with a focus on the epidemiology and the pathobiology of these viruses.

2. Overview of Porcine parvoviruses

2.1. History and Discovery

Porcine parvoviruses have had a significant importance in the swine population since the 1960s. The first known PPV was first detected in 1964 in Germany during an investigation of reproductive failure in pigs. Contaminating particles 22–23 nm in diameter, resembling rat parvovirus, were identified in primary porcine cell cultures used for isolating classical swine fever virus (CSFV). This virus, later reported in pigs and designated as PPV1 (*Ungulate protoparvovirus 1*) became recognized as a key pathogen responsible for reproductive issues in swine [1, 2]. PPV1 is now considered the primary agent responsible for the SMEDI complex, a syndrome characterized by stillbirths, mummification, embryonic death, and infertility.

Rapid advancement in molecular biology techniques in the early 2000s significantly impacted the research and identification of new PPVs. In 2001, PPV2 was first identified unintentionally in Myanmar during hepatitis-E surveillance study. The viral DNA was amplified from pig sera by PCR, revealing a genome closely related to PPV1 [3]. In 2008, PPV3 was discovered in Hong Kong while investigating phylogenetic links between Human Parvovirus 4 (PARV4) to pig and bovine parvoviruses. Samples were collected from the lymph nodes, serum, nasopharynx, and feces of both healthy and sick slaughterhouse pigs. The samples were analyzed via PCR, leading to the discovery of Porcine Hokovirus, which is now called PPV3, and Bovine Hokovirus (BHoV) as two novel parvoviruses [4]. In 2010, PPV4 was first identified in the USA from lung lavage samples of pigs coinfected with porcine circovirus 2 (PCV2). The phylogenetic analysis of PPV4 revealed its closer genetic relation to bovine parvovirus 2 (BPV2) rather than any other known PPV. The genomic characterization of PPV4 uncovered unique features, including an additional open reading frame (ORF), a characteristic of the *Bocavirus genus* [5]. In contrast, PPV5, detected in pig lung tissue in the USA in 2013, exhibited similarities to PPV4 but lacked ORF3 and had an extended ORF2 [6]. PPV6 was identified in 2014 from samples of aborted pig fetuses in China. It has the closest similarity to PPV4 as well, but it also lacks the extra ORF feature [7]. In 2016, PPV7 was first detected in the USA through metagenomic sequencing of rectal swab samples of pigs showing signs of reproductive failure [8]. The latest addition, PPV8, was discovered in 2022 in China using high throughput sequencing (HTS) of samples from porcine reproductive and respiratory syndrome virus (PRRSv)-positive pigs. The analysis of

PPV8 genomic sequence revealed that it is most closely related to PPV1 sharing 44.18% sequence identity, but dislayed only 16.23-24.17% identity with PPV2-7 [9].

2.2 Classification of PPVs

Parvoviruses are non-enveloped, single-stranded DNA viruses with a linear genome that typically ranges from 4 to 6 kilobases in length. They have an almost spherical, icosahedral capsid, 28 nm in diameter (Fig. 1) [10]. Their genome contains a 120-200 bp long complex palindromic hairpin structure at both ends, essential for initiating and regulating the viral DNA replication through rolling hairpin replication (RHR) [10]. Their genome contains two primary open reading frames (ORFs): ORF1, encoding non-structural proteins (NS1 and NS2) that are needed for viral replication, and ORF2, encoding structural capsid proteins (VP1 and VP2). To maximize coding efficiency within its compact genome, parvoviruses employ alternative splicing mechanisms. For instance, VP1 and VP2 are transcribed from the same RNA template, and VP2 is produced through splicing that removes a segment, that encodes an amino-terminal region unique to VP1. Additionally, in some cases post-translational modification of VP2 results in the formation of the third structural protein, VP3 [10]. Unlike the other PPVs, PPV4 contains an additional ORF, the ORF3, which is located between ORF1 and ORF2. The function of the ORF3-encoded protein remains unknown, and it shows no similarity to any other known ORF3-encoded proteins, suggesting a possible difference in the pathogenicity and replication mechanisms. While the size of the additional ORF3 in PPV4 is similar to ORF3-encoded proteins found in bocaviruses, their low nucleotide and amino acid identity differentiates them into different viral genus [8, 9].

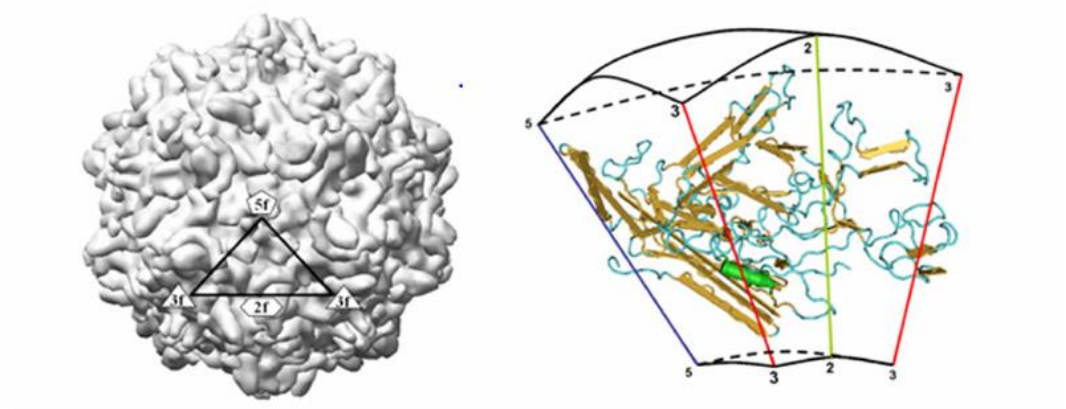

Figure 1. PPVs capsid structure. Left figure: Surface representations of the capsid calculated from X-ray coordinates. Right figure: 3D model of the PPV VP2 proteins, with a rocket (α-helix) and arrows (β-strands) representing the secondary structure. [1]

The *Parvoviridae* family is categorized into three subfamilies: *Parvovirinae*, which infects vertebrates and includes 11 genera; *Densovirinae*, primarily associated with arthropods and comprising 11 genera; and the recently identified *Hamaparvovirinae*, which includes both vertebrates and invertebrates and is divided into 5 genera (Fig.2). In swine, eight distinct parvoviruses have been identified, seven of which belong to the *Parvovirinae* subfamily. Specifically, PPV1 (*Protoparvovirus ungulate 1*) and PPV8 (*Protoparvovirus ungulate 4*) are classified under the *Protoparvovirus* genus, PPV2 (*Tetraparvovirus ungulate 3*) and PPV3 (*Tetraparvovirus ungulate 2*) under the *Tetraparvovirus* genus, and PPV4 (*Copiparvovirus ungulate 2*), PPV5 (still unclassified, but tentatively assigned to *Copioparvovirus* genus), and PPV6 (*Copiparvovirus ungulate 4*) are categorized within the *Copiparvovirus* genus. In contrast, PPV7 (*Chaphamaparvovirus ungulate 1*) is classified under the *Hamaparvovirinae* subfamily [12]. PPV7 exhibits the least genetic similarity to other PPVs but shows a closer evolutionary relationship with parvoviruses found in turkeys (turkey parvovirus, TuPV) and fruit bats (Eidolon helvum parvovirus 2, EhPV2), suggesting a distinct evolutionary lineage. Despite being part of the swine parvovirus family, PPV7 is differentiated from other PPVs in terms of its genetic, pathogenic, and epidemiological characteristics.

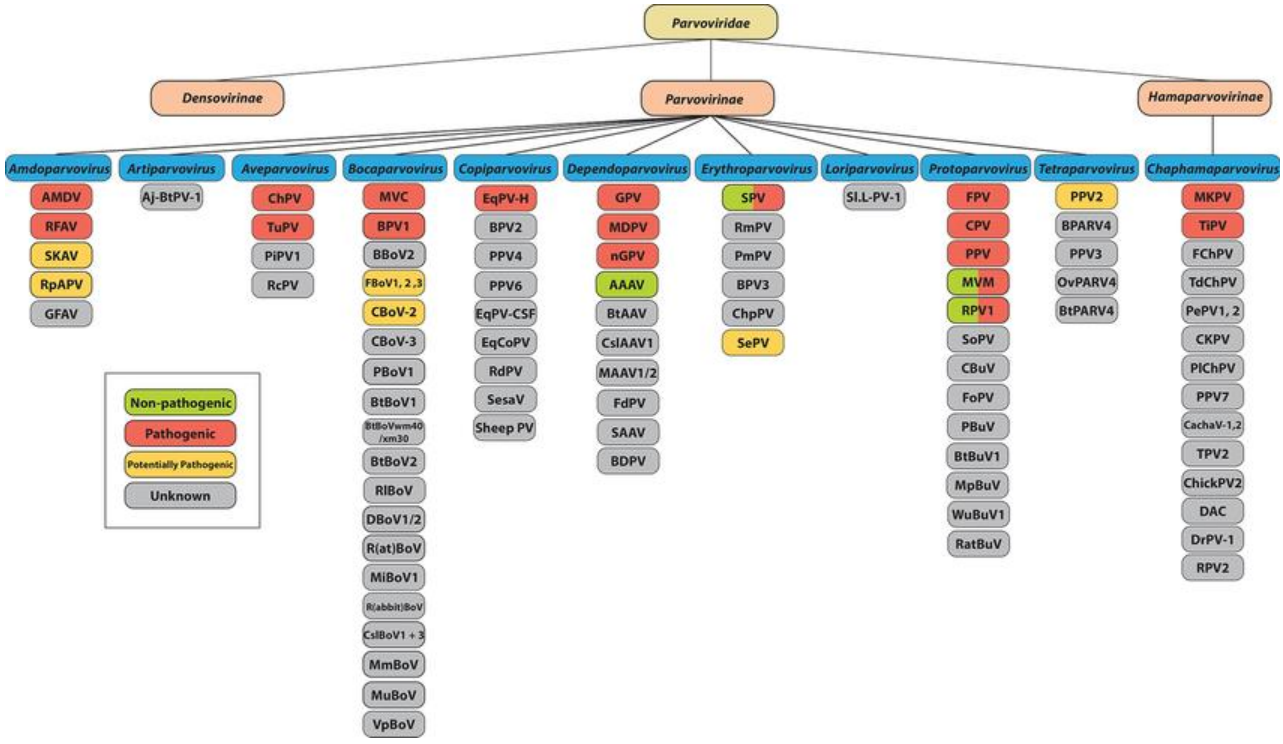

Figure 2. Taxonomy and classification of the *Parvoviridae* family. The pathogenicity of the viruses is color-coded: green indicates non-pathogenic, red indicates pathogenic, yellow indicates potentially pathogenic, and gray represents viruses of unknown pathogenicity. [11].

3. Epidemiology of PPV2–PPV8

3.1. Global Distribution`

Novel PPVs are widespread across multiple continents, affecting pig populations worldwide. They demonstrate remarkable adaptability to diverse climates and pig breeds. Figure 3 illustrates the global distribution of these viruese, while this chapter focuses on highlighting their presence in the most significant pig-producing countries, providing an overview of key regions.

PPV2 was first detected in Myanmar and rapidly spread throughout North America and Canada [5, 11], South America (Brazil and Colombia) [12, 13] and Europe, where it is present in Germany, Hungary, Poland, and Italy [14, 15, 16, 17]. It is also widely documented in Asia, including China, Japan, and South Korea [18, 19, 20].

Since the initial discovery of PPV3 in Hong Kong [4] it has been reported in other parts of Asia, including China and Korea [21, 22]. In Europe, PPV3 has been found in Poland, Hungary and in wild boars in Germany, Slovakia, and Romania [14, 16, 23, 24, 25]. Cases in North America [28] and Latin America, particularly in Brazil and Colombia further highlights its global distribution [13, 27].

PPV4 exhibits a similarly wide geographic distribution. Originally identified in the United States it has been since found in Mexico, Colombia [4, 28]. In Asia, PPV4 has been documented in China and Korea, while in Europe, it has been found in Italy, Poland, and Hungary [14, 16, 21, 29, 30]. Additionally, this virus has been also detected in wild boar populations in South Africa and Uganda, indicating its ability to adapt across diverse hosts and environments. [31, 32].

The distribution of PPV5 is comparatively narrower but still remains significant. It has been identified in the United States, China, and Korea [21, 33, 34]. It has also been detected in Eurpoe (Italy, Poland, and Hungary) and South America [13, 16, 28].

PPV6 was first reported in China [7] and later, in the United States, Mexico, and Colombia [28, 35, 36]. In Europe, this virus has been found in Poland and Russia while in Asia, cases have been recorded in China and Korea. [6, 21, 37, 38].

PPV7 was first detected in the United States [8], and it has since been widely identified in Europe, including Poland, Sweden, Hungary and Italy [9, 39, 40]. In addition, cases have also been recorded in Colombia, China and South Korea, affecting both domestic and wild boars [21, 41, 42, 43].

Lastly, after the initial detection of PPV8 in China in 2022, it has also been recently in Colombia [46] and in Europe, specifically in Hungary and Slovakia [47]. This extensive geographic distribution reflects the ability of PPVs to adapt to different environments, further emphasizing the importance of global surveillance and control measures,

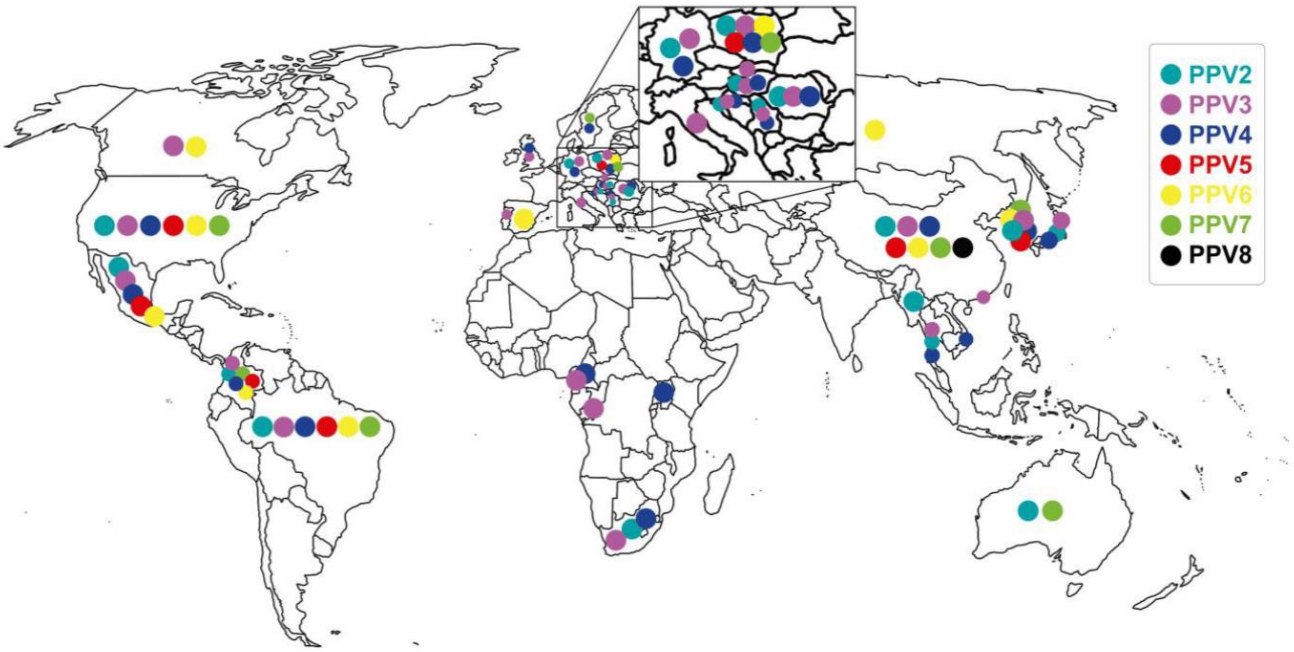

Figure 3. Global distribution of Porcine Parvoviruses (PPVs) (Vargas-Bermudez et al. 2023)

3.2. Transmission

The viruses in the *Parvoviridae* family have a wide range of transmission mechanisms. Novel PPVs exhibit similar ways of transmission to those of PPV1. Understanding their mechanism and gaining knowledge about their transmission is crucial for controlling the spread of these viruses, especially in the case of coinfections with other pathogens which may exacerbate their epidemiology and their pathobiology.

3.2.1. Horizontal Transmission

The primary transmission route for novel PPVs is believed to be horizontal, predominantly via the oronasal route [48]. Although specific studies on PPV2–8 transmission are limited, their detection in various tissues and sample types, combined with research on the closely related PPV1, suggests that these viruses are shed in bodily secretions, such as feces, nasal discharge, and oral fluids facilitating their spread among pigs

[48]. The presence of novel PPVs in respiratory tissues, tonsils, and the gastrointestinal system further supports the oronasal route as a significant mode of transmission [16, 46].

Indirect transmission is also likely to play a substantial role, as documented for PPV1. This includes the potential for spread via fomites, workers and animals such as rodents. Rodents can serve as mechanical vectors and can be responsible for the transmission of PPVs between herds or from one farm to the other. [49].

Additionally, PPVs demonstrate a remarkable ability to withstand environmental extremes, with PPV1 studies indicating the survival of the virus in dry heat up to 90°C. They can also withstand standard disinfectants, such as 70% alcohol solutions, which enables them to persist on tools, clothing, and other surfaces for extended periods, even for months. Particularly in high-density farms where animals are kept in close quarters and share common areas, their environmental resistance significantly increases the probability of infection in vulnerable pigs [50].

Furthermore, another possible route of infection is venereal. In PPV1 infections semen is a recognized infection pathway where semen from an infected boar can infect a sow during mating or artificial insemination in breeding farms. Semen serves as a direct pathway for introducing PPV1 infection in the sow's reproductive tract and potentially cause an infection. Less frequently, infection of boars with PPV1 from infected sows can also occur during mating. This bidirectional transmission highlights the importance of monitoring both male and female breeding animals for viral infections to prevent the spread within herds [47, 49]. The detection of novel PPVs in reproductive organs and also in clinical cases involving reproductive issues supports the possibility of similar venereal transmission pathways for PPV2–8 [52].

In summary, horizontal transmission is a crucial form of virus spreading within a swine population, occurring either through direct routes such as the fecal-oral pathway and venereal transmission or indirectly via fomites, equipment, and surfaces.

3.2.2. Vertical transmission

Vertical transmission is an established mode of infection for PPV1 which has been linked with substantial reproductive losses in swine populations. PPV1 is well known for causing SMEDI syndrome due to in-utero fetal infections. Similarly, novel PPVs may also utilize this transmission mechanism based on their detection in fetal tissues [48]. Multiple studies have confirmed the presence of these novel PPVs in cases of reproductive failure often in coinfection with other pathogens like PCV. Their presence in aborted fetuses and

reproductive organs of sows suggests that transplacental transmission from sows to fetuses is a likely route for the novel PPVs as well [28, 29, 40, 50, 51, 52]. Vertical transmission represents an important transmission pathway in breeding sows during the reproductive cycle. Understanding this route of transmission is essential for developing targeted interventions to mitigate reproductive losses and improve herd health management.

4. Prevalence of Novel Porcine Parvoviruses (PPV2—PPV8) Across Different Age Groups

The prevalence of novel PPVs varies significantly among pigs of different ages, reflecting distinct epidemiological patterns. According to a study conducted in Korea, the highest prevalence rate of PPVs was found in fattening pigs, with positivity rates ranging from 6.4% for PPV1 up to 36.5% for PPV6, indicating a persistent infection until the later stages of fattening. Among weaners (5-8 weeks of age), PPV2 was the most prevalent at 27.9%, followed by PPV6 at 21.5% and PPV7 at 18.6%. In contrast, piglets (up to 4 weeks of age) showed significantly lower prevalence of PPV2 (4.5%) and the other PPVs were also minimally detected in the youngest animals. This disparity suggests that maternal immunity may prevent piglets from contracting the infection. Adult sows and gilts displayed even lower PPV-prevalence rates than what was recorded in piglets, suggesting that pigs, particulary during fattening phase, are at the greatest risk of infection [23].

Similar patterns were observed in a study conducted in Mexico, where PPV2 and PPV6 were the most prevalent in weaned pigs aged between 8 to 11 weeks [30]. A study in Hungary found that almost all PPVs were detectable across age groups, with the exception of PPV4 and PPV7 in the youngest piglets (2-week-old) and PPV4 and PPV8 in the oldest age group (four-parity sows). The highest prevalence rates were recorded in fatteners and weaned pigs, with PPV2 dominating across all age groups, followed by PPV3 and PPV6, while PPV4, PPV5, PPV7 and PV8 had the lowest prevalence (Fig.4) [55].

In Polish farms, the highest detection rates occured in fattener pigs aged between 9 to 18 weeks with PPV2 being the most prevalent again. The results of this study also demonstrated a gradual decline in passive immunity from 2-6 weeks to 10-13 weeks of age, indicating that maternal immunity offers effective protection during early life [56]. These findings indicate that piglets are initially less affected but become more susceptible as they grow, especially during the fattening period [18].

The recently discovered PPV8 has been detected with higher prevalence at the beginning of the fattening period as well. According to a study conducted in Hungary and Slovakia 8–10 weeks of pigs had the highest PPV8 prevalence rate (8%), followed by those aged 14 weeks (7%) [47]. However, additional research is required to sufficiently support the specific age-related prevalence of PPV8 due to the lack of research on the virus.

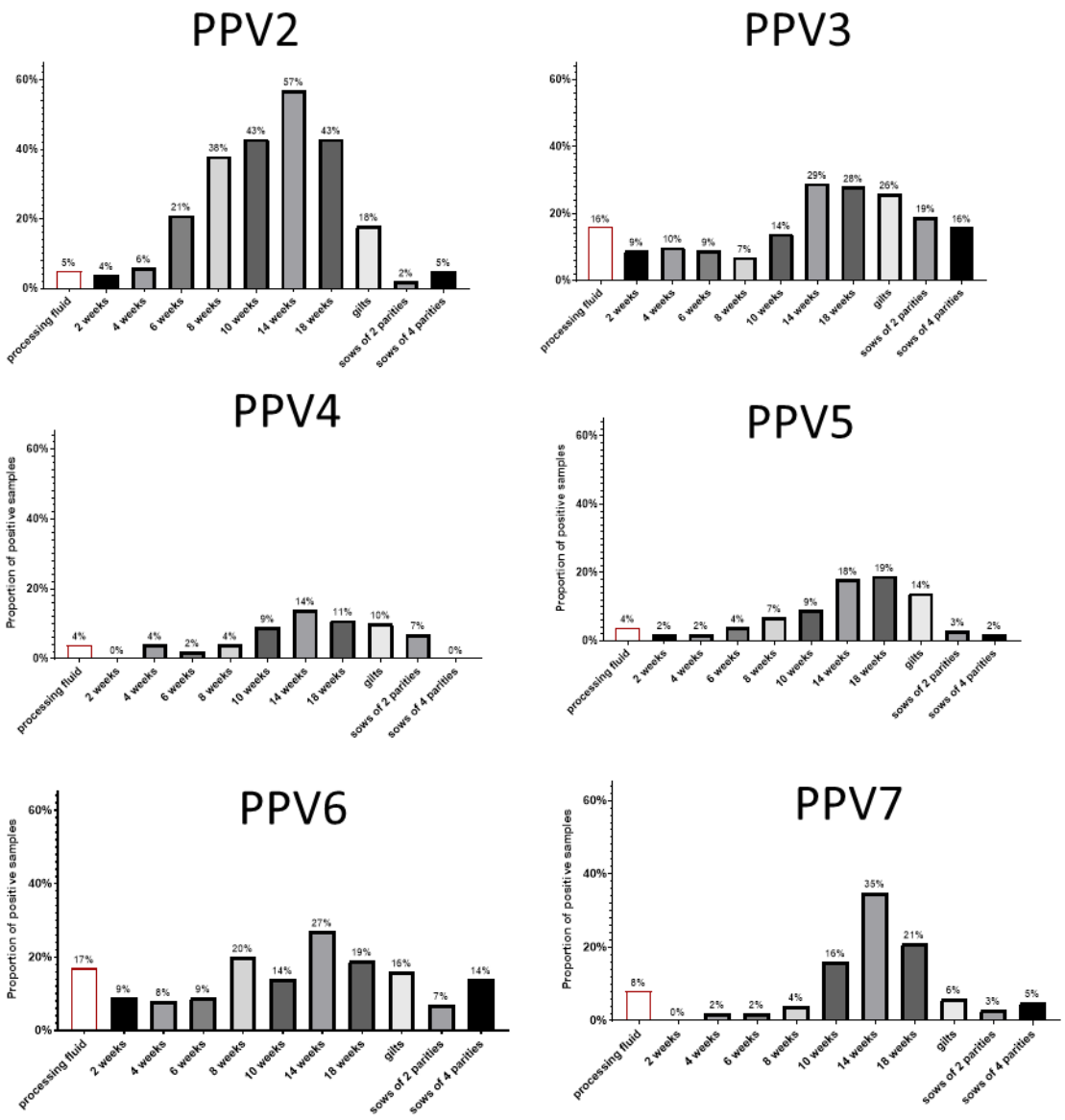

Figure 4. The percentages of PPV2–7-positive processing fluid and serum samples of different age groups detected in Hungary during a study including 26 large-scale pig farms [55].

5. Tissue tropism

The novel PPVs exhibit diverse tissue tropism across various organ systems, demonstrating their ability to adapt to different host environments. The primary target cells of PPV1 are rapidly dividing cells, significantly affecting tissues in the lymphoid and reproductive systems. The primary sites for replication are the lymphoid tissues which can lead to viremia and systemic spread [10]. Subsequently, the virus can replicate in macrophages and crosses the placenta to infect the fetus. In fetal samples, PPV1 has been detected in the liver, kidneys, brain, and lungs [57]. Although PPV1 is also detected in the respiratory and gastrointestinal systems, its primary pathogenic effects are observed in reproductive tissues. PPV1 replication has been observed in the heart, lung, kidney, spleen, endometrium, and intestines [58].

Unlike PPV1, the novel PPVs display a broader distribution across multiple organ systems. They have been detected in the serum, lymphatic tissues (such as the spleen and tonsils), and respiratory tract samples including nasal swabs, bronchoalveolar lavage, lung tissues, and bronchial lymph nodes [5, 44, 46, 51, 57]. Moreover, all of these viruses have been detected in gastrointestinal samples, including feces and oral fluids.

A study in Hungary tested PPVs in feces, blood serum, lungs, and tissues of different organs such as the liver, kidney, spleen, and lymph nodes, aborted fetuses and sperm samples. Out of all these samples, PPV4 was reported as the most prevalent in fecal samples, followed by PPV2 and PPV3 [16]. A study in Poland that used only oral fluids, serum, and fecal samples found PPV5 as the most prevalent PPV in fecal samples, followed by PPV2, PPV6, PPV4, and PPV3 [18]. Detection rates in oral fluids are notably high, indicating that this sample type is suitable for screening these pathogens in pig herds. Recent Hungarian studies found that PPV7 and PPV8 had the highest detection rates in oral fluid samples, as around half of the tested samples were positive for these viruses, but all other PPVs had significant prevalence in oral fluids (Fig.5) [55]. A Polish study also found high detetction rates in oral fluid samples, with PPV2 being the most prevalent followed by PPV6, PPV5, PPV3, and PPV4 respectively [18].

Reproductive tissues are another important site for novel PPVs, often associated with reproductive failure in sows as well. PPV2 and PPV6 have been frequently detected in fetal heart tissues in abortion cases, particularly in PCV2-positive samples [53]. PPV4 has been detected in the ovaries and uteri of sows with reproductive complications suggesting vertical transmission [52]. In addition, recently PPVs have been detected in processing fluids

collected during piglet castration with PPV6 and PPV3 being the most prevalent in this sample type (Fig.5) [55]. PPV4 and PPV7 have been detected in semen indicating a possible horizontal transmission route [14, 58]. Other novel PPVs, such as PPV3 and PPV8 have also been identified in liver and spleen samples [3, 44, 59]. Finally, PPV2, PPV4, and PPV8 have been detected in kidney tissues [30, 44, 51]. These findings emphasize the extensive tissue tropism and complex pathogenic potential of novel PPVs, pointing out their important role in diverse disease processes and their transmission across various organ systems (Fig. 6).

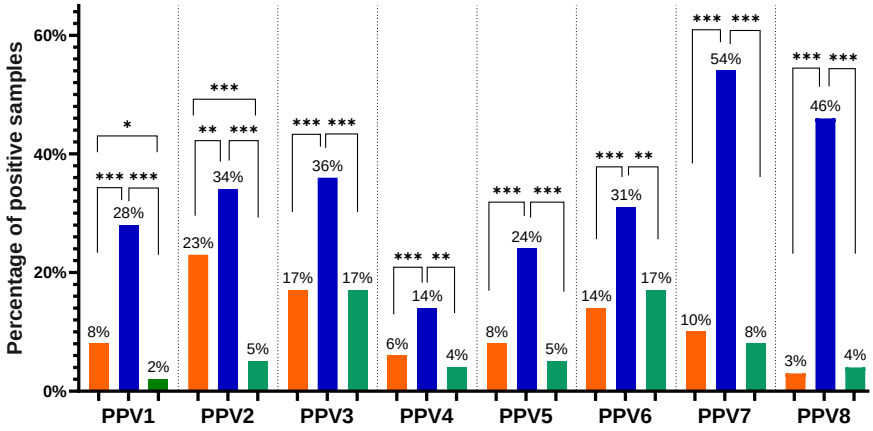

Figure 5. Percentages of porcine parvovirus 2–8 (PPV2–8)-positive serum pools, oral fluid, and processing fluid samples (Igriczi, 2024, Dissertation)

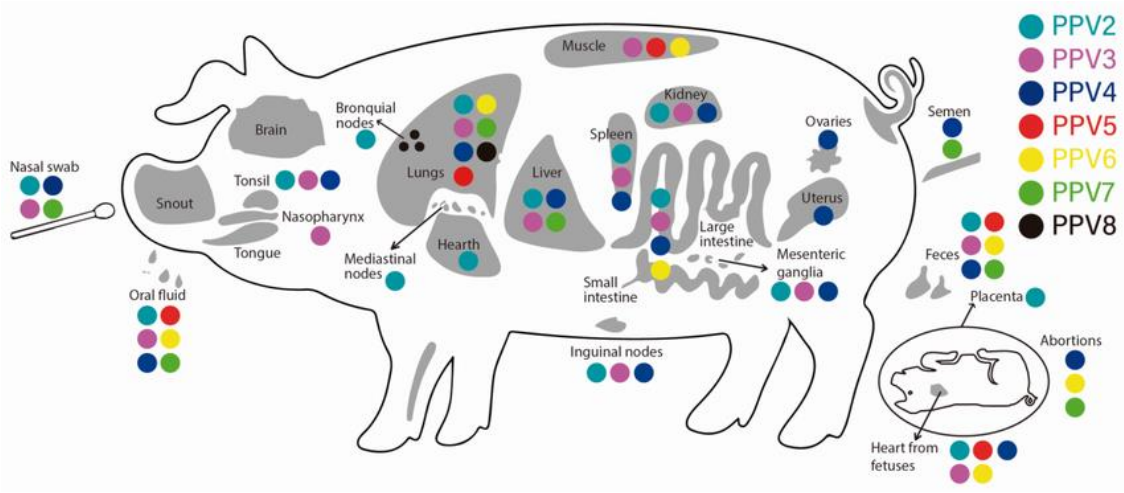

Figure 6. Detection of the novel PPVs (PPV2 through PPV8) in organs, excretions, and abortions of pigs, according to the reports found in the literature [53].

6. Clinical manifestations

Though less has been discovered about the pathogenesis of novel PPVs compared to PPV1, it is believed to involve factors, such as immune system weakness, persistent infections, and an increased risk of contracting further infections. PPVs are frequently detected in both healthy and sick pigs, which suggests that they may induce subclinical infections that, when paired with other pathogens, might worsen disease outcomes. These viruses appear to mostly replicate cells that are actively dividing, in organs connected to the immune system, such as the digestive and respiratory systems which result in persistent infection and a weakened immune system. Ni et al reported that these viruses may hide from the immune system, which enables them to remain in the body and potentially cause issues when the immune system is compromised [7]. Therefore, when additional pathogens or stressors are present, these novel viruses may exacerbate health difficulties, leading to respiratory, immunological, and reproductive concerns [7]. Since PPV2–8 infections might affect the health and productivity of pigs, it is essential to research the signs of emerging PPVs.

PPV2 has been linked with respiratory complications in pigs, especially in coinfections with other pathogens such as PRRSV and PCV2 [57, 60]. A study investigating the effect of PPV2 on the Porcine Respiratory Disease Complex (PRDC), found that it is present in 39% of PRDC-infected pigs and is more common in alveolar macrophages. PPV2 was found to be connected to interstitial pneumonia marked by the infiltration of macrophages and lymphocytes in lung tissues. Its presence was related to reduced alveolar spaces and lung inflammation contributing to breathing difficulties. However, it has also been discovered as a sole pathogen in respiratory diseases, which indicates that it might play a part in the development of diseases (Fig.7) [59]. In addition, another study exploring the localization of PPV2 in lung tissues using immunohistochemistry and *in situ* hybridization methods detected PPV2 primarily in lymphocytes and macrophages and was associated with typical histopathological lesions, including alveolar reduction, necrosis of bronchial epithelial cells, and inflammatory infiltrates [63]. This study suggested that PPV2 could have a tropism for immature B lymphocytes and or NK lymphocytes. The PPV2 virus is considered to potentially contribute to lung pathology, especially in cases of PCV2-SD. While evidence supports its role as a risk factor in respiratory disease, more studies are needed to determine PPV2 as an independent pathogenic agent in pigs [63].

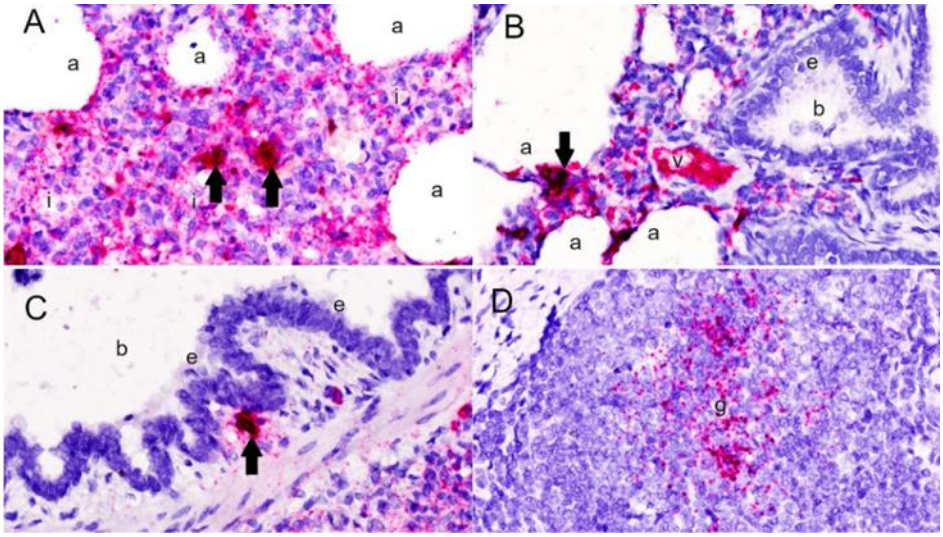

Figure 7. The presence of porcine parvovirus 2 (PPV2) nucleic acid in lung tissue was demonstrated using *in situ* hybridization (ISH, ×600). (A, B) PPV2 signals were observed as small pinpoint structures within the cytoplasm of monocytic cells in the alveolar interstitium (i), as well as in pneumonocytes, alveolar macrophages lining the alveoli (a), and endothelial cells of small blood vessels (v). (C) Intense PPV2 signals, both intracytoplasmic and intranuclear, were detected in large cells resembling macrophages or dendritic cells (arrows). Additionally, PPV2 signals were found in round cells surrounding the bronchus (b) but were absent in the bronchial epithelium (e). (D) Aggregated PPV2 signals were concentrated in the germinal center (g) of lymphoid follicles in peribronchial lymphoid tissues [59] .

Although PPV3 was detected in lymph nodes, liver, serum, and respiratory samples, its clinical manifestations are not well characterized [64]. However, it is usually identified in coinfection with other pathogens such as PCV2 and PRRSV, and thus, its direct contribution to the disease process is often unclear [21, 22]. Further investigation into PPV3 pathogenic role and clinical importance should be done in order to understand the impact it has in swine health.

PPV4-infection has been associated with reproductive disorders and is commonly detected in aborted pig fetuses, which provides a link to SMEDI syndrome [28] [50]. In China, PPV4 infection was recorded among pigs showing symptoms of trembling, fever, atrophy of testicles, abortion, and death. Infected adult pigs mainly had reproductive problems, while sick piglets showed neurological issues. PPV4 was detected in the heart, blood, lymph nodes, lungs, and kidneys. Interestingly, all PPV4-positive samples were detected in coinfection with porcine torque teno virus (PTTV1 and PTTV2) and in a few of them PCV2 was found as well [32]. Since PPV4 wasn't the only pathogen detected in these clinical cases, further research is needed to evaluate its ability to cause disease on its own.

PPV5 has been detected globally but lacks a definitive association with specific clinical signs. Garcia-Camacho et al has indicated that PPV5 has a substantial correlation with PCVAD, even though it has not been proven to be associated with particular clinical symptoms. The severity may increase if PPV5 is coinfected with other pathogens, particularly PCV2, suggesting that PPV5 is not a major pathogen but rather may contribute to disease complexes. The association between PPV5 and reproductive failure or other clinical symptoms alone is not well supported by the available data [28, 63].

PPV6 has been detected in aborted fetuses, although, its direct role in reproductive failure has not been determined. Subsequent research in North America found PPV6 in serum samples from pigs coinfected with PRRSV. However, there were no specific symptoms associated with PPV6 infection, so even though PPV6 is relevant in the pig population, current evidence does not clearly link it to specific clinical symptoms [35, 38].

PPV7 has also been associated with the reproductive syndrome SMEDI. Sows infected with PPV7 during gestation, usually suffer from reproductive failures, like early fetal death, and mummification of the fetuses [52, 64]. Though typically subclinical in adults, coinfections with pathogens like PCV2, PCV3 and PRRSV may worsen the disease's clinical manifestation and increase reproductive losses. However, PPV7 has been detected in other types of samples as well such as blood, faces, and saliva suggesting a systemic circulation of the virus in the organism and possibly being able to affect the overall health state of the animal [65, 66].

The most recently identified PPV, the PPV8 has been detected in pigs experiencing respiratory symptoms. A Colombian study identified PPV8 in 4.1% of lung samples collected from pigs having PRD (porcine respiratory disease) symptoms [46]. However, research carried out in Europe isolated PPV8 in samples from pigs that did not experience any clinical signs except one Slovakian farm that experienced respiratory problems in the nursery unit (personal communication) [47]. Even though there is evidence of PPV8 in connection with respiratory disease, it is very limited, and further research is necessary to determine the clinical manifestations of PPV8.

In summary, although the clinical signs of PPV1 are well-defined, the role of novel PPVs in pig health remains under investigation. Data collected so far indicate that these viruses are most likely associated with respiratory and systemic diseases, especially when coinfected with other viruses. Further research is needed to clarify their roles and implications for swine health.

7. Coinfections with novel porcine parvoviruses

Increasing evidence points to coinfections by novel PPVs (PPV2–PPV8) and other pathogens as an important contributing factor to the increasing complexity of swine diseases. These parvoviruses, often detected in asymptomatic animals, may play a role as opportunistic agents in exacerbating clinical manifestations of infection caused by more virulent pathogens. Coinfections of PPVs with other pathogens can lead to viral interference and recombination of the viruses which results in the emergence of new viral strains with altered pathogenicity. For example, a study detected PPV7 in a domestic pig which was a recombinant virus derived from two wild boar isolates. Such genetic changes may complicate disease dynamics and diagnosis of these viruses in swine populations [21].

7.2.Coinfection with PCV2

PCV2 is the primary causative agent of porcine circovirus-associated disease (PCVAD), for which PPV1 is considered to be one of the cofactors. PCV2 is associated with with PCV2 systemic disease, formerly called post-weaning multisystemic wasting syndrome (PMWS) in pigs. Coinfection of PCV2 with other agents usually increases the severity of the disease outcomes [69]. The relationship between PCVs, particularly PCV2 and PCV3, and PPVs remains a broadly discussed topic in the literature. A strong correlation has been observed between PCV2 and PPVs, such as PPV1, PPV2, and PPV7, which are known to exacerbate PCV2 infections. Among these, only PPV7 has been shown to increase PCV3 replication, contributing to PCV-3-related reproductive problems [21, 23, 31].

Various studies show slightly different prevalence rates and coinfection patterns, likely due to geographic variation, different age groups, and health conditions of the pigs sampled. A study carried out in Northern Italy investigating the PPV's presence in reproductive failure outbreaks, reported a higher frequency of PCV-2 coinfection with PPV5 [31]. In contrast, a four-year study (conducted between 2016 and 2020) in China examining the prevalence and coinfection status of PPV types 1 through 7 has reported associations between PCV2 and PPV1, PPV2, and PPV3, but no notable links with PPV2, PPV4, PPV5, or PPV7 [21]. Furthermore, a study conducted in Korea revealed that lung samples from PCVAD cases exhibited higher prevalence rates of PPV1, PPV3, and PPV6 with increased detection in affected pigs [23].

Thus, PPV coinfection with PCVs plays a significant role in the pathogenesis of PCVAD. Particularly coinfection of PPV1, PPV2, and PPV7, with PCVs might exacerbate the infection and the clinical manifestations. The prevalence rates and patterns of these coinfections are also shaped by the geographic distribution and sample variability. Further studies are necessary to understand the association between these diseases and how they influence the swine population.

7.3.Coinfections with PRRSV

Porcine Reproductive and Respiratory Syndrome virus (PRRSV) is one of the most important pathogens affecting populations of pigs worldwide. The virus has two clinical manifestations: reproductive failure in breeding animals and respiratory disease. Recent studies have reported that there is possibly a synergistic relationship between PRRSV and PPVs in pigs, aggravating the condition in coinfected pigs. A study performed in Korea showed that PPV2 and PPV7 were significantly more prevalent in PRRSV-positive samples than in PRRSV-negative samples. Also, cases where PRRSV1 and PRRSV2 were coinfected showed a higher prevalence of PPV5 and PPV6, compared to PRRSV-negative samples. No significant differences were observed for PPV1, PPV3, or PPV4 (Fig.8) [23].

This same coinfection of PPVs with PRRSV was also reported in a study conducted on gilts from Colombian swine herds. Their results indicated that the coinfection rate between PRRSV and PPV3 was higher, followed by PPV5 and PPV6. Results indicate that PPV and PRRSV coinfections are not rare in swine populations and may impact the dynamics of the diseases, especially in gilts. [38]

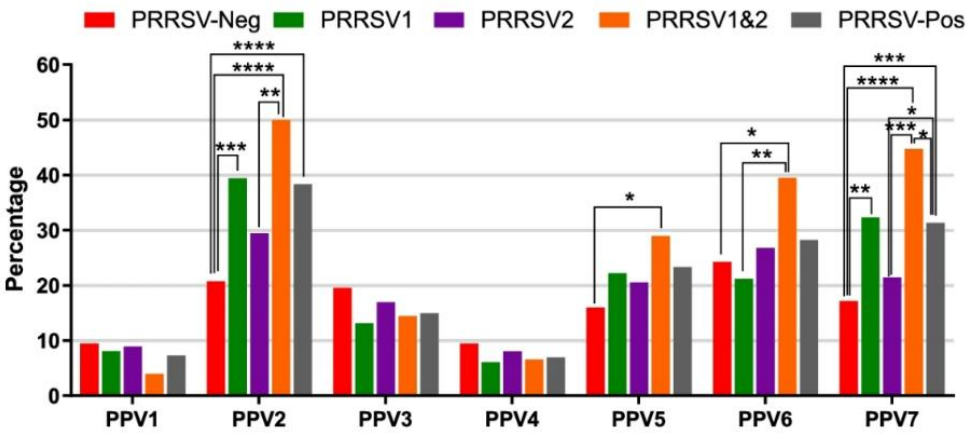

Figure 8. Prevalence PPV1-PPV7 and co-factor association with Porcine Reproductive and Respiratory Syndrome virus (PRRSV) [21]

8. Diagnosis of novel porcine parvoviruses

The diagnosis of novel PPVs is an important issue in the management of pig health, especially since these viruses, during the last years, have emerged as significant pathogens. Traditional diagnostic methods, such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) methods, have been widely used up to now for detection and identification because of their high sensitivity and specificity. New diagnostic methods have emerged, such as next-generation sequencing (NGS) methods, including Oxford Nanopore or Illumina, and an interesting novel CRISPR/Cas12a-based system, that enable more rapid and sensitive detection of PPVs. These recent techniques are expected to facilitate the control and management of diseases within swine herds.

8.2.Conventional methods for the identification and detection of PPVs

Polymerase chain reaction (PCR) is widely regarded as the gold standard in diagnostic virology, offering unmatched sensitivity, specificity, and versatility for detecting viral DNA. This technique, including its quantitative variant (qPCR), has become a cornerstone in identifying novel PPVs. It utilizes virus-specific primers to amplify its DNA making it highly reliable for distinguishing between different PPVs [16, 36]. Various studies have used duplex qPCR for the detection of PPVs. This technique uses two primers, each specific to different pathogens along with two different fluorescein probes which allow for the amplification of two DNA sequences simultaneously. Duplex qPCR was successfully used for the detection of PPV2–PPV7 from various samples in Hungarian pig farms [55]. A study carried out in Polish farms used both single and duplex qPCR to test the specificity of duplex PCR. The comparison of the results indicated that duplex PCR did not have any adverse effects on the test performance which confirmed its specificity [18].

A recent study has developed a multiplex PCR (mPCR) assay, that succesfully detected 7 PPVs (PPV1–7) simultaneously in clinical samples collected from different regions of China [19, 68]. This method enables the amplification of DNA from several PPVs in a single reaction, both time- and resource-efficient. The specificity of the mPCR has been validated against other common porcine pathogens to ensure accurate identification without cross-reactivity. The sensitivity was tested using serial dilutions of the seven PPVs to determine the minimum detection limit of each virus. Their detection threshold was

determined using plasmids of each PPV which contained specific genome sequences for each virus (Fig.9).

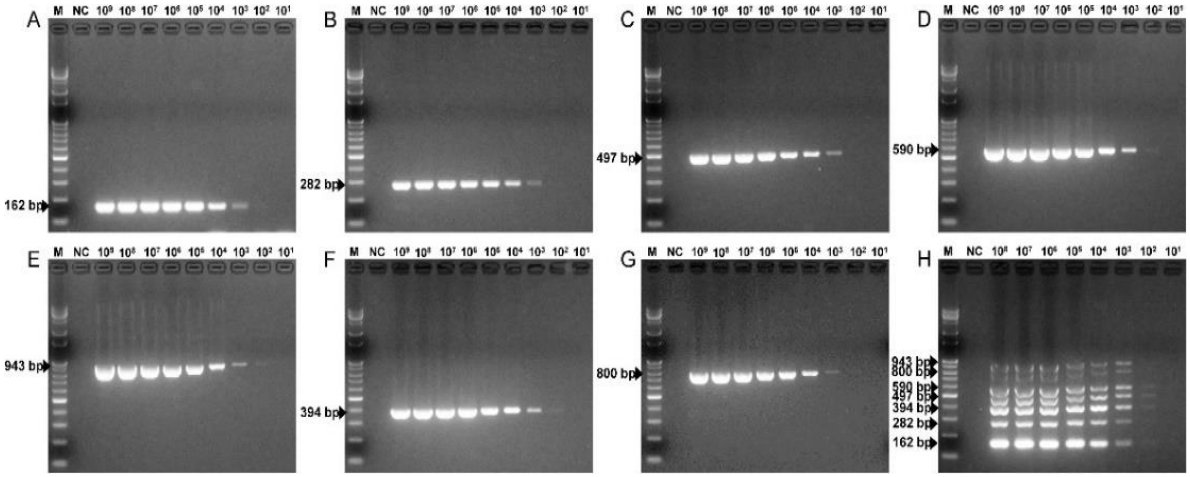

Figure 9. The seven PPV single plasmids, were used to determine the minimum detection limit of the PPV mPCR method. Agarose gel electrophoresis was used to visualize the results of the different PCR reactions: (A) Sensitivity for PPV1. (B) Sensitivity for PPV2. (C) Sensitivity for PPV3. (D) Sensitivity for PPV4. (E) Sensitivity for PPV5. (F) Sensitivity for PPV6. (G) Sensitivity for PPV7. (H) Sensitivity for PPV1-PPV7 [68].

Another important diagnostic tool is enzyme-linked immunosorbent assay (ELISA), which complements PCR by detecting the presence of antibodies against PPVs in swine serum. Serological profiles of infected herds can be examined with this method, which can give information on the immune responses against these viruses. For instance, an indirect ELISA specific to PPV2 revealed a decline in maternal antibody levels over time. The PPV2-specific antibodies started increasing between 28 to 43 days of age, which coincided with the observed respiratory signs in the herd [71].

8.3.New techniques for the identification and detection of PPVs

Advancements in molecular diagnostic tools have expanded the capabilities for identifying and characterizing novel PPVs. Among these, different NGS techniques and also CRISPR-based detection systems have emerged as innovative methods for PPV detection.

NGS platforms, sush as Oxford Nanopore or Illumina, have also been widely used to detect novel PPVs. Illumina was recently employed in China to identify the newest PPV, the PPV8 from pig lung samples. In this study, NGS was used to detect the presence of different porcine pathogens in PRRSV-positive clinical samples. This method involves extracting

viral DNA from samples and sequencing it to detect novel viruses. The genomic analysis revealed unique sequence motifs in NS1 and VP1 protein coding sequences, that were used for determining genetic relationships. The analysis showed that PPV8 was phylogenetically distinct from the rest of the PPVs and it belongs to the *Protoparvovirus* genus clustering together with PPV1 [9].

Nanopore sequencing has also been utilized to study viral genomes directly from clinical samples. This diagnostic tool is suitable for rapid detection and comprehensive genomic analysis, making it ideal for real-time diagnostics [72]. Whole-genome sequencing of PPV1 was successfully performed using the nanopore platform [73].

Another promising development is the application of CRISPR/Cas12a-based detection systems. These systems, combined with enzyme recombinase amplification (ERA) and lateral flow dipstick (LFD) technology, offer a rapid and cost-effective *in-situ* detection method of PPVs. The study demonstrated the efficacy of this system for detecting PPV1, and also tested the specificity of this method against a few other viruses, including PPV2. The ERA-CRISPR/Cas12a system targets the conserved VP2 gene and amplifies the DNA of PPV1. Results can then be obtained on-site by visual examination using an LFD, without the need for special equipment. This method reached a detection limit of 3.75×10^2 copies/mL surpassing the sensitivity of conventional PCR methods. This system also demonstrated high specificity for PPV1 without any cross-reactivity with other viruses. This test is practical and economical for field diagnosis which can improve disease management in pig herds [74]. Although its application to other PPV strains remains limited, this method seems promising for the future detection of different pig pathogens.

Altogether, these advanced techniques represent a significant leap forward in PPV detection, enabling more accurate, rapid, and accessible diagnostics, which are crucial for managing and controlling these infections in swine populations

9. Control and Prevention

The control and prevention of emerging PPVs require a multifaceted approach incorporating multiple farm management strategies. Implementing strict biosecurity procedures is one of the most essential actions to reduce the introduction and spread of these viruses. While vaccination programs are available only for PPV1 they are important for lowering the susceptibility of pigs against PPV1 and minimizing the adverse effects of the clinical signs. Frequent monitoring and surveillance are also crucial for the control and prevention of PPVs. Gathering important epidemiological data helps in the early diagnosis and control of the disease. All these strategies combined can create a successful management system for PPV prevention and control.

9.2. Biosecurity measures

To prevent PPV introduction, external biosecurity protocols are fundamental. This should include the installment of barriers, such as fencing around the farm to restrict the entrance of external wildlife that may introduce these viruses. Additionally, all farm visitors and staff should comply with strict sanitization protocols. Thorough disinfection of vehicles entering the premises and vehicles used for the transport of animals is also important to prevent the transmission of the disease between farms. Also, the semen used from external sources should be controlled and collected from PPV-free farms to reduce the risk of venereal infection in breeding farms. Implementing an all-in-all-out protocol is also essential where all animals are grouped and moved together through their production stages, with the farm facility undergoing strict cleaning and disinfection before introducing new groups. Furthermore, before the introduction of new animals on the farm, the animals should spend at least 14 days in quarantine before being introduced into the herd. Also, personnel movement between each herd is important. To prevent PPVs from spreading from one herd to another, staff members should take specific precautions. This can involve using gloves, foot baths, changing boots, and sanitization stations before entering each herd. Materials and equipment used in the farm are also very crucial factors in disease spreading. Appropriate cleaning of the equipment is important between each herd. Adequate ventilation is also necessary to decrease the environmental microbial load [73, 74].

9.3.Vaccination strategies

Vaccination strategies against PPVs have so far been developed exclusively for PPV1, the oldest and most well-characterized PPV. These vaccines have significantly reduced the clinical impact of infection, particularly reproductive losses, although they do not necessarily prevent the infection. Vaccinated herds present lower rates of reproductive losses, compared to non-vaccinated herds [10]. However, research regarding the vaccination strategies against the recently identified novel PPVs is limited. Due to the lack of knowledge regarding the pathogenicity, immunogenicity, and clinical impact of these novel viruses on swine health, no vaccines have been developed to protect swine populations against these strains. To date, there is no evidence to suggest that existing PPV1 vaccines provide cross-protection against novel PPVs. As the prevalence of these novel strains increases globally, their potential role in coinfections and disease complexes underscores the need for targeted vaccine research and development. Emerging research has begun to explore potential vaccination approaches for specific novel PPVs. For example, studies on PPV7 have utilized immune-informatics to evaluate possible epitopes of PPV7's CAP protein, which is the primary antigenic viral protein for vaccine production. These conserved B-cell and T-cell epitopes of the Cap protein have been shown to trigger immunological responses and could serve as the basis for developing effective vaccines. Moreover, these epitopes can potentially provide cross-protection against related PPV strains, providing a framework for broader protective strategies [77].

Further investigation into the immunological properties of novel PPVs, coupled with advancements in computational vaccinology and cross-protection studies, will be crucial for developing effective vaccines. Proactive vaccination strategies could mitigate the impact of novel PPVs on swine health and enhance herd immunity in the face of evolving viral challenges.

9.4.Monitoring and surveillance

Given the significant impact of PPVs on swine health and productivity, effective monitoring and surveillance systems are critical in controlling their effects on pig populations. These systems rely on comprehensive sample collection and analysis to assess the prevalence and distribution of these viruses across herds. Samples can be collected from pigs of all ages, utilizing various sample types, such as organ tissues, oral fluids, serum, and feces. For example, in a Polish study, oral fluids, serum, and fecal samples were collected

from pigs aged 3 to 21 weeks and tested using qPCR to determine the distribution of PPVs across pig farms [18]. This underscores the importance of collecting diverse samples to provide a complete epidemiological picture. Due to its high sensitivity and specificity, qPCR has become the gold standard diagnostic tool for detecting novel PPVs [16, 76]. The data obtained through such diagnostic methods form the backbone of monitoring programs, enabling the generation of critical epidemiological insights. For instance, it has been reported that some of the recently emerged PPVs can be found in several anatomical sites of pigs, and certain populations show greater susceptibility. The prevalence of such viruses might differ widely between geographical regions and among different age groups, hence, targeted surveillance can minimize the risk of infection in susceptible populations based on specific areas or age groups and help in the implementation of control measures and prevention of the infection [19, 77].

By integrating precise diagnostic techniques with well-designed surveillance strategies, swine health professionals can proactively manage the risks associated with novel PPVs, thereby improving herd health and productivity.

10. Conclusion

This review highlights the key aspects of novel PPVs and their impact on swine health. The global distribution and persistence of PPVs in many diverse pig populations and environments provide information on the robust mechanisms of transmission. Horizontal transmission via feces, oral fluids, and nasal discharge is of main importance, supplemented by vertical transmission, especially during gestation, which might lead to reproductive losses. The ability of novel PPVs to infect a wide range of tissues, including reproductive organs, fetal tissues, and the respiratory system, further justifies their adaptability and supports the potential for systemic infection.

The clinical significance of novel PPVs remains challenging to define due to their frequent coinfection with other pathogens such as PCVs (PCV2 and PCV3) and PRRSV. These coinfections often exacerbate disease severity, suggesting that novel PPVs may act as opportunistic pathogens. Compared to PPV1, their clinical manifestations still remain less defined. Moreover, their frequent subclinical presentations allow the viruses to circulate undetected, contributing to their spread and exacerbating disease severity under stress or in the presence of other pathogens. These complexities emphasize the urgent need for further research to find more information on the pathogenic mechanisms of these viruses and their interactions with other pathogens.

The traditional methods of diagnosis, like PCR and ELISA, are still useful tools for the diagnosis of PPVs, while NGS methods and novel CRISPR-based detection systems are promising emerging technologies for more precise and rapid identification. However, prevention methods for novel PPVs are limited at the moment, and no vaccination is available to reduce their impact. The current vaccination programs involve only PPV1, reducing reproductive losses but not solving the problems caused by the rest of the novel PPVs. The major challenge for their management is the limited knowledge of their pathogenicity, epidemiology, and wider implications. These viruses are considered to be emerging threats against the health of swine, yet much about their role in disease processes is still not known. This includes their interaction with other pathogens and their full impact on swine productivity and health. The novel PPVs can be prevented and controlled by the development of enhanced diagnostic technologies, enhanced biosecurity practices, and vaccination methodologies. It is further required that continued pathogenicity and epidemiological studies into these viruses are carried out to create the management protocols to reduce their impacts on the worldwide swine industry.

Further research on the pathogenicity, epidemiology, and transmission dynamics of these viruses is critically required. Further understanding of novel PPVs is essential for efficient management protocols to be created, contributing to a decrease in the influence they have on the health of swine populations. Understanding and mitigating these emerging threats requires proactive and collaborative attention to ensure the long-term sustainability and productivity of pig farming worldwide.

References

[1] A. Mayr and H. Mahnel, “Cultivation of hog cholera virus in pig kidney cultures with cytopathogenic effect,” *Zentralblatt Bakteriol. Parasitenkd. Infekt. Hyg. 1 Abt Med.-Hyg. Bakteriol. Virusforsch. Parasitol. Orig.*, vol. 195, no. 2, pp. 157–166, 1964.

[2] S. F. Cartwright, M. Lucas, and R. Huck, “A small haemagglutinating porcine DNA virus: I. Isolation and properties,” *J. Comp. Pathol.*, vol. 79, no. 3, pp. 371–377, 1969.

[3] M. Hijikata, K. Abe, K. Win, Y. Shimizu, N. Keicho, and H. Yoshikura, “Identification of new parvovirus DNA sequence in swine sera from Myanmar,” *Jpn. J. Infect. Dis.*, vol. 54, pp. 244–5, Jan. 2002.

[4] S. K. Lau *et al.*, “Identification of novel porcine and bovine parvoviruses closely related to human parvovirus 4,” *J. Gen. Virol.*, vol. 89, no. 8, pp. 1840–1848, 2008.

[5] A. K. Cheung, G. Wu, D. Wang, D. O. Bayles, K. M. Lager, and A. L. Vincent, “Identification and molecular cloning of a novel porcine parvovirus,” *Arch. Virol.*, vol. 155, no. 5, pp. 801–806, May 2010, doi: 10.1007/s00705-010-0646-8.

[6] C.-T. Xiao, P. F. Gerber, L. G. Gimenez-Lirola, P. G. Halbur, and T. Opriessnig, “Characterization of porcine parvovirus type 2 (PPV2) which is highly prevalent in the USA,” *Vet. Microbiol.*, vol. 161, no. 3–4, pp. 325–330, 2013.

[7] J. Ni *et al.*, “Identification and genomic characterization of a novel porcine parvovirus (PPV6) in china,” *Virol. J.*, vol. 11, no. 1, p. 203, Dec. 2014, doi: 10.1186/s12985-014-0203-2.

[8] R. M. Palinski, N. Mitra, and B. M. Hause, “Discovery of a novel Parvovirinae virus, porcine parvovirus 7, by metagenomic sequencing of porcine rectal swabs,” *Virus Genes*, vol. 52, no. 4, pp. 564–567, 2016.

[9] Y. Guo *et al.*, “Identification and genomic characterization of a novel porcine parvovirus in China,” *Front. Vet. Sci.*, vol. 9, p. 1009103, 2022.

[10] A. F. Streck and U. Truyen, “Porcine parvovirus,” *Curr. Issues Mol. Biol.*, vol. 37, no. 1, pp. 33–46, 2020.

[11] D. Miłek, A. Woźniak, and T. Stadejek, “The detection and genetic diversity of novel porcine parvovirus 7 (PPV7) on Polish pig farms,” *Res. Vet. Sci.*, vol. 120, pp. 28–32, Oct. 2018, doi: 10.1016/j.rvsc.2018.08.004.

[12] J. J. Pénzes *et al.*, “Reorganizing the family Parvoviridae: a revised taxonomy independent of the canonical approach based on host association,” *Arch. Virol.*, vol. 165, pp. 2133–2146, 2020.

[13] T. Opriessnig, C.-T. Xiao, P. F. Gerber, and P. G. Halbur, “Identification of recently described porcine parvoviruses in archived North American samples from 1996 and association with porcine circovirus associated disease,” *Vet. Microbiol.*, vol. 173, no. 1, pp. 9–16, 2014, doi: https://doi.org/10.1016/j.vetmic.2014.06.024.

[14] F. Campos *et al.*, “Complete genome sequence of porcine parvovirus 2 recovered from swine sera,” *Genome Announc.*, vol. 4, no. 1, pp. 10–1128, 2016.

[15] D. S. Vargas-Bermudez *et al.*, “u,” *Int. J. Mol. Sci.*, vol. 25, no. 19, p. 10354, 2024.

[16] A. Cságola, M. Lőrincz, D. Cadar, K. Tombácz, I. Biksi, and T. Tuboly, “Detection, prevalence and analysis of emerging porcine parvovirus infections,” *Arch. Virol.*, vol. 157, pp. 1003–1010, 2012.

[17] G. Faustini *et al.*, “Epidemiological survey of new porcine Parvovirus species in reproductive failure outbreaks in Italy.,” 2022.

[18] D. Miłek, A. Woźniak, M. Guzowska, and T. Stadejek, “Detection patterns of porcine parvovirus (PPV) and novel porcine parvoviruses 2 through 6 (PPV2–PPV6) in polish swine farms,” *Viruses*, vol. 11, no. 5, p. 474, 2019.

[19] A. F. Streck, T. Homeier, T. Foerster, S. Fischer, and U. Truyen, “Analysis of porcine parvoviruses in tonsils and hearts from healthy pigs reveals high prevalence and genetic diversity in Germany,” *Arch. Virol.*, vol. 158, pp. 1173–1180, 2013.

[20] J.-Y. Lee, E. Kim, I. Cho, K.-K. Lee, and Y.-K. Shin, “Complete genome sequences of porcine parvovirus 2 isolated from swine in the Republic of Korea,” *Genome Announc.*, vol. 5, no. 15, pp. 10–1128, 2017.
[21] J. Li *et al.*, “A Systematic Investigation Unveils High Coinfection Status of Porcine Parvovirus Types 1 through 7 in China from 2016 to 2020,” *Microbiol. Spectr.*, vol. 9, no. 3, pp. e01294-21, 2021, doi: 10.1128/Spectrum.01294-21.
[22] P. Saekhow, T. Mawatari, and H. Ikeda, “Coexistence of multiple strains of porcine parvovirus 2 in pig farms,” *Microbiol. Immunol.*, vol. 58, no. 7, pp. 382–387, 2014.
[23] S.-C. Kim, J.-H. Kim, J.-Y. Kim, G.-S. Park, C.-G. Jeong, and W.-I. Kim, “Prevalence of porcine parvovirus 1 through 7 (PPV1-PPV7) and co-factor association with PCV2 and PRRSV in Korea,” *BMC Vet. Res.*, vol. 18, no. 1, p. 133, Apr. 2022, doi: 10.1186/s12917-022-03236-1.
[24] S. Li, Y. Wei, J. Liu, Q. Tang, and C. Liu, “Prevalence of porcine hokovirus and its co-infection with porcine circovirus 2 in China,” *Arch. Virol.*, vol. 158, pp. 1987–1991, 2013.
[25] C. Adlhoch, M. Kaiser, H. Ellerbrok, and G. Pauli, “High prevalence of porcine Hokovirus in German wild boar populations,” *Virol. J.*, vol. 7, pp. 1–4, 2010.
[26] D. Cadar, A. Cságola, M. Lőrincz, K. Tombácz, M. Spînu, and T. Tuboly, “Distribution and genetic diversity of porcine hokovirus in wild boars,” *Arch. Virol.*, vol. 156, pp. 2233–2239, 2011.
[27] I. Sliz, M. Vlasakova, A. Jackova, and S. Vilcek, “Characterization of porcine parvovirus type 3 and porcine circovirus type 2 in wild boars (Sus scrofa) in Slovakia,” *J. Wildl. Dis.*, vol. 51, no. 3, pp. 703–711, 2015.
[28] C.-T. Xiao, L. G. Giménez-Lirola, P. G. Halbur, and T. Opriessnig, “Increasing porcine PARV4 prevalence with pig age in the US pig population,” *Vet. Microbiol.*, vol. 160, no. 3–4, pp. 290–296, 2012.
[29] C. K. Souza *et al.*, “Phylogenetic characterization of the first Ungulate tetraparvovirus 2 detected in pigs in Brazil,” *Braz. J. Microbiol.*, vol. 47, pp. 513–517, 2016.
[30] L. A. Garcia-Camacho, A. Vargas-Ruiz, E. Marin-Flamand, H. Ramírez-Álvarez, and C. Brown, “A retrospective study of DNA prevalence of porcine parvoviruses in Mexico and its relationship with porcine circovirus associated disease,” *Microbiol. Immunol.*, vol. 64, no. 5, pp. 366–376, 2020.
[31] G. Faustini *et al.*, “Molecular Survey on Porcine Parvoviruses (PPV1-7) and Their Association with Major Pathogens in Reproductive Failure Outbreaks in Northern Italy,” *Viruses*, vol. 16, no. 1, 2024, doi: 10.3390/v16010157.
[32] L. Huang, S.-L. Zhai, A. K. Cheung, H.-B. Zhang, J.-X. Long, and S.-S. Yuan, “Detection of a novel porcine parvovirus, PPV4, in chinese swine herds,” *Virol. J.*, vol. 7, pp. 1–4, 2010.
[33] K. O. Afolabi, B. C. Iweriebor, L. C. Obi, and A. I. Okoh, “Prevalence of porcine parvoviruses in some South African swine herds with background of porcine circovirus type 2 infection,” *Acta Trop.*, vol. 190, pp. 37–44, 2019.
[34] A.-L. Blomström *et al.*, “Viral metagenomic analysis of bushpigs (Potamochoerus larvatus) in Uganda identifies novel variants of Porcine parvovirus 4 and Torque teno sus virus 1 and 2,” *Virol. J.*, vol. 9, pp. 1–7, 2012.
[35] R. Wu *et al.*, “First complete genomic characterization of a porcine parvovirus 5 isolate from China,” *Arch. Virol.*, vol. 159, pp. 1533–1536, 2014.
[36] C.-T. Xiao, P. G. Halbur, and T. Opriessnig, “Complete genome sequence of a novel porcine parvovirus (PPV) provisionally designated PPV5,” *Genome Announc.*, vol. 1, no. 1, pp. 10–1128, 2013.
[37] E. E. Schirtzinger, A. W. Suddith, B. M. Hause, and R. A. Hesse, “First identification of porcine parvovirus 6 in North America by viral metagenomic sequencing of serum from pigs infected with porcine reproductive and respiratory syndrome virus,” *Virol. J.*, vol. 12, pp. 1–10, 2015.
[38] D. S. Vargas-Bermudez, A. Diaz, G. Polo, J. D. Mogollon, and J. Jaime, “Infection and Coinfection of Porcine-Selected Viruses (PPV1 to PPV8, PCV2 to PCV4, and PRRSV) in Gilts and Their Associations with Reproductive Performance,” *Vet. Sci.*, vol. 11, no. 5, p. 185, 2024.

[39] J. Cui *et al.*, “First identification of porcine parvovirus 6 in Poland,” *Virus Genes*, vol. 53, pp. 100–104, 2017.
[40] A. Komina, A. Anoyatbekova, N. Krasnikov, and A. Yuzhakov, “Identification and in vitro characterization of a novel porcine parvovirus 6 in Russia,” *Vet. Res. Commun.*, vol. 48, no. 1, pp. 417–425, 2024.
[41] A.-L. Blomström, X. Ye, C. Fossum, P. Wallgren, and M. Berg, “Characterisation of the virome of tonsils from conventional pigs and from specific pathogen-free pigs,” *Viruses*, vol. 10, no. 7, p. 382, 2018.
[42] S. Dei Giudici *et al.*, “First Molecular Characterisation of Porcine Parvovirus 7 (PPV7) in Italy,” *Viruses*, vol. 16, no. 6, 2024, doi: 10.3390/v16060932.
[43] G.-N. Park *et al.*, “Genetic analysis of porcine parvoviruses detected in South Korean wild boars,” *Arch. Virol.*, vol. 166, no. 8, pp. 2249–2254, 2021.
[44] D. S. Vargas-Bermudez, S. Rendon-Marin, J. Ruiz-Saenz, D. Mogollón, and J. Jaime, “The first report of porcine parvovirus 7 (PPV7) in Colombia demonstrates the presence of variants associated with modifications at the level of the VP2-capsid protein,” *Plos One*, vol. 16, no. 12, p. e0258311, 2021.
[45] X. Xing *et al.*, “First identification of porcine parvovirus 7 in China,” *Arch. Virol.*, vol. 163, no. 1, pp. 209–213, 2018.
[46] D. S. Vargas-Bermudez and J. Jaime, “The first report of porcine parvovirus 8 (PPV8) on the American continent is associated with pigs in Colombia with porcine respiratory disease,” *Arch. Virol.*, vol. 169, no. 9, p. 179, 2024.
[47] B. Igriczi, L. Dénes, K. Schönhardt, and G. Balka, “First Report of Porcine Parvovirus 8 in Europe: Widespread Detection and Genetic Characterization on Commercial Pig Farms in Hungary and Slovakia,” 2024.
[48] D. S. Vargas-Bermudez, J. D. Mogollon, C. Franco-Rodriguez, and J. Jaime, “The Novel Porcine Parvoviruses: Current State of Knowledge and Their Possible Implications in Clinical Syndromes in Pigs,” *Viruses*, vol. 15, no. 12, 2023, doi: 10.3390/v15122398.
[49] J. Szelei, Z. Zadori, and P. Tijssen, “Porcine parvovirus,” *Parvoviruses*, vol. 1, pp. 435–446, 2006.
[50] M. Eterpi, G. McDonnell, and V. Thomas, “Disinfection efficacy against parvoviruses compared with reference viruses,” *J. Hosp. Infect.*, vol. 73, no. 1, pp. 64–70, 2009.
[51] J. Kim, D. U. Han, C. Choi, and C. Chae, “Simultaneous detection and differentiation between porcine circovirus and porcine parvovirus in boar semen by multiplex seminested polymerase chain reaction,” *J. Vet. Med. Sci.*, vol. 65, no. 6, pp. 741–744, 2003.
[52] D. Gava *et al.*, “A TaqMan-based real-time PCR for detection and quantification of porcine parvovirus 4,” *J. Virol. Methods*, vol. 219, pp. 14–17, 2015.
[53] P. Lagan Tregaskis *et al.*, “Co-infection status of novel parvovirus’s (PPV2 to 4) with porcine circovirus 2 in porcine respiratory disease complex and porcine circovirus-associated disease from 1997 to 2012,” *Transbound. Emerg. Dis.*, vol. 68, no. 4, pp. 1979–1994, 2021.
[54] D. Wang, Q. He, N. Wang, and J. Mai, “Epidemiology and Genetic Characterization of Porcine Parvovirus 7 Recovered from Swine in Hunan, China,” *Animals*, vol. 14, no. 15, 2024, doi: 10.3390/ani14152222.
[55] B. Igriczi, L. Dénes, K. Schönhardt, A. Woźniak, T. Stadejek, and G. Balka, “Comparative Prevalence Estimation and Phylogenetic Analysis of Novel Porcine Parvoviruses (PPV2–7) in Hungarian Pig Herds,” *Transbound. Emerg. Dis.*, vol. 2024, no. 1, p. 5117884, 2024.
[56] J. Cui, K. Biernacka, J. Fan, P. F. Gerber, T. Stadejek, and T. Opriessnig, “Circulation of porcine parvovirus types 1 through 6 in serum samples obtained from six commercial polish pig farms,” *Transbound. Emerg. Dis.*, vol. 64, no. 6, pp. 1945–1952, 2017.
[57] C. Choi, T. W. Molitor, H. S. Joo, and R. Gunther, “Pathogenicity of a skin isolate of porcine parvovirus in swine fetuses,” *Vet. Microbiol.*, vol. 15, no. 1–2, pp. 19–29, 1987.
[58] I. Mészáros, F. Olasz, A. Cságola, P. Tijssen, and Z. Zádori, “Biology of porcine parvovirus (Ungulate parvovirus 1),” *Viruses*, vol. 9, no. 12, p. 393, 2017.

[59] A. Nelsen, C.-M. Lin, and B. M. Hause, “Porcine parvovirus 2 is predominantly associated with macrophages in porcine respiratory disease complex,” *Front. Vet. Sci.*, vol. 8, p. 726884, 2021.
[60] H.-C. Chung, V.-G. Nguyen, T.-M.-L. Huynh, Y.-H. Park, K.-T. Park, and B.-K. Park, “PCR-based detection and genetic characterization of porcine parvoviruses in South Korea in 2018,” *BMC Vet. Res.*, vol. 16, pp. 1–10, 2020.
[61] P. N. Bisimwa *et al.*, “First investigation on the presence of porcine parvovirus type 3 in domestic pig farms without reproductive failure in the Democratic Republic of Congo,” *Vet. Anim. Sci.*, vol. 13, p. 100187, 2021.
[62] G. Burrai *et al.*, “The Synergic Role of Emerging and Endemic Swine Virus in the Porcine Respiratory Disease Complex: Pathological and Biomolecular Analysis. Vet. Sci. 2023, 10, 595,” 2023.
[63] D. Novosel *et al.*, “Investigating Porcine Parvoviruses Genogroup 2 Infection Using in Situ Polymerase Chain Reaction.,” *BMC Vet. Res.*, vol. 14, pp. 1–8, 2018.
[64] M. C. Jager, J. E. Tomlinson, R. A. Lopez-Astacio, C. R. Parrish, and G. R. Van de Walle, “Small but mighty: old and new parvoviruses of veterinary significance,” *Virol. J.*, vol. 18, pp. 1–29, 2021.
[65] C.-T. Xiao, L. G. Gimenez-Lirola, Y.-H. Jiang, P. G. Halbur, and T. Opriessnig, “Characterization of a novel porcine parvovirus tentatively designated PPV5,” *PLoS One*, vol. 8, no. 6, p. e65312, 2013.
[66] I.-O. Ouh *et al.*, “First detection and genetic characterization of porcine parvovirus 7 from Korean domestic pig farms,” *J. Vet. Sci.*, vol. 19, no. 6, pp. 855–857, 2018.
[67] J. Mai *et al.*, “High co-infection status of novel porcine parvovirus 7 with porcine circovirus 3 in sows that experienced reproductive failure,” *Front. Vet. Sci.*, vol. 8, p. 695553, 2021.
[68] X. Zhang *et al.*, “Genetic and epidemic characteristics of porcine parvovirus 7 in the Fujian and Guangdong regions of southern China,” *Front. Vet. Sci.*, vol. 9, p. 949764, 2022.
[69] T. Opriessnig and P. G. Halbur, “Concurrent infections are important for expression of porcine circovirus associated disease,” *Virus Res.*, vol. 164, no. 1, pp. 20–32, 2012, doi: https://doi.org/10.1016/j.virusres.2011.09.014.
[70] S.-C. Kim *et al.*, “Evaluation of a multiplex PCR method for the detection of porcine parvovirus types 1 through 7 using various field samples,” *PLOS ONE*, vol. 16, no. 1, pp. 1–13, Jan. 2021, doi: 10.1371/journal.pone.0245699.
[71] A. Csagola, Z. Zádori, I. Meszaros, and T. Tuboly, “Detection of porcine parvovirus 2 (ungulate Tetraparvovirus 3) specific antibodies and examination of the serological profile of an infected swine herd,” *PLoS One*, vol. 11, no. 3, p. e0151036, 2016.
[72] S. Theuns *et al.*, “Nanopore sequencing as a revolutionary diagnostic tool for porcine viral enteric disease complexes identifies porcine kobuvirus as an important enteric virus,” *Sci. Rep.*, vol. 8, no. 1, 2018.
[73] N. Vereecke *et al.*, “Molecular epidemiology of Porcine Parvovirus Type 1 (PPV1) and the reactivity of vaccine-induced antisera against historical and current PPV1 strains,” *Virus Evol.*, vol. 8, no. 1, p. veac053, 2022.
[74] J. Wei *et al.*, “Rapid and visual detection of porcine parvovirus using an ERA-CRISPR/Cas12a system combined with lateral flow dipstick assay,” *Front. Cell. Infect. Microbiol.*, vol. 12, p. 879887, 2022.
[75] L. V. Alarcón, A. Allepuz, and E. Mateu, “Biosecurity in pig farms: a review,” *Porc. Health Manag.*, vol. 7, no. 1, p. 5, 2021.
[76] P. Assavacheep and R. Thanawongnuwech, “Porcine respiratory disease complex: Dynamics of polymicrobial infections and management strategies after the introduction of the African swine fever,” *Front. Vet. Sci.*, vol. 9, p. 1048861, 2022.
[77] D. Wang, J. Mai, Y. Yang, and N. Wang, “Porcine parvovirus 7: evolutionary dynamics and identification of epitopes toward vaccine design,” *Vaccines*, vol. 8, no. 3, p. 359, 2020.

[78] C. Song, C. Zhu, C. Zhang, and S. Cui, “Detection of porcine parvovirus using a taqman-based real-time pcr with primers and probe designed for the NS1 gene,” *Virol. J.*, vol. 7, pp. 1–4, 2010.
[79] K. O. Afolabi, B. C. Iweriebor, A. I. Okoh, and L. C. Obi, “Increasing diversity of swine parvoviruses and their epidemiology in African pigs,” *Infect. Genet. Evol.*, vol. 73, pp. 175–183, Sep. 2019, doi: 10.1016/j.meegid.2019.04.029.

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