THESIS

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Diagnostic methods for the detection of atypical porcine pestivirus

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Abbreviations

AA	Amino Acids
APPV	Atypical Porcine Pestivirus
ASFV	African Swine Fever
BDV	Border Disease Virus
BVDV-1	Bovine Viral Diarrhea Virus Type 1
BVDV-2	Bovine Viral Diarrhea Virus Type 2
CNS	Central Nervous System
CP	Cytopathic
CPE	Cytopathic Effect
CSFV	Classical Swine Fever Virus
CSF	Cerebrospinal Fluid
CT	Congenital Tremor
Cq	quantification Cycles
ELISA	Enzyme-linked Immunosorbent Assay
FFPE	Formalin-Fixed and Paraffin-Embedded
FISH	Fluorescent *in situ* Hybridization
HCV	Hepatitis C Virus
IH	Immunohistochemistry
ISH	*In situ* Hybridization
kDa	kiloDalton
MD	Mucosal Disease
NCP	Noncytopathic
NrPV	Norwegian Rat Pestivirus
NS	Nonstructural
ORF	Open Reading Frame
PCR	Polynucleotide Chain Reaction
PCV-2	Porcine Circovirus Type 2
PMWS	Post-weaning Multisystemic Wasting Syndrome
PRRSV	Porcine Reproductive and Respiratory Syndrome Virus
qRT-PCR	Real-Time Quantitative Reverse Transcription PCR

RNA	Ribonucleic Acid
USA	United States of America
VLP	Virus-like Particle
VNT	Virus Neutralization Test

1. Introduction

The family of *Flaviviridae* consists of four genera and includes diseases of economic relevance affecting mammals worldwide such as tick-borne encephalitis virus, yellow fever virus and dengue virus [1]. *Pestivirus*, one of the four genera includes diseases such as bovine viral diarrhea virus (BVDV, *Pestivirus A & B*), and classical swine fever virus (CSFV, *Pestivirus C*) [1]. In general, pestiviruses most notably infect livestock, but other non-ungulate hosts such as bats, rodents, whales, and pangolins have recently also been identified [2].

Atypical porcine pestivirus (APPV, *Pestivirus K*) is a newly discovered member of the genus *Pestivirus*, which was first identified in the United States of America (USA) in 2015, using a next-generation sequencing method [3]. Since then, APPV has been identified in Europe [4–14], Asia [15–17], North America [18], and South America [19].

In recent years, the virus has also been detected in wild boar samples in Germany, Serbia and Spain [20, 21]. Inoculation studies showed that APPV is one of the causative agents of congenital tremor A-II (CT), often complicated with splayed legs [13, 22, 23] and could be responsible for ~10% of piglet death [6, 7].

2. Viral taxonomy

2.1. The *Flaviviridae* family

The *Flaviviridae* family consists of enveloped viruses with a positive-sense, single-stranded ribonucleic acid (RNA) genome. Most viruses of the family infect mammals and birds [1].
Four genera make up the *Flaviviridae* family: *Flavivirus, Hepacivirus, Pegivirus, and Pestivirus.* The *Flaviviridae* family is an eponym derived from the yellow fever virus, with 'yellow' originating from the Latin word 'flavus,' which gave rise to the prefix 'flavi' used in the family and its genus names. The *Flavivirus* genus includes diseases such as West Nile virus, tick-borne encephalitis virus and dengue virus. The genus *Hepacivirus* includes several viruses that predominantly target the liver. *Pegiviruses* make up the third genera, they cause persistent infections but generally do not cause severe disease [1]. The *Pestivirus* genus, which has undergone significant expansion in recent years, is recognized as the fourth genus within the *Flaviviridae* family and is explained in further detail in the following paragraph [1, 2, 24].

2.2. The *Pestivirus* genus

Pestiviruses are enveloped, highly variable, single-stranded, positive-sense RNA viruses with a genome sized approximately 12.3 kb. The viral genome includes one single open reading frame (ORF), encoding a polyprotein of about 3900 amino acids. The genome organization of pestiviruses shows high similarity to the hepatitis C virus (HCV), which is also a member of the *Flaviviridae* family, therefore beside the structural proteins, the nonstructural proteins (NS) are also critical for virion morphogenesis [25]. Formerly this genus consisted of four species, bovine viral diarrhea virus 1 and 2 (BVDV-1 and 2), border disease virus (BDV) and classical swine fever virus (CSFV), which are causing economically relevant diseases in cattle, sheep, goat, and swine [26], respectively. In the last two decades, several additional pestiviruses have been described, like a unique pestivirus from a pronghorn antelope [27], Bungowannah virus of pigs [28], a Norwegian rat pestivirus (NrPV) [29], a putative pestivirus in *Rhinolophus affinis* bats (RaPestV-1) [30], giraffe and reindeer pestiviruses [31], other similar pestiviruses isolated from domestic and wild ruminants [32], atypical porcine pestivirus (APPV) [23] and Linda virus [33]. Therefore in 2017, through a proposed revision of their taxonomy, seven new species of the *Pestivirus* genus were created (*Pestivirus A–K*) [34].
Later, Postel et al. (2021) proposed a second revision to include the following eight new

species bringing the total *Pestivirus* count to 19 (*Figure 1*)*: Pestivirus L* (Linda virus), *Pestivirus M* (Phocoena pestivirus), *Pestivirus N* (Tunisian sheep-like pestivirus), *Pestivirus O* (ovine/IT virus), *Pestivirus P* (pangolin pestivirus), *Pestivirus Q* (rodent pestivirus), *Pestivirus R* (rodent pestivirus), *Pestivirus S* (bat pestivirus).

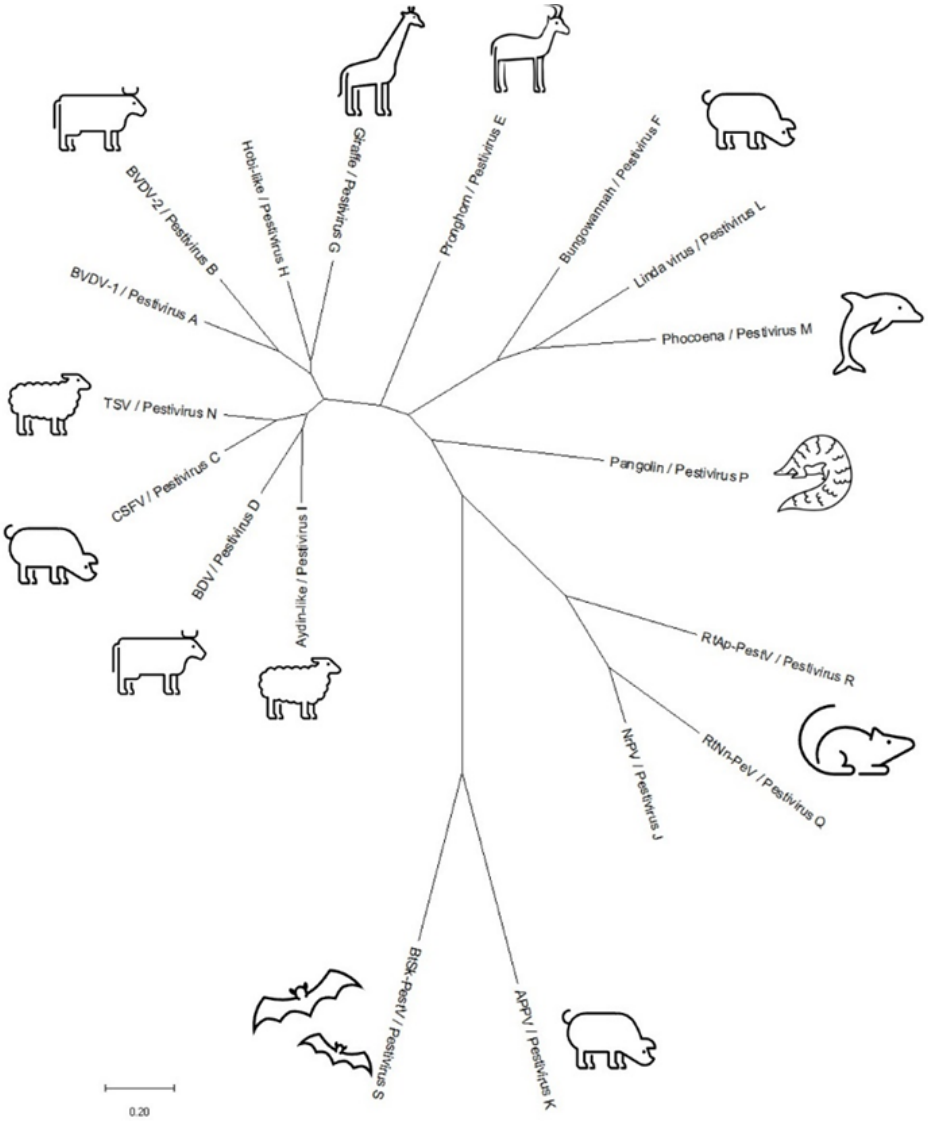

Figure 1: Phylogenetic comparison of pestiviruses (Denes, 2023, dissertation)

3. The genetic characterization of APPV

APPV was discovered by a research group in the USA in 2015, which performed metagenomic sequencing on a sample, which was quantitative reverse-transcription polymerase chain reaction (qRT-PCR) positive for porcine reproductive and respiratory syndrome virus (PRRSV). The reads were assembled de novo into 2167 contigs and six of them returned hits to pestiviruses with 68–98 % identity. They resequenced the sequencing library which resulted in an 11 276 bp long contig, encoding a 3635 amino acid (aa) polyprotein which shows 68–74% amino acid sequence identity to the partial polyprotein sequence of RaPV and 37–40% identity to complete polyprotein amino acid sequences of BVDV, BDV, and CSFV [3, 13]. They attempted to isolate the virus on MARC-145, Vero, Vero 76, HCT-8, BT, MDBK, ST, PK15, and MDCK cell lines, but after two passages on cells, all samples were qRT-PCR negative. No cytopathic effect (CPE) was discernible in any cell line [3]. Since then, similar approaches have been undertaken by other research groups with partial success [6, 35, 36]. Beer et al. (2017) managed to isolate the virus on porcine kidney cells (SPEV, cell line 0008, Collection of Cell Lines in Veterinary Medicine, FLI) but had no data on cell passage [4]. The research group of Schwarz et al. (2017) in Vienna was also able to isolate the virus, through SK-6 and PK-15 cell inoculation with an APPV-positive serum sample of a CT-affected piglet, taken before the first colostrum intake. Despite the positive results, the virus spread in the cell culture was still very inefficient [37], so further improvements are needed.

APPV is an enveloped virus with a positive-sense, single-stranded RNA genome [38]. The RNA polyprotein consists of twelve proteins of which four are structural proteins and eight are non-structural proteins. The structural proteins include C, E^{rns}, E1 and E2. The nonstructural proteins include: N^{pro}, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B (*Figure 2*) [39]. E^{rns} and N^{pro} are considered unique for pestiviruses. The first encoded protein is the unique N^{pro}, followed downstream by the structural proteins located in the amino-terminal part which are then followed by the non-structural proteins downstream [25].

3.1. Non-structural proteins

The non-structural protein N^{pro}, which functions as an autoprotease, is a distinguishing feature of the *Pestivirus* genus [3], apart from the novel *Phocoena pestivirus* which lacks this protein [40]. Additionally, N^{pro} plays an important role in blocking the host's antiviral response making

N^{pro} a crucial factor in promoting virus replication and survival [41]. The p7 protein functions as a viroporin which is crucial for viral virulence [3].

Nonstructural proteins of pestiviruses play a vital role in viral particle formation. NS2 functions as a cysteine autoprotease which is responsible for the cleavage of NS2–3, while NS3 has helicase, NTPase activity, and functions as a serine protease with its cofactor NS4A [42–44]. Based on their cytopathic effect, Pestiviruses are classified into two biotypes: noncytopathic (ncp) or cytopathic (cp). In the case of persistently BVDV-infected animals, the emergence of a cp-type virus is often caused by RNA recombination, which induces lethal mucosal disease (MD) [44]. The induction of this phenotype correlates with the production of free NS3, which is based on genomic alterations in the NS2–3 coding regions [43]. Uncleaved NS2–3 has also an essential, but not well-characterized function in virion morphogenesis [42]. It has been demonstrated that the insertion of certain fragments between the NS2 and NS3 genes abolishes infectious virus particle formation [45, 46]. Cp and ncp viruses are both capable of causing acute infections, but persistent infections are restricted to ncp viruses [47]. For pestiviruses, the active viral RNA replicase is the complex of the NS3, NS4A, NS4B, NS5A, and NS5B proteins and other, unknown host factors [42]. Among the nonstructural proteins of APPV NS2 showed 60%, NS3 74%, NS4A 61% similarity to RaPV, NS4B and NS5A 36–45 % and NS5A only 12–17% to other pestiviruses [23].

3.2. Structural proteins

Pestiviruses encode three envelope glycoproteins - E1, E2, and E^{rns} - with E^{rns} protein being exclusively found in pestiviruses and similar to N^{pro} being responsible for inhibiting the host's antiviral response. E2 exhibits a size of about 53–55 kilodaltons (kDa) and it represents the receptor-binding protein of pestiviruses, also is the main target for neutralizing antibodies and E1 also plays a key role in virus entry [42]. Both proteins showed less than 31% identity to other pestiviruses, although E2 was found to be 52% similar to RaPV, furthermore, APPV and RaPV E2 proteins are shorter with approximately 130 aa compared to other pestiviruses [23]. The

fourth structural protein is the capsid protein (C) which is responsible for RNA packaging into virions [3].

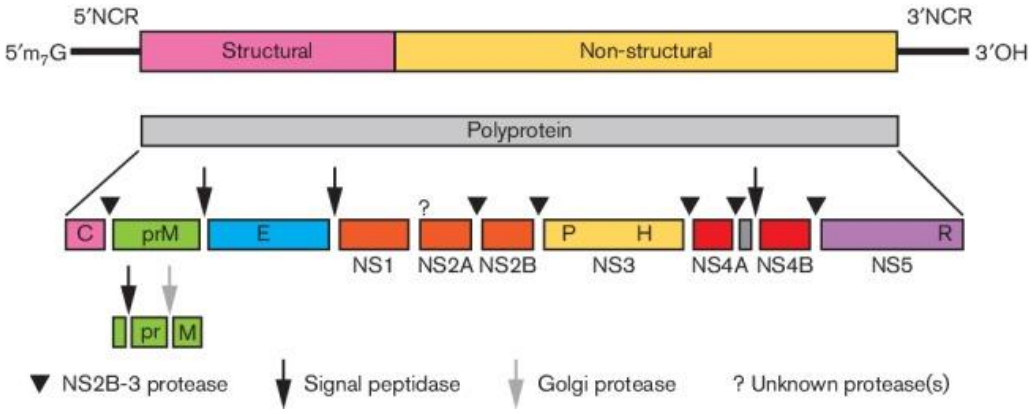

Figure 2: Genome organization and polyprotein processing of members of the genus *Flavivirus*. Boxes below the genome indicate viral proteins generated by proteolytic processing. NCR, non-coding region [1]

3.3. Genetic variability

After the first report of APPV in 2015 subsequent APPV sequences have been researched and released. Repeated genetic sequencing has enlarged the phylogenetic tree [2]. A Hungarian study showed that the genome of APPV is highly variable, with individual farms showing unique strains [10]. The phylogenetic analysis of representative partial APPV genomes downloaded from the GenBank showed that in unique cases, strains detected in the same country (Chinese and German sequences) form monophyletic groups. Sequences obtained between 2002 and 2015 from several Spanish farms formed different smaller monophyletic group, therefore it's possible that the same virus strain has been infecting pigs for over a decade. German strains isolated from wild boars during 2015-2017 form separate phylogenetic groups regardless of the time the samples were taken at. In addition, three of these German strains from 2015 and 2016 showed increased similarity to strains originating from Italy and the United Kingdom. Interestingly, these strains were also from 2015 and 2016. The average genetic distance of APPV strains in a country was found to be 9-12.3%, although Chinese strains have been shown to have the highest genetic distance with a genetic difference of 18.1% (*Figure 3*) [10].

Figure 3: Phylogenetic tree displaying relatedness of the NS2–3 polyprotein coding gene of APPV reference sequences from GenBank [48].

Denes et al. (2022) suggests the genetic diversity of strains be caused by local, divergent evolution of APPV strains in addition to the trade of clinically healthy, persistently infected animals worldwide.

4. Clinical and histological presentation

The clinical presentation of pigs infected with APPV is mainly characterized by CT [39]. Nevertheless, a few other clinical signs such as splayed legs have also been associated with APPV [6, 36].

4.1. Congenital tremor

Congenital tremor is a neurological disease that manifests in visible spasms of the head and limbs of newborn piglets which was first described over one hundred years ago in the United States by Kinsley who described “dancing pigs“ [6]. In 1976 pregnant sows were inoculated with tissue homogenates from the central nervous system (CNS) and spleen from piglets affected with CT of unknown aetiology and successfully produced piglets with CT symptoms [49]. This has been the first successful inoculation study concerning CT symptoms. Nevertheless, the causative agent responsible for these symptoms was unknown by researchers at the time of performing the tests [39].

Tremors can be differentiated into two groups, “resting tremors” and “action tremors”. The first one occurs when the animal is as the name suggests relaxing or sleeping and seizing when the muscle is actively used. Congenital tremors belonging to the second group, called “action tremors”, occur during muscle activation and stimulation such as in times of movement or stress. These neurological signs are of decreased strength or even absent when the piglets are resting and or sleeping in the case of action tremors [50].

Congenital tremor is usually non-lethal and piglets show signs of spontaneous recovery about three months after birth. Nonetheless, it has also been documented that some piglets exhibit signs past the first 3 months and develop chronic symptoms [13, 51]. Piglets that no longer show clinical signs and have recovered have been found to start having spasms after external stimuli. External stimuli and stress factors such as transport, sudden noises or slaughterhouse waiting areas can all be potential causes of relapse [37, 51].

4.1.1. Classification of congenital tremors

Congenital tremor can be subcategorized further based on their causative agents and the presence (type A I-V) or absence (type B) of histopathological hypomyelination and vacuolization of the CNS and spinal cord, which is thought to be due to retarded myelination

during fetal development [50].

Transplacental infection of the fetus with CSFV is the cause of CT type A-I [52]. CT type A-II has only recently been associated with APPV, with the help of inoculation studies, it has been shown that APPV is the causative agent responsible for CT type A-II [13, 22, 23]. Type A-III and A-IV are genetic defects only found in British Landrace and Saddleback breeds [39, 53]. CT type A-V is linked to organophosphate intoxication with metrifonate (international nonproprietary name) also known as trichlorfon (United States adopted name) [54]. It has previously been used as a feedstuff pesticide as well as an ectoparasiticide given to pregnant sows [6, 39].

Type B with the absence of CNS and spinal cord lesions has not yet been linked to a causative agent but it has been speculated to be of non-infectious or idiopathic origin [39].

4.1.2. Differential diseases of CT

The main clinical sign of spasms and tremors is not limited to APPV or uncommon in piglets. A wide range of possible other causes exists that can lead to the same neurological deficits. A common cause of shivering, possibly misinterpreted as tremors in neonatal piglets, is hypoglycaemia often but not necessarily in combination with hypothermia. Unlike CT these shivers will not seize during times of rest or sleep [39]. Furthermore, other infectious agents or intoxications can also cause neurological symptoms seen in APPV, these include but are not limited to aflatoxicosis, Aujeszky's, african swine fever (ASFV), CSFV, post-weaning multisystemic wasting syndrome (PMWS) which is caused by porcine circovirus type 2 (PCV-2) or salt intoxication. Unlike APPV these diseases are often accompanied by severe multisystemic disease [39].

4.2. Other clinical signs

Apart from tremors mentioned previously other clinical symptoms have also been described. Splay leg, which is referred to as a temporary muscle dysfunction of the posterior legs can be seen as well in infected newborn piglets (*Figure 4*). It has been linked to APPV with an occurrence rate of up to 40% [6, 7, 36]. Splay leg can contribute to piglets having difficulty walking and standing and thus impede their ability to properly suckle on the sow's teat. In severe cases, the reduced ability to suckle, ingest colostrum and lowered competitiveness with other littermates can lead to increased mortality due to starvation. In addition, the inability to move

can lead to piglets being crushed by the sows [55]. Moreover, facial dermatitis has also been reported in connection with APPV [13].

Figure 4: A 6-days-old piglet with splay leg syndrome [55]

4.3. Histopathological lesions

To characterise the histopathological lesions Schwarz et al. (2017b) evaluated piglets from Austrian farms. Three groups of piglets were distinguished: piglets affected by CT, their healthy littermates and healthy piglets as a control group.

Hypomyelination was not evident in samples of the cerebral and cerebellar white matter when results were compared between the three groups (*Figures 5A–C*). However, hypomyelination was visible in the white matter of the spinal cord of affected piglets when compared to the other groups of piglets (*Figures 5D–F*). Compared to the healthy control group, neither the affected nor unaffected piglets showed a reduced number of oligodendrocytes. Nevertheless, the staining intensity of oligodendrocytes in affected animals was observed to be higher than that of the healthy control group (*Figures 5G–I*). Moderate, partially perivascular, focal gliosis was observed in the brain and spinal cord of three piglets, two of which exhibited clinical signs while the third belonged to the group of unaffected littermates. One piglet that exhibited clinical signs was found to have dilated myelin sheaths and vacuolization of the white matter in the medulla oblongata and spinal cord. However, all affected animals showed a distinct decrease in the

thickness of their myelin sheaths [37].

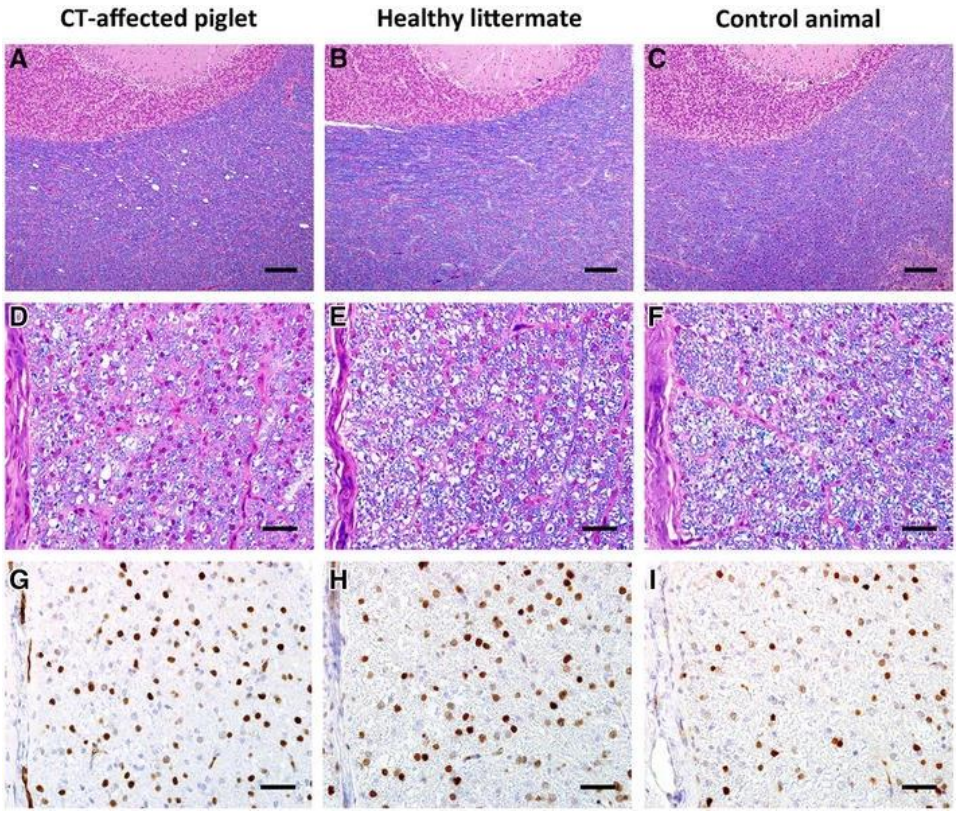

Figure 5: Histological lesions in a CT-affected animal compared to a healthy littermate and a healthy control.

Vacuoles in cerebellar white matter in affected animal (A), normal white matter in littermate (B) and control (C), LFB-HE, bar = 150 µm. Hypomyelination in the white matter of the thoracic spinal cord in affected animal (D), normal myelination in littermate (E) and control (F), LFB-HE, bar = 40 µm. Detection of oligodendrocytes, increased staining intensity in affected animal (G), less intense staining in littermate (H) and control (I), Olig2-IHC, bar = 40µm [37]

5. Pathogenesis

5.1. Cellular tropism

Understanding the cellular targets of a pathogen is of high importance, which can provide insight into the exact pathogenesis and mechanisms of infection. As the precise mechanism of transmission and spread of APPV has not been thoroughly researched yet, comprehending the cell types that are preferentially infected can aid in gaining insight into the virus's life cycle, creating preventative measures such as vaccines and manifesting further possible theories.

Several studies have performed necropsies and sampled different organs of piglets affected by CT to determine the severity of viral load in varying tissues. Results of these studies indicate that the main targets of APPV might be the cerebellum and lymph nodes [6, 13, 38, 41].

Postel et al. (2016) sampled serum, cerebrospinal fluid (CSF), cerebellum, spinal cord and the cerebrum of six piglets affected by CT and two clinically healthy. Piglets with CT tested positive for the APPV genome in every sample whereas clinically healthy piglets were negative in all samples listed above. To further evaluate the systemic distribution of APPV the piglet with the lowest quantification cycle (Cq) value, measured with qRT-PCR was evaluated further. 26 samples from different organs were taken and analysed with qRT-PCR and fluorescent *in-situ* hybridization (FISH). The results were consistent over both methods except for the thymus sample which was positive with qRT-PCT but negative with FISH. The highest genome load with Cq values of 24.3 and 24.4 were detected in the glandular epithelial cells of the arcus palatoglossus and the lymphonodus mandibularis. Viral load in samples of the stomach, nasal glands and cerebellum all had similar Cq values between 27-28. Out of the different CNS tissue sampled, the inner granular cell layer of the cerebellum, the spinal ganglia and the trigeminal ganglia were found to be positive. Whereas the cervical spinal cord, the hippocampus and the cerebral cortex had Cq values of 40 and can be regarded as negative. Lymphoid organs also showed to be infected, a more intense signal was visible via FISH in the follicular centres of the lymphoid nodes [13].

Similar results from Pan et al. were published in 2019, with the highest viral load being detected in the cerebellum and the lowest values in the brainstem. The viral load of submandibular and inguinal lymph nodes was higher when compared to other lymphoid organs such as the spleen, the thymus and the liver [41].

In addition, lymphoid organs such as the thymus, the lymph nodes, the tonsils, and the fibrous capsule and trabeculae of the spleen have also been shown to be positive for APPV in other studies [38, 56].

In (2021) Buckley et al. performed necropsies on CT-affected piglets that originated from experimentally infected sows via intravenous, intranasal, and injection of fetal amniotic vesicles. RNA *in situ* hybridisation (ISH) was used on the different organ samples for the visualisation of the APPV genome. Visualisation of APPV was possible in all three vascular tunics (intima, media, adventitia), the fibroblasts and fibromuscular stroma of varying organs such as the liver, kidney, colon, lung, lymph nodes, tonsil, thymus, and thyroid (*Figures 6A-B*). In tissue samples of the heart, APPV was able to be distinguished in the epicardial mesothelium and the endocardial endothelium. The small and large intestines were also analysed with RNA ISH and positive labeling for APPV was seen in the smooth muscle layers (*Figure 6B*) [38]. Buckley et al. (2021) observed the areas positive for APPV to be similar between piglets and adult boars (*Figures 6C-D*), however, piglets showed more intense labeling. It should be noted that only tissues from the cerebellum, the large intestine, and the lymph nodes were tested from boars. Furthermore, they also observed the highest viral load to be found in the cerebrum and cerebellum, regardless of age. Boars tested had CT when they were piglets, which resolved and at the age of 11 months they were humanly euthanised. As mentioned above distribution of APPV in tissues was like that of piglets. However, at the time of slaughter, the boars were asymptomatic even though high amounts of the APPV genome were detectable in the CNS tissue [38]. It is worth bearing in mind that only two boars were tested, further testing might be needed to substantiate the findings.

De Groof et al. (2016) necropsied seven piglets affected by CT. A wide range of organs were sampled (heart, small intestine, large intestine, brain, thoracic spinal cord, lumbar spinal cord, liver, inguinal lymph node, lung, gallbladder, urinary bladder, kidney, tonsil, spleen) as well as peripheral blood lymphocytes and serum. All samples were found to be positive for APPV, with the highest viral load being attributed to tonsils, inguinal lymph nodes and serum. Which is in line with the findings of previously mentioned studies. All samples from control piglets unaffected by CT were negative [6].

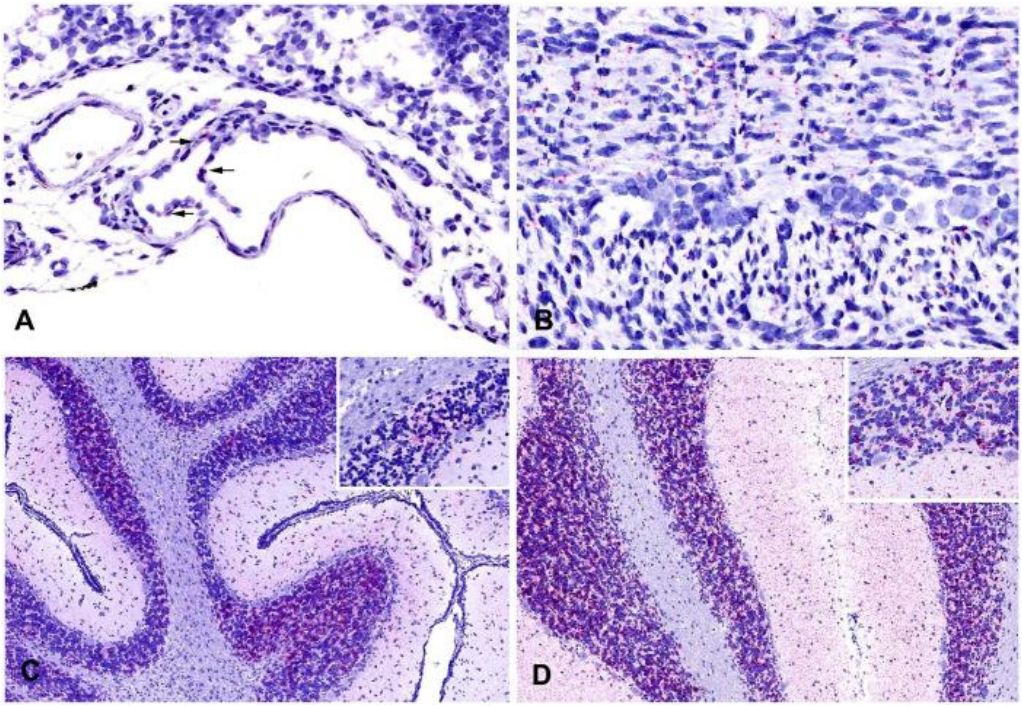

Figure 6: Piglets and boars experimentally infected with atypical porcine pestivirus (APPV). RNAScope in situ hybridization. A. Piglet lymphatic vessel in mesenteric lymph node with labeling (red dots, arrows) for APPV RNA in endothelial cells of vessel wall and valves. B. Piglet colon with abundant APPV RNA labeling (red dots) in circular and longitudinal muscle layers. C. Piglet cerebellum with multifocal labeling for APPV in granular and molecular layers. Inset: positive labeling (red dots) of APPV RNA. D. Boar cerebellum with diffuse labeling for APPV RNA in granular and molecular layers. Inset: positive labeling (red dots) of APPV RNA [38].

5.1.1. Testicular tropism

Only a few articles and studies have been published regarding testicular targets of pestiviruses. The male reproductive tract has been shown to be particularly vulnerable and act as a viral reservoir, as it was previously shown for CSFV [57] and BVDV [58]. This may also apply to APPV [56].

Little is known about the exact cellular targets of APPV in the testicles with only recent research focusing on this topic [38, 56]. However, it has been shown that APPV exists in the genital tract of boars and has been confirmed to be present in samples of preputial fluids as well as semen [6, 7, 19].

According to Dénes et al. (2022), the genome of APPV has been detected in two different types of cells within the testicular interstitium: Leydig cells and peritubular myoid cells (*Figure 7*). Leydig cells are responsible for the production of testosterone, which is essential for male reproductive function, as well as the maintenance of spermatogenesis. Peritubular myoid cells, on the other hand, are involved in the transport of spermatozoa and the regulation of spermatogenesis. Buckley et al. (2021) also noted APPV positivity in seminiferous tubule lumens together with a strong positivity of the tunica albuginea of the testicles. Furthermore, Dénes et al. (2022) have identified the virus in endothelial cells of medium-sized arteries in the testicles as well (*Figure 7*). The identification of APPV in these cells suggests a potential role for the virus in disrupting normal testicular function, male fertility, and transmission.

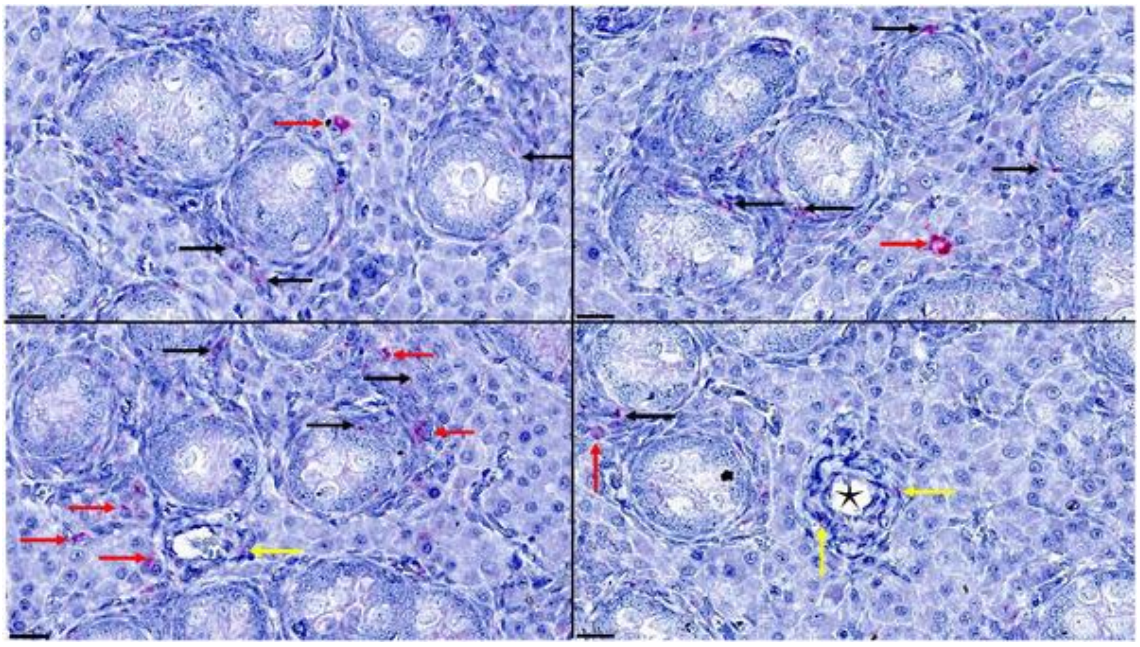

Figure 7: Representative pictures of atypical porcine pestivirus (APPV) RNAScope in situ hybridization (ISH) genome on the testicle samples. Black arrows point at positive peritubular myoid cells, and red arrows show the signal in the interstitial Leydig cells, whereas the yellow arrows indicate positive endothelial cells in the wall of medium-sized arteries marked by black stars. 600×, bar = 20 μm [56]

6. Diagnostic methods

The systemic identification of the APPV genome addressed in chapter 5.1: Cellular Tropism, indicated that a wide range of tissues could be positive for APPV and can theoretically be used for diagnostics. Samples suitable for genome detection are among others: serum, lymphoid organs and CNS tissue, whereas the cerebellum and lymph nodes are of increased interest as the viral load has been documented to be the highest in these [59]. Processing fluids have been used as a cost-effective way to obtain pooled samples of animals after castration, in other viral outbreaks. This approach allows for no further stress to the animal and is effective for APPV identification [56].

6.1. Virus isolation

In contrast to CSFV, which isolation on cell culture has been documented, efficient isolation of APPV has been challenging due to its limited ability to grow on cell cultures [3, 6, 36] with only a few groups have reported virus isolation for APPV [4, 37, 60]. However, a variant of APPV has been developed that exhibits superior replication *in vitro* and can be cultivated on the porcine kidney cell line named SPEV. This breakthrough has enabled further research into the life cycle and molecular mechanisms of APPV, as well as the establishment of virus-neutralization assays. The improved ability to grow the virus *in vitro* is a promising development for future detection and research efforts [61].

6.2. qRT-PCR

Quantitative reverse-transcription polymerase chain reactions (qRT-PCR) have been routinely used ever since the complete genome of APPV has been available [41]. qRT-PCR allows for genome replication, detection and quantification of genome load in a sample based on previously selected primer pairs. Commonly targeted areas include conserved sequences of non-structural proteins such as NS3, NS4B or NS5B [36, 60, 62]. Mósena et al. (2018) sequenced partial NS5B gene fragments from positive pigs originating from two farms in Brazil. The two sequences from farm A, when compared to each other, shared 92.97% and the three strains of farm B, shared 98.65%–99.73% nucleotide sequence identity. Nucleotide identity of sequences between farms A and B was 83.24% and 88.92%. Additionally, nucleotide samples were entered into the Nucleotide BLAST software to compare to other previously identified partial or

absolute genomes. Nucleotide samples from Farm A showed 89% identity to a strain from the USA and strains from Farm B showed 92.3% and 93.2% identity to a strain from USA and Germany respectively [62].

6.3. Serology techniques

6.3.1. ELISA

Enzyme-linked immunosorbent assay (ELISA) is a rapid method used for the detection of viral proteins and antigens and can be used to detect APPV antibodies such as the proteins NS3, E^{rns} and E2 [3, 7, 60, 63].

Rosell et al. (2014) monitored Spanish piglets from a herd tested positive for a routine CSFV specific ELISA. These samples were further investigated with comparative VNT (virus neutralization test). The results showed substantially higher titres for BDV and CSFV-infection has been ruled out, therefore the study proved cross-reaction of BDV and CSFV antibodies. This false diagnosis could be attributed to the fact that pestiviruses share similar immunological and genetic structures, subsequently, identification of specific virus strains can prove to be difficult and cause false positives during sampling [64].

Diagnostic methods for the detection of antibodies against the APPV E^{rns} protein have been developed and used in several studies [3, 9, 60]. Hause et al. (2015) expressed and purified two APPV E^{rns} proteins with *Escherichia coli*. A subsequent ELISA was established and tested on commercial pigs from three farms and specific pathogen-free (SPF) pigs. Results of the E^{rns}-based ELISA for the first farm showed 100% and for the second farm 96% positivity. The third farm and all SPF pigs were negative.

Postel et al. (2017b) established an indirect ELISA, targeting the E^{rns} glycoprotein of APPV, with the help of a protozoan *Leishmania tarentolae*, which was used for glycoprotein expression. The study concluded that the E^{rns}-specific APPV antibody showed no cross-reactivity with diagnostic tests commonly used for CSFV detection. A later study by Postel et al. (2017a) then applied their ELISA to European and Asian swine samples. A total of 40% of the pigs were negative by APPV-specific polymerase chain reaction (PCR), out of these 10% were positive for E^{rns} ELISA.

In 2017 Schwarz et al. (2017) used NS3 blocking ELISA, which is normally used for the diagnostics of CSFV and BVDV, the antibodies used showed cross-reactivity with APPV.

BALB/c mice were immunized with recombinant proteins against structural and non-structural proteins of CSFV, BDVD and Bungowannah virus. The monoclonal antibodies (mAb) expressed by the mice were screened for APPV cross-reactivity. Two antibodies against the NS3-helicase protein showed cross-reactivity when subjected to APPV-positive samples. Both antibodies originated from mice that were simultaneously immunized against the NS3H protein of CSFV and BVDV [37].

Incidentally, a recent study measured the immune response of piglets and compared three indirect ELISAs using anti-E^{rns}, -NS3, and -E2 antibodies, qRT-PCR was used to verify and support results. Piglets born by caesarean section, inoculated with APPV and deprived of colostrum were sampled for 70 days post-inoculation, together with a negative control group mock inoculated with a synthetic cell culture medium (minimal essential medium, MEM). Samples taken were serum, nasal swabs and oral saliva. The most sensitive antibody detection was achievable with an E^{rns}-based ELISA used on saliva. This study was the first to use saliva for APPV antibody detection and interestingly the saliva samples gave the most sensitive results of all samples [63].

Currently, there is no ELISA test available for the detection of APPV in a commercial setting. Nevertheless, NS3, E^{rns} and E2-specific ELISAs show promising signs of becoming methods for routine APPV diagnosis, further research should be done to determine other possible targets as well as further inquiries on already valid targets to improve sensitivity and specificity.

6.3.2. VNT

Virus neutralization tests are used to quantify the capacity of a sample to neutralize a virus, this can give valuable information on the current antibody status of tested animals. Difficulties of viral isolation and culturing of APPV have hindered techniques such as VNT being researched [61]. As part of an experiment to test horizontal infection of APPV Cagatay et al. (2019) sampled the sera of piglets and their gilts. The serum samples were then tested with VNTs. Additionally, to provide data on specific antibodies, samples were also tested via APPV-specific E2 and E^{rns} antibody ELISAs. VNTs evaluate the presence or absence of cytopathogenic changes to determine viral neutralization. Natural occurring APPV strains lack cytopathogenic features. For this reason, an immunofluorescent stain of the viral antigen was applied and used for visualization of viral neutralization. Results of the study showed high neutralizing titers, indicating protective humoral immunity to be established in tested pigs. When compared to the

accompanying ELISA results, data indicated the E2 antigen correlated with and be responsible for the high neutralization titers [61].

6.4. Hybridization techniques

6.4.1. RNA *in situ* hybridization

RNA *in situ* hybridization (ISH) techniques such as RNAScope and FISH are powerful tools used to study the expression and localization of specific RNA molecules in cells and tissues [65]. *In situ* diagnostics allow for the visualisation of a pathogen in its normal surrounding and to accentuate lesions, for this histological samples can be used. Detection of the viral proteins and nucleic acids at the site of the lesion, can help in understanding viral targets. For example, with the use of Luxol Fast Blue staining, the APPV typical demyelination of the CNS tissue can be highlighted [59]. The use of different methods in consecutive histological slides can allow for additional information to be gained [56]. Knowledge of viral load and cellular preferences of APPV can help clarify disease-related processes and contribute to a better understanding of the pathogenesis of APPV [66].

6.4.2. RNAScope

RNAScope is a newly produced type of RNA *in situ* hybridization technique which achieves high specificity and selectivity via a three-part target probe unique to RNAScope and uses chromogenic or fluorescent labels for staining [67]. Successful use of this technique, applied to APPV, can be seen in a study by Dénes et al. (2022) and Buckley et al. (2021). Dénes et al. (2022) used probes targeting regions of the proteins NS2-3 whereas Buckley et al. (2021) used probes targeting the N^{pro}-E^{rns} coding region of APPV. In both studies commercially available RNAScope kits were used which were fabricated by Advanced Cell Diagnostics.

Target probes consist of three segments: a region complementary and specific to the target RNA followed by a spacer sequence and finally a tail segment. Visually these three components create a “Z”. Two target probes hybridized next to each other; one continuous tail segment will be created from both “Z” segments which form a target region for a preamplifier to hybridize with. The preamplifier allows amplifier regions to attach, with in turn can all accept specific labeling probes which will allow for visualization later (*Figure 8*) [67]. This double “Z” complex allows target-specific signals to be amplified without interference and false binding of unwanted

sequences. False hybridization of probes with unwanted sequences can lead to background noise and subsequently make differentiation of cell borders more difficult to distinguish. By choosing different target probes that have the same labels or different target probes with different labels, multiple target RNAs can be marked. This results in either an intensified signal or the ability to visualise different RNA targets with different colours. Samples that can be used for RNAScope analysis include formalin-fixed paraffin-embedded (FFPE) tissue samples. FFPE samples have routinely shown the ability to partially degrade RNA, the ample amount of target probe pairs used should provide high sensitivity to the method [67].

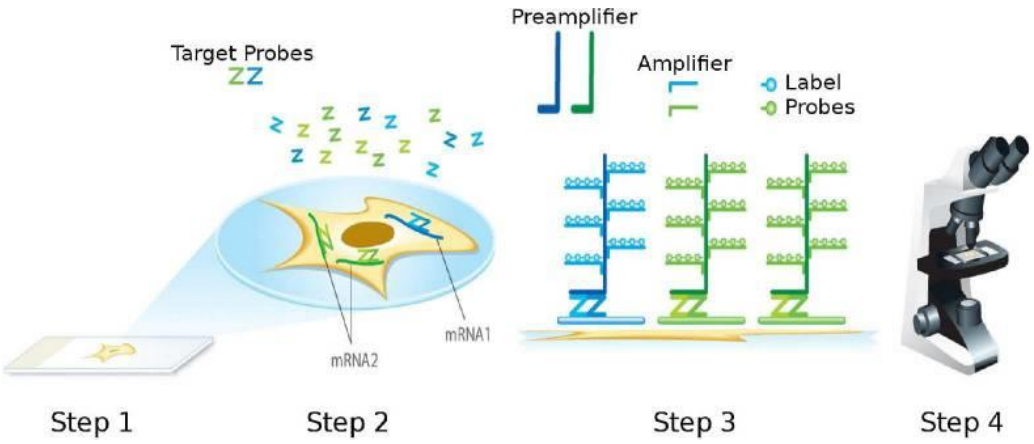

Figure 8: Schematic of the RNAScope assay procedure. In step 1, cells or tissues are fixed and permeabilized to allow for target probe access. In step 2, target RNA-specific oligonucleotide probes (Z) are hybridized in pairs (ZZ) to multiple RNA targets. In step 3, multiple signal amplification molecules are hybridized, each recognizing a specific target probe, and each unique label probe is conjugated to a different fluorophore or enzyme. In step 4, signals are detected using a fluorescence microscope (for a fluorescent label) or standard bright-field microscope (for enzyme label) [67].

The simplified steps mentioned above are complex and involve several precise steps. Expensive and specialized equipment is needed which might not be available in all laboratories. This can lead to potentially expensive prices compared to other methods of gene expression analysis [68].

6.4.3. Immunohistochemistry

Immunohistochemistry (IHC) uses labelled antibodies that can bind to specific cellular antigens

of interest. When used in connection with histological samples this method allows for the visualization of specific cells *in situ*. In the study partly addressed in the previous chapter, IHC was applied in combination with RNAScope for APPV detection *in situ*. Markers used in the study by Dénes et al. (2022) were: anti-von Willebrand Factor to mark endothelial cells, anti-c-kit to mark Leydig and germ cells and anti-alpha smooth muscle actin to mark contractile smooth muscle components of varying cells. The information gained by RNAScope was overlayed with information gained by immunohistochemistry to conclude which specific cells were positive for the APPV genome [56].

7. Epidemiology

7.1. Worldwide emergence of APPV

After the discovery of APPV in 2015 in the United States [3], several current and retrospective studies have shown the worldwide emergence of APPV in: the Netherlands [6], Germany [4, 13], Austria [7], Denmark [12], Spain [8], Hungary [10], Sweden [5], Switzerland [11] and Italy [13, 14], Brazil [19], Canada [18], China [15], Great Britain [9], Republic of Serbia [21], South Korea [69] and Japan [16].

One of the first studies attempting to retrospectively identify APPV in samples was published in 2017, stored samples in Spain were analysed for APPV and the results provided proof of APPV having existed as early as 1997 [8].

A study performed by Dénes et al. (2018) tested samples of Hungarian farms where piglets were affected by CT. The oldest sample tested predated the official discovery of APPV by 10 years, other samples used were from 2007, 2010 and 2016-2018. All samples from CT-affected piglets were positive for APPV and proved that APPV has existed on Hungarian farms since at least 2005.

A retrospective study on 1080 sera originating from Swiss farms coming from a time frame between 1986 and 2018 were also tested for APPV. From all samples tested an average of 13% of the samples tested positive for APPV. A total of 97 serum samples originated from the year 1986, out of these a total of six (7%) tested positive for APPV. This makes 1986 the oldest known case of APPV worldwide [11].

These studies show that APPV has been present in swine herds substantially longer than since its time of discovery and could have affected commercial swine herds without notice for decades and possibly causing unaccounted-for economic losses.

7.2. Economic importance

It is still unclear what exact economic impact APPV and its clinical symptoms have and further research is probably helpful in evaluating its full impact on the swine industry [70]. Nevertheless, CT may influence the health of piglets and the efficiency of pig farms all over the world, because asymptomatic carriage, increased mortality and decreased reproductive ability has been linked with APPV [6, 37, 70].

Furthermore, increased mortality has been documented in several establishments affected by CT

with a mortality rate of 10% to 30% [7, 36]. De Groof et al. (2016) even attributed 60% of the total deaths reported on a farm to be due to CT [6]. Death of affected piglets is mainly caused by the reduced movement ability and ataxia seen in piglets with APPV due to CT and splay leg. This can lead to reduced feed intake, inability to suckle, inadequate nutrition, and starvation [53, 70–72]. The reproductive performance of sows has dropped by 10% in connection with APPV [37]. Studies of pig herds from Germany and the USA have shown asymptomatic carriage of APPV to reach up to 22% of healthy pigs of affected farms [3, 4, 13]. In a more recent publication by Gatto et al. (2018) commercial swine boars from the USA were assessed for APPV genome in samples of semen, preputial swabs and preputial fluids with the positivity of samples averaging 34%, 23% and 28% respectively [73]. Additionally, increased piglet mortality has been associated with piglets infected with both APPV and porcine teschovirus [74].

These findings show it is of significance to further study both the impact of APPV as well as its impact as part of coinfections.

7.3. Transmission of the virus

Transmission of pestiviruses can either occur horizontally via direct contact between animals through body fluids and secretions or via indirect contact with contaminated food or materials. Furthermore, pestiviruses can also be transmitted vertically, which means intrauterine infection [25].

7.3.1. Horizontal

Horizontal transmission of the virus, such as through the orofecal route, is a plausible mechanism for the spread of APPV. This is supported by the detection of significant amounts of the APPV genome in various organs of pigs, including the colon, duodenum, pancreas, and salivary glands [13].

In 2019, Cagatay et al. describes a study to research the horizontal transmission of APPV. In this study piglets free of disease were mixed with piglets proven to be APPV-positive. These previously free-of-disease piglets showed viraemia for six weeks and high amounts of specific antibodies when tested after ten and twenty-three weeks. 100% of these piglets were horizontally infected and tested positive for the APPV genome but clinical signs could not be observed.

Studies have demonstrated that semen serves as a critical vehicle for virus transmission in other pestiviruses, including CSFV and BVDV-type 1 & 2 [56]. This aligns with the evidence showing the presence of APPV in preputial fluid and semen of boars [6, 37, 73, 75]. However, horizontal spread among pigs via semen has not been officially studied for APPV.

7.3.2. Chronic and persistent infections

Pestiviruses such as CSFV, BVDV and BDV have been demonstrated to cause persistent infections (PI), especially after intrauterine or early post-natal infection. Persistently infected animals play a key role in viral transmission as they act as reservoirs and shed the virus for a long time. These animals are immune-tolerant to specific/endemic viral strains and thus show no signs of disease [76].

It is currently not known if PI animals play a crucial role in APPV infection. However, specific proteins are involved in initiating PI in pestiviruses, these are the non-structural protein N^{pro} and structural protein E^{rns}. Interestingly these proteins are also present in the genome of APPV as discussed in chapter 3: The genetic characterization of APPV. This gives rise to the possibility that APPV is also capable of causing PI [41]. Identification and removal of PI animals are of high importance for eradication programs of other pestiviruses [76]. Postel et al. (2017a) tested 1460 clinically healthy swine, the samples originated from European and Asian pigs. Both indirect APPV ELISAs and APPV-specific PCRs were applied. Only 8.9% of all samples were positive by PCR, in comparison 60% were APPV-positive by ELISA. Interestingly, out of the remaining 40% of pigs with negative antibody results, 10% of these were positive by PCR. The Authors of the study hypothesize this to have two possible reasons: Either sample were taken from acutely infected animals which have not yet responded by antibody production to the virus or samples could originate from PI animals which are immunotolerant and lack a humoral immune response and subsequent antibodies [9]. When testing piglets for APPV, de Groof et al. (2016) found their clinically unapparent control piglets to be positive for APPV upon PCR testing. The study documented 9% of a total of 66 control piglets to be positive for the APPV genome [6].

Longitudinal studies have been performed that continuously tested the serum, faeces, and saliva of piglets affected by CT. The samples were taken over several months and tested positive for APPV. This finding supports the idea of chronic carriage and spread. Two longitudinal studies are briefly explained below [6, 7]. For six months piglets were sampled. Naturally infected,

genome APPV-positive piglets as well as clinically apparent piglets were selected to monitor. Clinical signs were absent after 14 weeks of monitoring, but the APPV genome was still detectable at month 6 in both saliva and semen [7]. Ten different farms, all affected by CTs, underwent long-term sampling and testing of their piglets. Both clinically sick and asymptomatic piglets were sampled. Serum samples were collected from their first week after birth till 4.5 and 8.5 months of age. Seven of twenty piglets were positive after 4.5 months. All five of the piglets that were tested for longer, tested positive in faecal samples after 8.5 months [6].

7.3.3. Vertical

Pestiviruses such as CSFV, BVDV-type 1 & 2 and BDV have been demonstrated to cross the placenta of pregnant animals and subsequently cause prenatal infections in fetuses [76]. To investigate whether APPV is affected similarly, researchers conducted studies where they experimentally inoculated pregnant gilts. Inoculum containing APPV sera was used and successfully induced clinical signs in the following farrowed piglets [6, 36]. Arruda et al. (2016b) inoculated fetal amniotic vesicles and additionally, gilts were administered inoculum via intravenous and intranasal routes. All piglets inoculated with APPV sera showed signs of CT after birth, and all control piglets were negative. Despite inoculation of the sows via intravenous and intranasal methods, no viraemia or measurable levels of the virus could be detected.

On the other hand, de Groof et al. (2016) performed intramuscular injections. Inoculated sows all had negative serum and faecal tests for APPV at the time of farrowing. Interestingly, one of the three litters had no piglets with CT, and all piglets of this third litter were negative upon PCR testing for APPV. The authors of the study de Groof et al. (2016) concluded that "trans-placental transmission of APPV and subsequent infection of the foetuses is a very likely cause of congenital tremor type A-II in piglets."

One should be aware that clinical symptoms and CT only develop in newly born piglets infected via the transplacental route. Since fetal immunity develops after around ten weeks of gestation, an infection before this time is likely needed to cause clinical signs in the piglets [6, 7, 36]. Considering that piglets typically show clinical symptoms only during the pre-weaning period, virus elimination from the body is likely after reaching weaning age [76].

Nevertheless, a combination of both host-related and environmental factors is likely to play a critical role in the transmission and persistence of APPV. This is evidenced by the observation that even with proactive measures such as active removal of infected animals and regular testing, the virus can persist on farms for extended periods, making eradication difficult to achieve [77].

7.3.4. Gilt predisposition

Congenital tremor associated with APPV has been detected in piglets without any gender bias [6]. Piglets originating from gilts have shown a higher morbidity rate than piglets originating from sows. Possibly indicating that an immune status is developed after having a litter with APPV-infected piglets [6, 19]. Morbidity and severity of CT symptoms within individual litters themselves have shown to vary [6, 7, 19, 36]. Even though APPV has been identified in adult pigs, CT in adult pigs as well as older post-weaning piglets is not common, as symptoms mostly cease when piglets reach weaning age [6, 19].

7.4. APPV in wild boars

Throughout Europe wild boar populations are widely distributed, they are known to act as crucial reservoirs and can transmit disease to domestic herds, for example in the case of CSFV, a devastating pig disease of worldwide economic importance [21]. Infection of the wild population with APPV has been proven by several studies in the following countries: Germany, Italy, Sweden, the Republic of Serbia, South Korea and Spain [14, 20, 21, 69, 78].

Cagatay et al. (2018) and Stenberg et al. (2022) sampled a total of 456 and 595 wild boars respectively. Both reported rather high levels of antibodies and APPV genome. Samples by Cagatay et al. (2018) were 52% positive for APPV antibodies and 19% were positive for the APPV genome. Stenberg et al. (2022) sampled wild Swedish pigs and 72% of the samples were antibody positive and 12% genome positive.

In contrast to this, studies on wild boars by Choe et al. (2020), Colom-Cadena et al. (2018) and Sozzi et al. (2019) documented rather low rates of APPV in their samples. Choe et al. (2020) analysed 2297 blood samples from wild South Korean swine, 18 (0.78%) strains of APPV were able to be identified [69]. Colom-Cadena et al. (2018) tested a total of 437 wild boar samples from Spain, samples were taken between 2012 and 2016. Interestingly, only one sample was positive for the APPV genome, this was a sample of a male boar with an estimated age between 12–18 months [20]. Sozzi et al. (2019) tested a total of 430 blood samples from boars in Italy,

and only three samples (0.6%) were found to be positive for the APPV genome [14].

Thought-provokingly, APPV genomes isolated from domestic pigs have been shown to have high genetic variability [9], whereas, in contrast to this, phylogenetic analysis among isolated genomes of wild boars shows rather low genetic diversity [21]. APPV sequences from wild boars isolated from several German hunting regions formed a single phylogenetic clade. These were compared to APPV reference strains of European, American and Chinese domestic pigs. The domestic strain most like the German wild strains had a genetic variability of 8.1% and originated from southern Germany [21].

Nevertheless, no studies have proven the transmission and spread of APPV from wild boars to domestic pigs and vice versa till now. However, the fact that APPV is found worldwide in wild swine populations with high rates of genome and antibody positivity, as well as in domestic populations, indicates it is of great importance to conduct further research. Knowledge of the exact method of viral spread and transmission is a necessity for the establishment of effective and successful control measures [21].

8. Treatment

As addressed in chapter 7.1: Worldwide emergence of APPV, APPV is a disease of worldwide relevance for the swine industry with morbidity reaching 100% in affected litters [59, 70]. Under normal circumstances, piglets recover from signs of CT, 3 months after birth [39]. Nevertheless, CT has been linked to the occurrence of splay leg syndrome with high incidence (40%), together these can lead to increased mortality rates [6, 7, 36].

Unfortunately, treatment for active APPV infections and CT symptoms is currently not available [39, 59].

However, there are methods of assisting piglets affected by clinical symptoms. To prevent underdevelopment and starvation of affected piglets, assistance should be provided with colostrum intake, so piglets ingest an adequate amount of fluid and nutrients. It should be made sure that piglets receive sufficient heating. If piglets are affected by splay leg the affected hind limbs can be bound together, in a normal physiological standing position, to improve stability. Other aspects such as non-slippery flooring should also be considered in affected establishments [55].

9. Prevention

Due to the worldwide distribution of APPV, together with the high rate of subclinical infections addressed in chapter 7: Epidemiology, preventative measures should be of high relevance [6, 7] because there are currently no drugs available to prevent APPV infections [59].

9.1. Vaccines

Subunit vaccines targeting the E2 antigen have been used for CSFV vaccines. The structural protein E2 of APPV has shown to be a target of neutralising antibodies in APPV infections, subsequently, a possible subunit vaccine may be of interest for APPV prevention as well [76]. Currently, there is no available APPV vaccine on the market for commercial vaccination of domestic pigs. Nevertheless, a new vaccine candidate reducing the clinical signs of congenital tremor in piglets was recently patented applying a pestivirus strain which has at least 95% similarity to a porcine pestivirus isolate previously described by Arruda et al. [KU194229, 2016] The authors recommend the vaccination of gilts and pregnant sows to prevent in utero infection of the fetus. Unfortunately, the large-scale manufacturing of inactivated APPV vaccines proves to be difficult as *in vitro* cell lines of APPV are hard to maintain [79].

Recently two vaccine approaches have been described that successfully induced an immune response in experimental animals. One approach successfully induced a higher antibody response in BALB/c mice compared to the control group, virus-like particles (VLP) were used that were based on the interaction of E2 and E^{rns} proteins [80]. A second group developed an Fc-mediated E2 dimer subunit vaccine. They measured a strong humoral and cellular immune response in piglets achieved by an APPV E2 subunit vaccine fusing with Fc fragments emulsified with an ISA 201VG adjuvant [81].

9.2. Herd management techniques

CT-affected piglets are predominantly farrowed by gilts, this is speculated to be because gilts are often newly introduced into farms from external sites as naïve pigs. At their new farm, they infect themselves with an endemic APPV strain. The naïve gilts do not have the time to build up an adequate immune protection before the piglets are infected by the transplacental route. For this reason, herd management techniques should rather focus on gilts rather than sows. Sows can farrow CT piglets as well, for example by encountering a different strain of APPV which

they have not gained immunity against yet [6, 19, 76]. Farms introducing new gilts should allow time for proper acclimatization before insemination, this could allow for contact with APPV and the possibility for a humoral immune response to be established. In addition, prior to insemination, gilts should be checked regarding antibody status and possible APPV genome detection. However, the introduction of naive sows may lead to the possible introduction of new APPV strains and can be a cause of new cases of CT [76].

Like gilts, before insemination boars and their semen should be evaluated for the presence of APPV. Boars established as positive should be disregarded as breeding material and additionally, they should also be removed from the establishment to avoid possible horizontal infection. Semen from external sources intended for artificial insemination (AI) should as well be evaluated prior to and only be bought from healthy farms [59].

As chronic carriage of APPV has been documented and discussed previously in 7.3 Transmission of the virus, CT-affected male piglets should be disregarded as possible breeding pigs. These piglets may be free of symptoms at the time of insemination, but no risk should be taken if they were previously documented to show signs of CT as they might still be shedding APPV [56].

Preventing piglets from infection with APPV lies partly on preventive measures of sows and boars. Nevertheless, piglets are capable of horizontal transmission regardless of whether clinical symptoms are present or absent [56]. However, horizontally infected piglets that were subclinical have been shown to establish protective immunity. Post-weaning infection of piglets could thus be able to establish herd immunity. Additionally, a stronger immune response could be achievable if piglets are weaned later than 21 days as the immune system of swine is fully matured within four weeks [76].

General biosecurity measures and hygiene should not be overlooked regarding the prevention of APPV, overall herd health and reducing viral damage. Replacement animals should only be bought from healthy herds and subsequently quarantined before entering the herd. This is especially true for the introduction of new gilts. Proper acclimatization protocols should be in place to ensure gilts are infected at a relatively young age to ensure adaptive immunity is established and subsequent infections of piglets via cross-placental route are hindered [13]. Personal hygiene and regulation of workers and vehicles should follow protocols like regular disinfection and proper cleaning of facilities and tools [76]. As wild boars act as reservoirs for

APPV, correct biosecurity measures should be established to avoid possible infection of domestic herds, this should also contribute to reducing rodents, pests and other wild animals [21, 59].

10. Conclusion

Even though APPV is a rather newly discovered virus it is evident that it had an impact on the swine industry on a worldwide level. Since the first discovery of APPV in 2015, research has been done concerning APPV in several directions and a lot has already been discovered [3]. APPV had been detected on several continents around the world, and its genome has been detected in domestic and wild boar populations as well [70].

The reason for varying results of positivity published on wild boar population should be investigated further [14, 21], as this information could aid in understanding the extent of wild boar to domestic swine transmission. Domestic herds have been shown to have a high percentage of asymptomatic animals which were positive for the APPV genome [9]. If and to what extent persistent infections play a critical role in APPV transmission has not been fully established and should be the focus of future studies [41].

The ability of APPV to spread both via horizontal and vertical routes has been shown and is of great importance to know, nevertheless one should not forget that horizontally infected piglets do not show symptoms. And in the case of clinically apparent piglets after vertical infection symptoms reside when piglets reach weaning age [76]. The reason for both absence after horizontal and disappearance after vertical transmission should be the target of future research. Knowledge of this could help in understanding APPV's pathogenesis better.

Systemic viral load of APPV has been shown in several studies with higher concentrations detected in lymphoid cells and the cerebellum [6, 13, 38, 41]. Additionally, the identification and visualisation of APPV in testicular cells [56] has laid the ground for further research concerning the sexual transmission of the disease. This could be of high importance as vertical infection results in symptoms which have been shown to cause increased mortality [6, 36].

Many aspects of APPV’s life cycle, transmission, proteins, cellular tropism and epidemiology are characteristic to the *Pestivirus* genus [76] genomic research is needed to identify further similarities and differences to other pestiviruses. As this knowledge could help in establishing proper diagnostic measures that could be used in domestic swine herds regularly for large-scale identification of APPV.

Prevention and eradication of APPV in domestic and wild boars are important for the worldwide swine industry but can only be established if further research is done on the transmission, life cycle and epidemiology of APPV.

11.Abstract

The *Flaviviridae* family consist of 4 genera. The *Pestivirus* genus is one of these four and includes the novel Atypical porcine pestivirus which was first detected in 2015 by Hause et al. in the USA. In subsequent studies, APPV has been shown to exist worldwide in both domestic and wild swine populations. Many other new *Pestiviruses* have been detected over the past decade and a total of 19 pestiviruses have been proposed as part of the genus. Research shows that APPV has similar attributes to other economically important diseases belonging to the *Pestivirus* genus such as CSFV, BVDV 1 and 2, and BVD.

APPV causes clinical signs in piglets born to infected gilts, at weaning age the symptoms are mostly absent. Inoculation studies have shown that APPV is a causative agent of CT type AII which is often complicated with splay leg, a muscle dysfunction of the hind legs.

Congenital tremor is a neurological disease, it manifests itself as spasms of the head and body. Together with splay leg, CT can lead to complications concerning movement and feed intake. Increased mortality rates of 10% to 30% have been documented in connection with CT-affected piglets.

APPV has been detected systemically in affected piglets. However lymphoid organs and the cerebellum have shown the highest concentrations in several studies. Hypo- and demyelination, especially prominent in the spinal cord, is a common histopathological lesion seen in conjunction with CT and could be a possible reason for associated neurological symptoms. Horizontal and vertical transmission of APPV has been shown to exist, however, horizontal infection of piglets does not resolve in clinical symptoms. Chronic and asymptomatic carriage of APPV has been established in other pestiviruses, important proteins playing a role in persistent infections (PI) are also present in APPV, but the exact impact of PI on the transmission of APPV has not been established. Wild boars play a critical role in the epidemiology of other swine diseases, and studies have tried to determine their impact on APPV. However, results have varied as some studies have reported high levels of genome detection (17%, 19%) whereas others have reported genome detection rates of <1% in the wild boars sampled.

Currently, there is no treatment method available for CT, standard biosecurity measures may help in the prevention of APPV, but more research is needed to better understand viral transmission, life cycle and determine possible vaccines and specific prevention methods.

12. Bibliography

1. Simmonds P, Becher P, Bukh J, Gould EA, Meyers G, Monath T, Muerhoff S, Pletnev A, Rico-Hesse R, Smith DB, Stapleton JT (2017) ICTV virus taxonomy profile: Flaviviridae. Journal of General Virology 98:2–3. https://doi.org/10.1099/jgv.0.000672
2. Postel A, Smith DB, Becher P (2021) Proposed update to the taxonomy of pestiviruses: Eight additional species within the genus pestivirus, family flaviviridae. Viruses 13:. https://doi.org/10.3390/v13081542
3. Hause BM, Collin EA, Peddireddi L, Yuan F, Chen Z, Hesse RA, Gauger PC, Clement T, Fang Y, Anderson G (2015) Discovery of a novel putative atypical porcine pestivirus in pigs in the USA. J Gen Virol 96:2994–8. https://doi.org/10.1099/jgv.0.000251
4. Beer M, Wernike K, Dräger C, Höper D, Pohlmann A, Bergermann C, Schröder C, Klinkhammer S, Blome S, Hoffmann B (2017) High Prevalence of Highly Variable Atypical Porcine Pestiviruses Found in Germany. Transbound Emerg Dis 64:e22–e26. https://doi.org/10.1111/tbed.12532
5. Blomström A-L, Fossum C, Wallgren P, Berg M (2016) Viral Metagenomic Analysis Displays the Co-Infection Situation in Healthy and PMWS Affected Pigs. PLoS One 11:e0166863. https://doi.org/10.1371/journal.pone.0166863
6. de Groof A, Deijs M, Guelen L, van Grinsven L, van Os-Galdos L, Vogels W, Derks C, Cruijsen T, Geurts V, Vrijenhoek M, Suijskens J, van Doorn P, van Leengoed L, Schrier C, Hoek L (2016) Atypical Porcine Pestivirus: A Possible Cause of Congenital Tremor Type A-II in Newborn Piglets. Viruses 8:271. https://doi.org/10.3390/v8100271
7. Schwarz L, Riedel C, Högler S, Sinn LJ, Voglmayr T, Wöchtl B, Dinhopl N, Rebel-Bauder B, Weissenböck H, Ladinig A, Rümenapf T, Lamp B (2017) Congenital infection with atypical porcine pestivirus (APPV) is associated with disease and viral persistence. Vet Res 48:1–14. https://doi.org/10.1186/s13567-016-0406-1
8. Muñoz-González S, Canturri A, Pérez-Simó M, Bohórquez JA, Rosell R, Cabezón O, Segalés J, Domingo M, Ganges L (2017) First report of the novel atypical porcine pestivirus in Spain and a retrospective study. Transbound Emerg Dis 64:1645–1649. https://doi.org/10.1111/tbed.12699
9. Postel A, Meyer D, Cagatay GN, Feliziani F, De Mia GM, Fischer N, Grundhoff A, Milićević V, Deng M-C, Chang C-Y, Qiu H-J, Sun Y, Wendt M, Becher P (2017) High Abundance and Genetic Variability of Atypical Porcine Pestivirus in Pigs from Europe and Asia. Emerg Infect Dis 23:2104–2107. https://doi.org/10.3201/eid2312.170951
10. Dénes L, Biksi I, Albert M, Szeredi L, Knapp DG, Szilasi A, Bálint Á, Balka G (2018) Detection and phylogenetic characterization of atypical porcine pestivirus strains in Hungary. Transbound Emerg Dis 65:2039–2042. https://doi.org/10.1111/tbed.12981
11. Kaufmann C, Stalder H, Sidler X, Renzullo S, Gurtner C, Grahofer A, Schweizer S, Schweizer M (2019) Long-term circulation of atypical porcine pestivirus (APPV) within switzerland. Viruses 11:1–14. https://doi.org/10.3390/v11070653
12. Pedersen K, Kristensen CS, Strandbygaard B, Bøtner A, Rasmussen TB (2021) Detection of atypical porcine pestivirus in piglets from Danish sow herds. Viruses 13:. https://doi.org/10.3390/v13050717
13. Postel A, Hansmann F, Baechlein C, Fischer N, Alawi M, Grundhoff A, Derking S, Tenhündfeld J, Pfankuche VM, Herder V, Baumgärtner W, Wendt M, Becher P, Baumgrtner W, Wendt M, Becher P (2016) Presence of atypical porcine pestivirus

(APPV) genomes in newborn piglets correlates with congenital tremor. Sci Rep 6:27735. https://doi.org/10.1038/srep27735
14. Sozzi E, Salogni C, Lelli D, Barbieri I, Moreno A, Alborali GL, Lavazza A (2019) Molecular Survey and Phylogenetic Analysis of Atypical Porcine Pestivirus (APPV) Identified in Swine and Wild Boar from Northern Italy. Viruses 11:. https://doi.org/10.3390/v11121142
15. Yuan J, Han Z, Li J, Huang Y, Yang J, Ding H, Zhang J, Zhu M, Zhang Y, Liao J, Zhao M, Chen J (2017) Atypical Porcine Pestivirus as a Novel Type of Pestivirus in Pigs in China. Front Microbiol 8:1–6. https://doi.org/10.3389/fmicb.2017.00862
16. Kasahara-Kamiie M, Kagawa M, Shiokawa M, Sunaga F, Fukase Y, Aihara N, Shiga T, Kamiie J, Aoki H, Nagai M (2021) Detection and genetic analysis of a novel atypical porcine pestivirus from piglets with congenital tremor in Japan. Transbound Emerg Dis 69:1761–1769. https://doi.org/https://doi.org/10.1111/tbed.14149
17. Seung-Chai K, Gi JC, Yoon S-M, Lee K, Yang M-S, Kim BJ, Lee S-Y, Kang S, Won-il K (2017) Detection of atypical porcine pestivirus (APPV) from a case of congenital tremor in Korea
18. Dessureault F, M C, C P, CA. G (2018) First report of atypical porcine pestivirus in piglets with congenital tremor in Canada. Can Vet J 59:429–432
19. Gatto IRH, Harmon K, Bradner L, Silva P, Linhares DCL, Arruda PH, de Oliveira LG, Arruda BL (2018) Detection of atypical porcine pestivirus in Brazil in the central nervous system of suckling piglets with congenital tremor. Transbound Emerg Dis 65:375–380. https://doi.org/10.1111/tbed.12824
20. Colom-Cadena A, Ganges L, Muñoz-González S, Castillo-Contreras R, Bohórquez JA, Rosell R, Segalés J, Marco I, Cabezon O (2018) Atypical porcine pestivirus in wild boar (Sus scrofa), Spain. Veterinary Record vetrec-2017-104824. https://doi.org/10.1136/vr.104824
21. Cagatay GN, Antos A, Meyer D, Maistrelli C, Keuling O, Becher P, Postel A (2018) Frequent infection of wild boar with atypical porcine pestivirus (APPV). Transbound Emerg Dis 65:1087–1093. https://doi.org/10.1111/tbed.12854
22. Arruda BL, Arruda PH, Magstadt DR, Schwartz KJ, Dohlman T, Schleining JA, Patterson AR, Visek CA, Victoria JG (2016) Identification of a Divergent Lineage Porcine Pestivirus in Nursing Piglets with Congenital Tremors and Reproduction of Disease following Experimental Inoculation. PLoS One 11:e0150104. https://doi.org/10.1371/journal.pone.0150104
23. Hause BM, Collin EA, Peddireddi L, Yuan F, Chen Z, Hesse RA, Gauger PC, Clement T, Fang Y, Anderson G (2015) Discovery of a novel putative atypical porcine pestivirus in pigs in the USA. Journal of General Virology 96:2994–2998. https://doi.org/10.1099/jgv.0.000251
24. Smith DB, Meyers G, Bukh J, Gould EA, Monath T, Muerhoff AS, Pletnev A, Rico-Hesse R, Stapleton JT, Simmonds P, Becher P, Scott Muerhoff A, Pletnev A, Rico-Hesse R, Stapleton JT, Simmonds P, Becher P (2017) Proposed revision to the taxonomy of the genus Pestivirus, family Flaviviridae. Journal of General Virology 98:2106–2112. https://doi.org/10.1099/jgv.0.000873
25. Tautz N, Tews BA, Meyers G (2015) The Molecular Biology of Pestiviruses. In: Advances in Virus Research, 1st ed. Elsevier Inc., pp 47–160
26. Dietzgen RG, Ghedin E, Jiāng D, Kuhn JH, Song T, Vasilakis N, Wang D (2017) ICTV virus taxonomy profile: Nyamiviridae. Journal of General Virology 98:2914–2915.

https://doi.org/10.1099/jgv.0.000973
27. Vilcek S, Ridpath JF, Van Campen H, Cavender JL, Warg J (2005) Characterization of a novel pestivirus originating from a pronghorn antelope. Virus Res 108:187–193. https://doi.org/10.1016/j.virusres.2004.09.010
28. Kirkland PD, Frost MJ, King KR, Finlaison DS, Hornitzky CL, Gu X, Richter M, Reimann I, Dauber M, Schirrmeier H, Beer M, Ridpath JF (2015) Genetic and antigenic characterization of Bungowannah virus, a novel pestivirus. Vet Microbiol 178:252–259. https://doi.org/10.1016/j.vetmic.2015.05.014
29. Firth C, Bhat M, Firth MA, Williams SH, Frye MJ, Simmonds P, Conte JM, Ng J, Garcia J, Bhuva NP, Lee B, Che X, Quan PL, Ian Lipkin W (2014) Detection of zoonotic pathogens and characterization of novel viruses carried by commensal rattus norvegicus in New York city. mBio 5:1–16. https://doi.org/10.1128/mBio.01933-14
30. Wu Z, Ren X, Yang L, Hu Y, Yang J, He G, Zhang J, Dong J, Sun L, Du J, Liu L, Xue Y, Wang J, Yang F, Zhang S, Jin Q (2012) Virome Analysis for Identification of Novel Mammalian Viruses in Bat Species from Chinese Provinces. J Virol 86:10999–11012. https://doi.org/10.1128/JVI.01394-12
31. Avalos-Ramirez R, Orlich M, Thiel H-J, Becher P (2001) Evidence for the Presence of Two Novel Pestivirus Species. Virology 286:456–465. https://doi.org/10.1006/viro.2001.1001
32. Becher P, Orlich M, Shannon AD, Horner G, Konig M, Thiel H-JJ, König M, Thiel H-JJ (1997) Phylogenetic analysis of pestiviruses from domestic and wild ruminants. J Gen Virol 78 (Pt 6):1357–66. https://doi.org/10.1099/0022-1317-78-6-1357
33. Lamp B, Schwarz L, Högler S, Riedel C, Sinn L, Rebel-Bauder B, Weissenböck H, Ladinig A, Rümenapf T (2017) Novel Pestivirus Species in Pigs, Austria, 2015. Emerg Infect Dis 23:1176–1179. https://doi.org/10.3201/eid2307.170163
34. Smith DB, Meyers G, Bukh J, Gould EA, Monath T, Scott Muerhoff A, Pletnev A, Rico-Hesse R, Stapleton JT, Simmonds P, Becher P, Muerhoff AS, Pletnev A, Rico-Hesse R, Stapleton JT, Simmonds P, Becher P (2017) Proposed revision to the taxonomy of the genus Pestivirus, family Flaviviridae. Journal of General Virology 98:2106–2112. https://doi.org/10.1099/jgv.0.000873
35. Lamp B, Riedel C, Wentz E, Tortorici M-A, Rümenapf T (2013) Autocatalytic Cleavage within Classical Swine Fever Virus NS3 Leads to a Functional Separation of Protease and Helicase. J Virol 87:11872–11883. https://doi.org/10.1128/jvi.00754-13
36. Arruda BL, Arruda PH, Magstadt DR, Schwartz KJ, Dohlman T, Schleining JA, Patterson AR, Visek CA, Victoria JG (2016) Identification of a Divergent Lineage Porcine Pestivirus in Nursing Piglets with Congenital Tremors and Reproduction of Disease following Experimental Inoculation. PLoS One 11:e0150104. https://doi.org/10.1371/journal.pone.0150104
37. Schwarz L, Riedel C, Högler S, Sinn LJ, Voglmayr T, Wöchtl B, Dinhopl N, Rebel-Bauder B, Weissenböck H, Ladinig A, Rümenapf T, Lamp B (2017) Congenital infection with atypical porcine pestivirus (APPV) is associated with disease and viral persistence. Vet Res 48:1–14. https://doi.org/10.1186/s13567-016-0406-1
38. Buckley AC, Falkenberg SM, Palmer M V., Arruda PH, Magstadt DR, Schwartz KJ, Gatto IR, Neill JD, Arruda BL (2021) Distribution and persistence of atypical porcine pestivirus in experimentally inoculated pigs. Journal of Veterinary Diagnostic Investigation 33:952–955. https://doi.org/10.1177/10406387211022683
39. Stenberg H, Jacobson M, Malmberg M (2020) A review of congenital tremor type A-II

in piglets. Anim Health Res Rev 21:84–88. https://doi.org/10.1017/S146625232000002X
40. Jo WK, van Elk C, van de Bildt M, van Run P, Petry M, Jesse ST, Jung K, Ludlow M, Kuiken T, Osterhaus A (2019) An evolutionary divergent pestivirus lacking the Npro gene systemically infects a whale species. Emerg Microbes Infect 8:1383–1392. https://doi.org/10.1080/22221751.2019.1664940
41. Pan S, Mou C, Chen Z (2019) An emerging novel virus: Atypical porcine pestivirus (APPV). Rev Med Virol 29:. https://doi.org/10.1002/rmv.2018
42. Tautz N, Tews BA, Meyers G (2015) The Molecular Biology of Pestiviruses. In: Advances in Virus Research, 1st ed. Elsevier Inc., pp 47–160
43. Klemens O, Dubrau D, Tautz N (2015) Characterization of the Determinants of NS2-3-Independent Virion Morphogenesis of Pestiviruses. J Virol 89:11668–11680. https://doi.org/10.1128/JVI.01646-15
44. Becher P, Tautz N (2011) RNA recombination in pestiviruses: Cellular RNA sequences in viral genomes highlight the role of host factors for viral persistence and lethal disease. RNA Biol 8:216–224. https://doi.org/10.4161/rna.8.2.14514
45. Moulin HR, Seuberlich T, Bauhofer O, Bennett LC, Tratschin J-D, Hofmann MA, Ruggli N (2007) Nonstructural proteins NS2-3 and NS4A of classical swine fever virus: Essential features for infectious particle formation. Virology 365:376–389. https://doi.org/10.1016/j.virol.2007.03.056
46. Agapov E V, Murray CL, Frolov I, Qu L, Myers TM, Rice CM (2004) Uncleaved NS2-3 is required for production of infectious bovine viral diarrhea virus. J Virol 78:2414–25. https://doi.org/10.1128/JVI.78.5.2414
47. Brownlie J (1991) The pathways for bovine virus diarrhoea virus biotypes in the pathogenesis of disease. Arch Virol Suppl 3:79–96
48. Dénes L, Balka G (2022) Az atipikus sertés-pestivírus és az általa okozott reszketőkór Irodalmi összefoglaló. Magy állatorvosok lapja 144:591–602
49. Patterson DSP, Done JT, Foulkes JA, Swfasey D (1976) NEUROCHEMISTRY OF THE SPINAL CORD IN CONGENITAL TREMOR OF PIGLETS (TYPE AII), A SPINAL DYSMYELTMOGENESIS OF INFECTIOUS ORIGIN. J Neurochem 26:481–485. https://doi.org/10.1111/J.1471-4159.1976.TB01499.X
50. Done JT, Woolley J, Upcott DH, Hebert CN (1986) Porcine congenital tremor type AII: Spinal cord morphometry. British Veterinary Journal 142:145–150. https://doi.org/10.1016/0007-1935(86)90090-4
51. Done JT (1976) The congenital tremor syndrome in pigs. Veterinary Annual (1976) v. 16
52. Vannier P, Plateau E, Tillon JP (1981) Congenital tremor in pigs farrowed from sows given hog cholera virus during pregnancy. Am J Vet Res 42:135–7
53. Done JT (1968) Congenital nervous diseases of pigs: a review. Lab Anim 2:207–217. https://doi.org/10.1258/002367768781082861
54. Bölske G, Kronevi T, Lindgren NO (1978) Congenital tremor in pigs in Sweden. A case report. Nord Vet Med 30:534–7
55. Papatsiros VG (2012) The splay leg syndrome in piglets: A review. Am J Anim Vet Sci 7:80–83. https://doi.org/10.3844/ajavsp.2012.80.83
56. Dénes L, Ruedas-Torres I, Szilasi A, Balka G (2022) Detection and localization of atypical porcine pestivirus in the testicles of naturally infected, congenital tremor affected piglets. Transbound Emerg Dis 69:e621–e629. https://doi.org/10.1111/tbed.14355
57. Choi C, Chae C (2002) Localization of classical swine fever virus in male gonads during subclinical infection. Journal of General Virology 83:2717–2721.

https://doi.org/10.1099/0022-1317-83-11-2717
58. Voges H, Horner GW, Rowe S, Wellenberg GJ (1998) Persistent bovine pestivirus infection localized in the testes of an immuno-competent, non-viraemic bull. Vet Microbiol 61:165–175. https://doi.org/10.1016/S0378-1135(98)00177-1
59. Dall Agnol AM, Alfieri AF, Alfieri AA (2020) Pestivirus K (Atypical Porcine Pestivirus): Update on the virus, viral infection, and the association with congenital tremor in newborn piglets. Viruses 12
60. Postel A, Meyer D, Petrov A, Becher P (2017) Recent emergence of a novel porcine pestivirus: Interference with classical swine fever diagnosis? Emerg Microbes Infect 6:e19-2. https://doi.org/10.1038/emi.2017.5
61. Cagatay GN, Meyer D, Wendt M, Becher P, Postel A (2019) Characterization of the humoral immune response induced after infection with atypical porcine pestivirus (APPV). Viruses 11:13–23. https://doi.org/10.3390/v11100881
62. Mósena ACS, Weber MN, da Cruz RAS, Cibulski SP, da Silva MS, Puhl DE, Hammerschmitt ME, Takeuti KL, Driemeier D, de Barcellos DESN, Canal CW (2018) Presence of atypical porcine pestivirus (APPV) in Brazilian pigs. Transbound Emerg Dis 65:22–26. https://doi.org/10.1111/tbed.12753
63. Arruda BL, Falkenberg S, Mora-Díaz JC, Matias Ferreyra FS, Magtoto R, Giménez-Lirola L (2022) Development and Evaluation of Antigen-Specific Dual Matrix Pestivirus K ELISAs Using Longitudinal Known Infectious Status Samples. J Clin Microbiol 60:. https://doi.org/10.1128/jcm.00697-22
64. Rosell R, Cabezón O, Pujols J, Domingo M, Muñoz I, Núñez JI, Ganges L (2014) Identification of a porcine pestivirus as a border disease virus from naturally infected pigs in Spain. Veterinary Record 174:18. https://doi.org/10.1136/vr.101920
65. Cassidy A, Jones J (2014) Developments in in situ hybridisation. Methods 70:39–45. https://doi.org/10.1016/j.ymeth.2014.04.006
66. Liu J, Li Z, Ren X, Li H, Lu R, Zhang Y, Ning Z (2019) Viral load and histological distribution of atypical porcine pestivirus in different tissues of naturally infected piglets. Arch Virol 164:2519–2523. https://doi.org/10.1007/s00705-019-04345-3
67. Wang F, Flanagan J, Su N, Wang LC, Bui S, Nielson A, Wu X, Vo HT, Ma XJ, Luo Y (2012) RNAScope: A novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. Journal of Molecular Diagnostics 14:22–29. https://doi.org/10.1016/j.jmoldx.2011.08.002
68. Atout S, Shurrab S, Loveridge C (2022) Evaluation of the Suitability of RNAScope as a Technique to Measure Gene Expression in Clinical Diagnostics : A Systematic Review. Mol Diagn Ther 26:19–37. https://doi.org/10.1007/s40291-021-00570-2
69. Choe SE, Park GN, Cha RM, Park BK, Hyun BH, An DJ (2020) Prevalence and Genetic Diversity of Atypical Porcine Pestivirus (APPV) Detected in South Korean Wild Boars. Viruses 12:. https://doi.org/10.3390/v12060680
70. Gatto IRH, Sonálio K, de Oliveira LG (2019) Atypical Porcine Pestivirus (APPV) as a New Species of Pestivirus in Pig Production. Front Vet Sci 6:. https://doi.org/10.3389/fvets.2019.00035
71. Kinsley A (1922) Dancing pigs? Vet Med 17:123
72. Larsson EL (1955) Om skaksjuka hos smågrisar. Sv Svinavelsför Tidskrft 9: 149-151.
73. Gatto IRH, Arruda PH, Visek CA, Victoria JG, Patterson AR, Krull AC, Schwartz KJ, de Oliveira LG, Arruda BL (2018) Detection of atypical porcine pestivirus in semen from commercial boar studs in the United States. Transbound Emerg Dis 65:e339–e343.

https://doi.org/10.1111/tbed.12759
74. Possatti F, Headley SA, Leme RA, Dall Agnol AM, Zotti E, de Oliveira TES, Alfieri AF, Alfieri AA (2018) Viruses associated with congenital tremor and high lethality in piglets. Transbound Emerg Dis 65:331–337. https://doi.org/10.1111/tbed.12807
75. Houston GE, Jones CK, Woodworth JC, Palinski R, Paulk CB, Petznick T, Gebhardt JT (2022) Detection and investigation of atypical porcine pestivirus in a swine production system. Front Vet Sci 9:998344. https://doi.org/10.3389/fvets.2022.998344
76. Cagatay GN, Becher P, Postel A, Baumgärtner W, Tautz N (2019) Genetic and Biological Characterization of Atypical Porcine Pestivirus (APPV) Associated with Congenital Tremor in Newborn Piglets Submitted in partial fulfillment of the requirements for the degree (PhD). Stiftung Tierärztliche Hochschule Hannover
77. Folgueiras-González A, van den Braak R, Simmelink B, Deijs M, van der Hoek L, de Groof A (2020) Atypical porcine pestivirus circulation and molecular evolution within an affected swine herd. Viruses 12:1–16. https://doi.org/10.3390/v12101080
78. Stenberg H, Leveringhaus E, Malmsten A, Dalin AM, Postel A, Malmberg M (2022) Atypical porcine pestivirus—A widespread virus in the Swedish wild boar population. Transbound Emerg Dis 69:2349–2360. https://doi.org/10.1111/tbed.14251
79. Patterson AR, City S, Arruda BL, Us IA, Henrique P, Arruda E, Us IA, Magstadt DR, Us IA, Schwartz KJ (2018) PESTIVIRUS VACCINES FOR CONGENITAL TREMORS. 2
80. Liu J, Zhang P, Chen Y, Zhong W, Li B, Pi M, Ning Z (2021) Vaccination with virus-like particles of atypical porcine pestivirus inhibits virus replication in tissues of BALB/c mice. Arch Virol 166:2733–2741. https://doi.org/10.1007/s00705-021-05185-w
81. Ren X, Qian P, Liu S, Chen H, Li X (2021) Fc-mediated e2-dimer subunit vaccines of atypical porcine pestivirus induce efficient humoral and cellular immune responses in piglets. Viruses 13:. https://doi.org/10.3390/v13122443

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