

REVIEW

An emerging novel virus: Atypical porcine pestivirus (APPV)

Shuonan Pan¹ | Chunxiao Mou¹ | Zhenhai Chen^{1,2,3} ¹ College of Veterinary Medicine, Yangzhou University, Yangzhou, China² Jiangsu Co-Innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou, China³ Joint International Research Laboratory of Agriculture and Agri-Product Safety, the Ministry of Education of China, Yangzhou University, Yangzhou, China**Correspondence**Zhenhai Chen, College of Veterinary Medicine, Yangzhou University, Yangzhou, China.
Email: zhenhai@yzu.edu.cn**Funding information**

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Summary

Emerging porcine pestivirus diseases frequently challenge prevention and control strategies in the swine industry. Over the past decade, a few novel pestiviruses have been identified in pigs. This article focuses on the recently emerging atypical porcine pestivirus (APPV) that potentially threatens global swine herd health security. The virus was first identified in 2016, in the United States and thereafter, accumulated evidence shows that it is currently distributed in three continents. The clinical presentation of APPV-infected pigs is characterized by congenital tremor (CT) type A-II in piglets, while adult pigs may become persistent carriers and shedders. Here, a literature review is conducted to summarize the published findings in the virus genomic biology, transmission, epidemiology, pathogenesis, and diagnosis, which would shed light on acceleration of development of anti-APPV strategies.

KEYWORDS

congenital tremor, pestivirus, porcine, prevention and control

1 | INTRODUCTION

Pestiviruses are a group of enveloped viruses in the genus pestivirus of family Flaviviridae,¹ possessing a single-stranded and positive-sense RNA (+ssRNA) genome. They infect a range of target animal species including pigs, ruminants, and wild animals. Four typical virus species, comprising classical swine fever virus (CSFV), border disease virus (BDV), bovine viral diarrhoea virus (BVDV1), and BVDV2, are officially recognized in the genus Pestivirus.¹ Over the past decades, new members of pestiviruses have frequently been found in a number of domestic and wild animal species with different clinical manifestations or without clinical signs. These unclassified new members include Bungowannah virus, pestivirus of the giraffe, sheep pestivirus (Aydin-like), rat pestivirus, Pronghorn antelope virus, atypical ruminant pestivirus (Hobi-like; BVDV3), atypical porcine pestivirus (APPV), and Lateral-shaking Inducing Neurodegenerative Agent (Linda) virus (Figure 1).¹⁻⁶ The whole genome sequences among the pestivirus species are more than 25% divergent, which

has been used for demarcation of a new species in the genus. As one of the atypical pestiviruses, Bungowannah virus has been identified to cause cardiac failure, stillbirth, and sudden death in piglets in Austria without clinical signs of congenital tremor (CT).^{3,7} Recently, two other atypical pestiviruses, APPV and Linda virus, were reported to be associated with CT in piglets.^{5,6} Phylogenetic analysis has shown that these atypical pestiviruses (Bungowannah virus, Linda virus, and APPV) are highly divergent from the other members.

CT is the phenomenon of "shaker pigs" or "dancing pigs," which is characterized with clinical signs of muscular tremor and incapability of standing and feeding on sow's milk in newborn piglets.^{8,9} Severely affected piglets may die later because of inadequate colostrum intake and starvation. Referring to the differences in the clinical signs, causes of diseases, and mortality, CT is divided into types A and B.^{8,10} Based on distinct causes of diseases, the type A is further subdivided into five subgroups (types A-I, II, III, IV, and V).⁹ The types A-III and IV occur rarely, caused by inherited factors including

Abbreviations: aa, amino acids; APPV, atypical porcine pestivirus; BDV, border disease virus; BVDV, bovine viral diarrhoea virus; CDD, Conserved Domain Database; CSFV, classical swine fever virus; CT, congenital tremor; FISH, fluorescence in situ hybridization; Linda, Lateral-shaking Inducing Neurodegenerative Agent; NCBI, National Center for Biotechnology Information; NSP, non-structural protein; nt, nucleotides; Olig 2, oligodendrocyte transcription factor 2; PI, persistent infection; RdRp, RNA-dependent RNA polymerase; UTR, untranslated region; qRT-PCR, Quantitative reverse transcriptase PCR
Shuonan Pan and Chunxiao Mou contributed equally to this article.

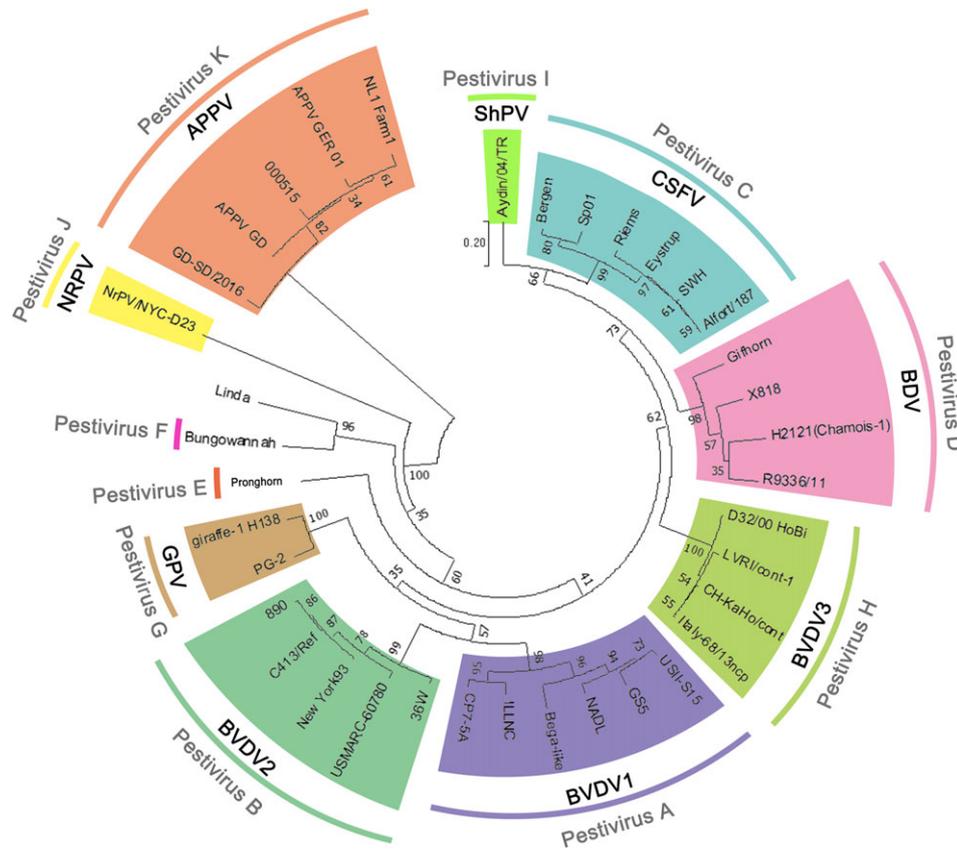


FIGURE 1 Phylogenetic analyses based on the N^{pro} gene sequences of pestivirus members. The tree was constructed using MEGA7 applying the Maximum Likelihood for 1000 bootstrap replications. The new names of species, pestiviruses A-K recently proposed by ICTV,¹⁹ were presented. Abbreviations are as follows with the GenBank accession numbers in parentheses: 1) ShPV, sheep pestivirus: Aydin/04/TR (NC_018713); 2) CSFV: Bergen (KJ619377), Sp01(FJ265020), Riems (AY259122), Eystrup (NC_002657), SWH (DQ127910), Alfort/187(X87939); 3) BDV: Gifhorn (KF925348), X818(NC_003679), H2121(Chamois-1)(GU270877), R9336/11(MF102261); 4) BVDV3 (Hobi-like pestivirus): D32/00_“HoBi”(AB871953), LVRI/cont-1(KC297709), CH-KaHo/cont (JX985409), Italy-68/13ncp(KJ627179); 5) BVDV1: USII-S15(KU159365), GS5(KJ541471), NADL (NC_001461), Bega-like (KF896608), ILLNC(U86600), CP7-5A(AF220247); 6) BVDV2: 36 W(KT875141), USMARC-60780(KT832823), New York’93(AF502399), C413/Ref (NC_002032), 890(U18059); 7) GPV, giraffe pestivirus: PG-2(KJ660072), giraffe-1 H138(AF144617); 8) Pronghorn antelope pestivirus (NC_024018); 9) Bungowannah virus (NC_023176); 10) Linda virus (NC_035432); 11) NRPV, Norway rat pestivirus: NrPV/NYC-D23, (KJ950914); 12) APPV: GD-SD/2016(KY475593), APPV_GD(KY624591), 000515(KR011347), APPV_GER_01(LT594521), NL1 Farm1(KX929062)

a sex-linked inherited defect in Landrace pigs (Type A-III) and an inherited recessive genetic defect in Saddleback pigs (Type A-IV), while another type is caused by exposure of specific toxins to the pregnant sows (type A-V). In comparison, CT are commonly caused by infectious agents including mild CSFV strain (Type A-I) and previously unidentified causative agent (Type A-II). The CT type A-II is characterized with its sporadic and occasional occurrence in gilt litters, demyelination of the spinal cord and brain in piglets, and low-moderate mortality of piglets caused by malnutrition.^{9,11-13} The clinical signs of CT type A-II could be reproduced on the piglets, farrowed by sows infected with inoculum containing the potential agent 40 years ago,¹⁴ which has provided a powerful evidence to support the assumption that an infectious agent may be responsible for the tremor. Until recently, researchers from both the United States and the Netherlands found that the APPV may be the causative agent of CT type A-II in neonatal piglets during the same period. Of these, APPV was first identified by next-generation sequencing from the samples in the United States in 2015.⁵ Shortly thereafter, it was reported in pigs with CT in the United States, Germany, the Netherlands, Spain, Austria, China, and Brazil (Table 1).^{9,11-13,15-18}

The emergence of APPV, Bungowannah virus, and Linda virus in the pestivirus genus suggests that there are possibly more “atypical” pestiviruses circulating in domestic animals and wild animals that remain to be identified (Figure 1). Nevertheless, it has to be clarified here that the proposed term “atypical porcine pestivirus, APPV” is questionable because the “atypical” has already been used to name other new pestiviruses. It is predictable that the genus pestivirus will expand with discovery of new members, and a rational nomenclature would be beneficial to recognize each virus species. Recently, the International Committee on Taxonomy of Viruses (ICTV) has proposed to name the APPV as Pestivirus K,¹⁹ which would clearly differentiate it from the other members within the pestivirus genus.

Thus far, accumulated evidence has shown that the newly discovered APPV has epidemic potential and threatens global swine herd health security. However, information about its molecular biology, pathogenesis, epidemiology, carriage, and transmission is largely unknown. This dearth of information hampers implementation of intervention and preventive measures. Therefore, it would be worthwhile, at this time, to review these important advancements with limited scientific literature and thereby, provide insights for further research on the APPV.

TABLE 1 Detection of atypical porcine pestivirus infection in pigs in different countries

Country	Year Emerged (Reference)	Porcine Samples	Genomic Detection	Clinical Diseases in Pigs
United States	2015 (Hause et al ⁵)	Serum	NGS	NA
United States	2016 (Arruda et al ¹⁵)	Various tissues	NGS	CT
Germany	2016 (Postel et al ²⁰)	Various tissues	RT-PCR	CT; healthy pigs
Netherlands	2016 (de Groof et al ⁹)	Serum	NGS	CT
Sweden	2016 (Blomström ⁴⁵)	Lymph node	NGS	PMWS
Austria	2017 (Schwarz et al ¹²)	Various tissues	RT-PCR	CT
Germany	2017 (Beer et al ¹⁶)	Tonsil, Serum	RT-PCR	NA
China	2017 (Zhang et al ¹⁷)	Cerebellum	RT-PCR	CT
China	2017 (Yuan et al ¹³)	Various tissues	RT-PCR	CT
Spain	2017 (Muñoz-González et al ¹¹)	Various tissues	RT-PCR	CT; healthy pigs
Germany, Great Britain, Italy, Serbia, Switzerland, China, Taiwan	2017 (Postel et al ²¹)	Serum	RT-PCR; NGS	healthy pigs
China	2018 (Zhang et al ⁴⁶)	Serum and brain	RT-PCR	CT
USA	2018 (Gatto et al ²⁶)	Semen, preputial fluid	RT-PCR	NA
Germany	2018 (Cagatay et al ²²)	Serum	RT-PCR	NA, wild boar
Brazil	2018 (Gatto et al ⁴⁷)	Cerebellum, spinal cord	RT-PCR	CT
Brazil	2018 (Possatti et al ²⁸)	Various tissues	RT-PCR	CT-associated lethality
Brazil	2018 (Mósená et al ¹⁸)	Various tissues	RT-PCR	CT

Abbreviations: CT, congenital tremors; NA, not available; NGS, next generation of sequencing; PMWS, postweaning multisystemic wasting syndrome; RT-PCR, reverse transcription polymerase chain reaction; Various tissues, including serum, cerebrum, cerebellum, spinal cord, cerebrospinal fluid, lung, etc.

2 | EPIDEMIOLOGY AND TRANSMISSION

To date, APPV has been detected in the North America, South America, Asia, and Europe (Table 1). It has been shown that APPV has been circulating in Spain as early as at least 1997,¹¹ which is currently the earliest time that the APPV isolate can be tracked back to. An early study identified an APPV strain from the samples of piglets with CT and found an APPV prevalence of 6% by quantitative reverse transcriptase PCR (qRT-PCR) in the US pig herds.⁵ APPV was then reported to be present in all of the CT-affected piglets from South China, which further indicates the potential relationship between APPV and CT type A-II.¹³ Moreover, it has been reported that detection rates of 2.4% to 22% for APPV genomes were found in apparently healthy pigs in the United States and Germany.^{5,16,20} In a recent similar study with serum samples from apparently healthy pigs, APPV prevalence reached from 2.3% to 17.5% in Europe and from 5% to 11% in China.²¹ All of the results demonstrated that APPV was highly prevalent in both apparently healthy pigs and CT-affected pigs, suggesting the virus may have spread worldwide. A most recent finding shows that presence of APPV genome in the serum samples of wild boars in Germany reached 19%, suggesting that the wild boars may be an important APPV reservoir.²² Therefore, an intensive epidemiological investigation on domestic pigs and wild boars in a wider geographical area is urgently needed in the future.

The natural routes of APPV transmission have been investigated by several research groups. To address whether vertical transmission is a natural route of infection causing CT type A-II in the offspring, sows during gestation have been infected with APPV-containing inocula in utero. The results show that the farrowed piglets developed clinical signs of CT.^{9,15} In these studies, APPV was consistently detected in multiple tissues of piglets with CT by RT-PCR. The results provide a

strong evidence that the CT type A-II is possibly caused by APPV. During gestation, the piglets are infected by APPV while the central nervous system is developing. This infection results in changes in nerve fibers and the appearance of farrowed piglets with CT symptoms.

Porcine semen is considered as a vehicle for viral disease transmission.²³ Pestiviruses, such as CSFV and BVDV have been detected in the boar and bull semens.^{24,25} APPV was detected in the preputial fluids and in the semen of 15% boars, which shows that the disease may be transmitted sexually.^{9,12} In the United States and Europe, the semens were collected from the "healthy" boars without a clinical history of CT. However, the APPV infection has been reported in a number of cases in the abovementioned regions (Table 1), suggesting that either transiently infected or persistently infected boars without symptoms may shed APPV in semen. Furthermore, phylogenetic analysis shows that different APPV strains may stem from the same batch of boar studs,^{20,26} which may be produced by the boars from different locations. Undoubtedly, it would increase the complexity of preventing the APPV infection in the pig farms. Recently, Postel et al reported that APPV genome was found in the porcine samples from salivary glands, duodenum, pancreas, and colon,²⁰ suggesting that the orofecal may be another transmission route for the virus to spread. This is in accordance with a later observation of virus presence in porcine stool.⁹

3 | GENETIC DIVERSITY AND EVOLUTION OF APPV

The APPV strains were found to be significantly distinct from the other pestiviruses in the genomic sequence. Phylogenetic analysis shows that it has a high similarity of 68% to *Rhinolophus affinis*

pestivirus, as contrasted with the low similarity of 25% to 28% to the well-known pestiviruses including CSFV, BVDV, and BDV.⁵ All of the APPV isolates shared a relatively high degree of nucleotide sequence similar to each other than the other pestiviruses. However, the genetic variability of 81% to 87% among the APPV isolates is still remarkably high even within a region.^{16,20} For example, cases of CT type A-II have been monitored in the Netherlands since 2012, and a number of APPV strains were identified in the study.⁹ Follow-up phylogenetic analysis shows that there is a large genetic variation of more than 10% nucleotides (nt) difference among the APPV strains, and the strains could be classified into more than three clusters. Other works have been also involved in investigating the genetic diversity of APPV strains within a farm.^{16,20} The results indicate a high degree of genetic diversity between strains among farms in the same country, potentially posing a big challenge against the development of diagnostic tests and vaccines.

Phylogenetic analysis of APPV has been fraught with low availability of a sufficient number of strains during a certain period. Although a number of APPV clusters with high variability within it have been proposed, the origin and evolution of APPV remain largely unclear. In one study, 1615 nt of APPV NS2-3 region were used to calculate the nucleotide substitutions per site per year (s/s/y) among the Spanish strain 2001, Spanish strain 2015, and German strain 2015. The results show that during the past 14 years, a mutation rate of 2.65×10^{-3} s/s/y has occurred between the Spanish strains 2001, and 2005, while it is 3.62×10^{-3} s/s/y between the Spanish strain 2001, and the German strain 2015.¹¹ Both high-mutation rates were comparable with that of CSFV reported previously,²⁷ suggesting that the APPV may have been evolving within the range of mutation rate as pestiviruses have. In addition, another study utilized the Bayesian analysis to analyze the origin and dissemination of current APPV strains.¹³ The analysis demonstrated that APPV strains may originate in Germany, and then spread from Germany to the United States in one way, while transmitting from Germany to the Netherlands and China in another way. Notably, the conclusions about the geographic origin and dissemination of APPV would be more reliable when more virus strains become available in the future.

4 | PATHOGENESIS AND PATHOLOGY

4.1 | Morbidity and mortality

APPV-infected newborn piglets develop clinical disease of CT, which then interferes with the piglets' ability to suckle milk resulting in increased preweaning mortality. It is also shown that the viability of piglets with persistent tremor has been heavily affected. As described from two reports, the losses of 2.5 piglets per sow have occurred on a farm in Austria,¹² and the death of more than 700 pigs after an outbreak of tremors on a farm in the United States.⁵ The economic losses in the APPV-affected pig herds remain to be determined, but it was estimated to have a 10% drop in pig reproductive performance.¹² The clinical signs of CT type A-II-affected piglets may change dramatically both in one litter and between

litters. Outbreaks generally occur in piglets of around 1 week to 2 months old. Of note, it has only one initial outbreak of CT type A-II in the litters from the same sow but without any subsequent recurrences. A correlation between RNA load and age distribution has been identified that the highest-RNA load is in the CT type A-II-affected piglets below 1 week of age,¹¹ which would contribute to understanding why the disease is more prevalent in piglets than in sows. Furthermore, a recent study shows that coinfection of APPV and porcine teschovirus (PTV) increased piglet mortality,²⁸ which reveals the clinical significance of APPV infection in pigs and necessitates further study on this virus and its coinfections with those important porcine pathogens.

4.2 | Tissue tropism

Currently, tissue tropism of APPV was primarily determined by qRT-PCR. In one study from the 2-day-old piglets, high-APPV RNA loads were found in the tonsil, spleen, and thymus samples, while moderate RNA loads in the sera, nasal/rectal swabs, cerebrospinal fluid, and cerebrum.¹¹ In another study, the APPV can be detected in nearly all tissues with the highest-RNA levels in the submaxillary lymph nodes, and moderate RNA levels in the peripheral lymphoid organs (spleen, tonsil, and inguinal lymph node), nervous system (brain stem, brain, and cerebellum), and digestive system (duodenum).¹³ Both studies show that a high abundance of APPV RNA were found in the lymphoid organs, which provides important information about the major sites of APPV replication in pigs and suggests that APPV may have a role in the suppression of the host immune system as well. Additionally, a study of fluorescence in situ hybridization (FISH) found that moderate levels of APPV RNA were detected in the inner granular cell layer of the cerebellum, which provides a direct evidence that the virus could infect the central nervous system.²⁰ The results may partially explain why the neurological symptoms could be caused by this virus.

4.3 | Postmortem lesions

Histologic lesions of CT type A-II-affected pigs were characterized with vacuolization in the cerebellar white matter.¹² Further ultrastructural examination found that not only hypomyelination was present in the spinal cord but also myelin breakdown in the cerebellar white matter of CT type A-II-affected pigs. These alterations differ somewhat from the lesions identified in CSFV-infected piglets and BVDV-infected calves.^{29,30} The immunohistochemical analysis indicated that the numbers of oligodendrocytes in the spinal cord were not changed, but the oligodendrocyte transcription factor 2 (Olig 2) was found to express increasingly in the CT type A-II-affected piglets.¹² The results indicate that APPV infection may affect myelin development and result in disruption of the myelin, which sheds light on understanding pathogenesis of the disease. Further investigation on this direction is essential to understand the exact mechanisms responsible for occurrence of CT in APPV-infected piglets.

5 | VIROLOGY, IMMUNOLOGY, AND PERSISTENT INFECTION

5.1 | Virus culture in vitro

Extensive efforts have been put into growing APPV on primary swine kidney cells and continuous swine kidney cell lines (PK15, IBRS, SK6, and ST), but all have failed.^{5,9,15} Recently, the results from three research groups demonstrated that APPV could be passaged on a few porcine cell lines, though the virus titers were low.^{12,16,21} Considering the high variability of APPV strains, it could not be excluded that some specific virus strains may be robust enough to be successfully cultured in vitro. Accordingly, it is worth investigating many more virus isolates in multiple cell lines for establishment of an APPV culture system.

Furthermore, two CT reproduction studies were performed with the porcine serum samples containing APPV.^{9,15} Both studies could reproduce CT in the farrowed piglets, suggesting that APPV is most possibly the causative agent of CT type A-II. However, the two studies have not been finished with the pure virus without other potential infectious agents. Therefore, it is urgent to work out how to culture the virus in vitro and then completely perform the Koch's postulates with the pure APPV.

5.2 | Virus genome

APPV has a positive-stranded and nonpolyadenylated RNA genome with a length of about 11 500 nt. The genomic RNA contains 5' and 3' untranslated regions (UTRs) at both ends and has a large open-reading frame encoding a 3635-aa polyprotein, which is then processed to yield the following single proteins: N^{pro}-C-E^{rn5}-E1-E2-p7-NS2-3(NS2, NS3)-NS4A-NS4B-NS5A-NS5B (Figure 2). Notably, it seems unusual that this polyprotein is 250 aa residues shorter than those of the other pestiviruses.⁵ Currently, the sequences of mature APPV proteins were determined by identification of the N- and C-

terminus of each virus protein via alignment with known cleavage sites of the other pestivirus polyproteins. However, these putative N- and C-terminus of virus proteins warrant further experimental confirmation.^{31,32}

So far, the 5' and 3' UTRs of APPV have been determined to be as long as 378 nt and 276 nt, respectively. In comparison, the 5' and 3' UTRs of the other classical pestiviruses are within the ranges of 369 to 498 nt and 206 to 503 nt in sizes. These observations indicate that the 5' UTR and 3' UTR sequences currently available for APPV may be intact or close to be complete. Therefore, further research is needed to confirm its authentic 5' and 3' terminal sequences. To address this issue, it is critical to grow sufficient quantities of APPV through establishment of efficient virus-cell culture systems in vitro. N^{pro} is unique to the genus Pestivirus. As a nonstructural protein (NSP), APPV-N^{pro} possesses autoprotease activity responsible for self-cleavage at the N^{pro}/C site from the polyprotein, which has been confirmed in our lab (unpublished data). A conserved catalytic dyad of cysteine protease was identified at His69 and Cys89 by pairwise alignment with the N^{pro} proteins of other pestiviruses.³³ Despite the existence of conservation of the catalytic and cleavage sites in the APPV-N^{pro}, it has no significant similarity to any known proteins in the sequence. In addition, there is no discernible TRASH motif in the APPV-N^{pro} as found in the other pestivirus N^{pro} proteins,³⁴ suggesting that the APPV-N^{pro} may have novel functions distinct from the ones of other pestiviruses.

As far as we know, the structural proteins of pestivirus commonly include a capsid protein and three envelope glycoproteins comprising E^{rn5}, E1, and E2. The three glycoproteins are involved in viral entry into target cells. E^{rn5}, which uniquely appears in pestiviruses, is a structural protein with an RNase T2 domain involved in ribonuclease activity.³⁵ E1 and E2 heterodimers are crucial for viral infectivity.³⁶ E2 is usually considered as the major protective antigen eliciting the production of neutralizing antibodies against the pestivirus, in addition to being the key factor in determining cell tropism. Following APPV-N^{pro}, a capsid protein is encoded, which is a small and basic protein and plays a role in RNA packaging into mature virions. It is currently determined to

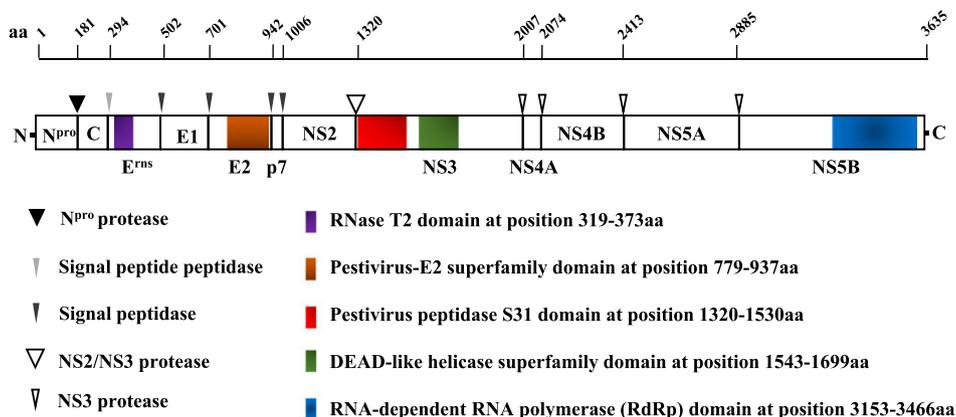


FIGURE 2 Polyprotein cleavage map of atypical porcine pestivirus. The complete genome of APPV-000515 strain encodes a polyprotein that is co and posttranslationally cleaved into the structural proteins including the capsid protein (C) and envelope glycoproteins (E^{rn5}, E1, and E2) and the NSPs comprising the N^{pro}, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B. As reviewed previously,⁴³ structural proteins and p7 are potentially cleaved by cellular signal peptide peptidase and signal peptidases. The N^{pro} cleaves itself from the polypeptide, which has been confirmed in our lab. The remaining NSPs are cleaved by the NS2/NS3 and NS3 proteases. The colored boxes show a number of protein domains identified by CDD search service of NCBI in this study

have a length of 111 aa, which is slightly longer than the other pestiviruses (97–102 aa).⁵ In the putative APPV-E^{ms} region, an RNase T2 domain was found at position 319 to 373 aa in the APPV polyprotein through the Conserved Domain Database (CDD) search service of National Center for Biotechnology Information (NCBI) (Figure 2). This is the evidence showing the existence of E^{ms} gene in the APPV genome. In the putative APPV-E2 region, a pestivirus-E2 superfamily domain was identified at position 779 to 937 aa in the APPV polyprotein by alignment with pfam16329 of pestivirus glycoprotein E2. Interestingly, there seems a large “deletion” within the N-terminal region of the putative APPV-E2 protein, leading to that the size (241aa) of APPV-E2 is significantly shorter than the ones of other pestiviruses (373–378 aa).⁵ However, a known fusion peptide region is conservatively located in the abovementioned “deletion” region, which may significantly affect the APPV's ability in entry of host cells.³⁷ Further research is required to determine if the “deletion” in the N-terminal region of APPV-E2 would alter virus property in receptor utilization or entry of cells.

Other putative NSPs, including p7, NS2-3, NS4A, NS4B, NS5A, and NS5B, are encoded following the E2-coding region. Overall, these NSPs lack significant similarity to any corresponding proteins of other pestiviruses (12%–74% identity).⁵ Among these, a cysteine autoprotease domain could not be identified in the putative APPV-NS2, which is reported to be responsible for cleavage of NS2 from NS3 in the other pestiviruses.³⁸ Furthermore, it is known that pestivirus NS3 is a chymotrypsin-like serine protease having both cis- and trans-cleavage activities.³⁹ In the putative APPV-NS3 region, a pestivirus peptidase S31 domain is found at position 1320 to 1530 aa in the APPV polyprotein, with a DEAD-like helicase superfamily domain at position 1543 to 1699 aa. In the putative APPV-NS5B region, an RNA-dependent RNA polymerase (RdRp) domain is also identified at position 3153 to 3466 aa.

5.3 | Immunity

It is still a mystery about the immunity against APPV infection. As mentioned before, if the sow farrows a litter of CT type A-II-affected piglets in the first pregnancy, the subsequent litters from the same sow will not display symptoms of the disease.⁹ This is an indication that protective immunity has developed against APPV infection in pigs. Other evidence also shows that the interferon α was detected in the sera of APPV-infected piglets,¹¹ which suggests that host innate immune system could be activated once APPV infection happens in pigs. Interestingly, the presence of high-APPV RNA load in lymphoid organs suggests that the virus may be able to act on the host immune system.^{11,13} Therefore, further studies need to clarify the roles of the innate and adaptive immune response to this virus.

5.4 | Persistent infection

Persistent infections (PIs) have been reported for CSFV, BVDV, BDV, and Hobi-like pestivirus.^{40–42} Research has demonstrated that the pestivirus N^{pro} and E^{ms} play critical roles in suppression of type I interferon response, which are involved in establishing virus PI.^{42,43}

Now that APPV contains the two proteins, it is plausible that the novel virus may have the capability of establishing PI in pigs. Our preliminary data have also confirmed that the APPV-N^{pro} has the function of type I interferon antagonism (unpublished data). However, it is yet unclear about the potential role of PI in APPV transmission and virus maintenance in a swine herd. Some data have shown that a number of piglets with 14 weeks old did not display any symptoms of CT type A-II but were APPV viremic.¹¹ The piglets of PI could develop without any clinical signs or antibody response against the virus, while virus shed in their saliva and semen. Considering that a population of as low as 1% BVDV PI-affected calves can maintain the virus existence in a cattle herd,⁴⁴ APPV PI-affected pigs in the absence of CT can very possibly play an important role in the virus spread.

6 | DIAGNOSIS

Metagenomic sequencing is a powerful technique in identification of novel virus pathogens that has successfully been applied in discovering the APPV for the first time in the samples of swine in the United States.⁵ With availability of APPV genome sequence, a few RT-PCR protocols that target conserved regions within NS3, NS4B, or NS5B have then been developed to detect the APPV and quantify the copies of virus genome in the clinical samples.^{15,18,21} Preferred specimens include brain, lymph node, and whole blood. For detection of APPV antibody, an indirect ELISA has been established with APPV-E^{ms} as coating antigens, and the results show that 94% of pig serum samples in the United States are positive for APPV-E^{ms} antibody.⁵ However, another study from Austria found that 35.3% serum samples are positive by APPV-NS3 blocking ELISA.¹² The big difference of results between both assays suggests that it warrants further research to validate the results and even develop more efficient and reliable diagnostic methods.

7 | CONCLUSIONS

Although it is unknown about how APPV emerges in swine, the pandemic potential of the virus should be paid attention with the facts that APPV has already been found to be distributed in a number of countries on three continents. Efforts to estimate the exact economic impact of the virus on swine industry require more information about the virus epidemics. APPV was found to be distinct from other species in the pestivirus genus. Although there is so far no evidence showing that APPV negatively influences the established diagnosis and surveillance programs for important pestiviruses, the issues of cross-antigenicity and cross-protection between APPV and the other pestiviruses are worth investigating further. The high divergence of APPV genome may cause failure in the detection of virus by RT-PCR and in the assay of antibodies against APPV. This problem increases the importance of knowing the virus epidemiology and genetic diversity in order to improve diagnostic methods. Furthermore, it is urgent to resolve the important problem of APPV culture *in vitro*, aiming to fully prove Koch's postulates in animal trials and address the questions related to APPV molecular biology and

immune response against virus infection. Increasing knowledge on transmission, pathogenesis and prevalence of APPV will enable the development of effective control programs to prevent introduction and dissemination of the virus.

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CONFLICT OF INTEREST

The authors have no competing interest.

ORCID

Zhenhai Chen  <http://orcid.org/0000-0002-6704-7546>

REFERENCES

- Blome S, Beer M, Wernike K. New leaves in the growing tree of pestiviruses. *Adv Virus Res.* 2017;99:139-160.
- Avalos-Ramirez R, Orlich M, Thiel HJ, Becher P. Evidence for the presence of two novel pestivirus species. *Virology.* 2001;286(2):456-465.
- Kirkland PD, Frost MJ, Finlaison DS, King KR, Ridpath JF, Gu X. Identification of a novel virus in pigs—Bungowannah virus: a possible new species of pestivirus. *Virus Res.* 2007;129(1):26-34.
- Postel A, Schmeiser S, Ogozogluc TC, et al. Close relationship of ruminant pestiviruses and classical swine fever virus. *Emerg Infect Dis.* 2015;21(4):668-672.
- Hause BM, Collin EA, Peddireddi L, et al. Discovery of a novel putative atypical porcine pestivirus in pigs in the USA. *J Gen Virol.* 2015;96(10):2994-2998.
- Lamp B, Schwarz L, Högl S, et al. Novel pestivirus species in pigs, Austria, 2015. *Emerg Infect Dis.* 2017;23(7):1176-1179.
- Kirkland PD, Read AJ, Frost MJ, Finlaison DS. Bungowannah virus—a probable new species of pestivirus—what have we found in the last 10 years? *Anim Health Res Rev.* 2015;16(1):60-63.
- Done JT, Woolley J, Upcott DH, Hebert CN. Porcine congenital tremor type AI: Spinal cord morphometry. *Br Vet J.* 1986;142(2):145-150.
- de Groof A, Deijs M, Guelen L, et al. Atypical porcine pestivirus: a possible cause of congenital tremor type A-II in newborn piglets. *Viruses.* 2016;8(10):E271.
- Done JT, Harding JD. Congenital tremor in pigs (trembling disease of piglets): lesions and causes. *Dtsch Tierarztl Wochenschr.* 1967;74(13):333-336.
- Muñoz-González S, Canturri A, Pérez-Simó M, et al. First report of the novel atypical porcine pestivirus in Spain and a retrospective study. *Transbound Emerg Dis.* 2017;64(6):1645-1649.
- Schwarz L, Riedel C, Högl S, et al. Congenital infection with atypical porcine pestivirus (APPV) is associated with disease and viral persistence. *Vet Res.* 2017;48(1):1.
- Yuan J, Han Z, Li J, et al. Atypical porcine pestivirus as a novel type of pestivirus in pigs in China. *Front Microbiol.* 2017;8:862.
- Patterson DS, Done JT, Foulkes JA, Sweasey D. Neurochemistry of the spinal cord in congenital tremor of piglets (type AI), a spinal dysmyelination of infectious origin. *J Neurochem.* 1976;26(3):481-485.
- Arruda BL, Arruda PH, Magstadt DR, et al. Identification of a divergent lineage porcine pestivirus in nursing piglets with congenital tremors and reproduction of disease following experimental inoculation. *PLoS One.* 2016;11(2):e0150104.
- Beer M, Wernike K, Dräger C, et al. High prevalence of highly variable atypical porcine pestiviruses found in Germany. *Transbound Emerg Dis.* 2017;64(5):e22-e26.
- Zhang K, Wu K, Liu J, et al. Identification of atypical porcine pestivirus infection in swine herds in China. *Transbound Emerg Dis.* 2017;64(4):1020-1023.
- Mósen ACS, Weber MN, da Cruz RAS, et al. Presence of atypical porcine pestivirus (APPV) in Brazilian pigs. *Transbound Emerg Dis.* 2018;65(1):22-26.
- King AMQ, Lefkowitz EJ, Mushegian AR, et al. Changes to taxonomy and the international code of virus classification and nomenclature ratified by the international committee on taxonomy of viruses (2018). *Arch Virol.* 2018. [Epub ahead of print]
- Postel A, Hansmann F, Baechlein C, et al. Presence of atypical porcine pestivirus (APPV) genomes in newborn piglets correlates with congenital tremor. *Sci Rep.* 2016;6(1):27735.
- Postel A, Meyer D, Cagatay GN, et al. High abundance and genetic variability of atypical porcine pestivirus in pigs from Europe and Asia. *Emerg Infect Dis.* 2017;23(12):2104-2107.
- Cagatay GN, Antos A, Meyer D, et al. Frequent infection of wild boar with atypical porcine pestivirus (APPV). *Transbound Emerg Dis.* 2018. [Epub ahead of print]
- Maes D, Van Soom A, Appeltant R, Arsenakis I, Nauwynck H. Porcine semen as a vector for transmission of viral pathogens. *Theriogenology.* 2016;85(1):27-38.
- Choi C, Chae C. Detection of classical swine fever virus in boar semen by reverse transcription-polymerase chain reaction. *J Vet Diagn Invest.* 2003;15(1):35-41.
- Givens MD, Heath AM, Carson RL, et al. Analytical sensitivity of assays used for detection of bovine viral diarrhoea virus in semen samples from the Southeastern United States. *Vet Microbiol.* 2003;96(2):145-155.
- Gatto IRH, Arruda PH, Visek CA, et al. Detection of atypical porcine pestivirus in semen from commercial boar studs in the United States. *Transbound Emerg Dis.* 2018;65(2):e339-e343.
- Díaz de Arce H, Núñez JI, Ganges L, Barreras M, Teresa Frías M, Sobrino F. Molecular epidemiology of classical swine fever in Cuba. *Virus Res.* 1999;64(1):61-67.
- Possatti F, Headley SA, Leme RA, et al. Viruses associated with congenital tremor and high lethality in piglets. *Transbound Emerg Dis.* 2018;65(2):331-337.
- Bradley R, Done JT, Hebert CN, et al. Congenital tremor type AI: light and electron microscopical observations on the spinal cords of affected piglets. *J Comp Pathol.* 1983;93(1):43-59.
- Porter BF, Ridpath JF, Calise DV, et al. Hypomyelination associated with bovine viral diarrhoea virus type 2 infection in a longhorn calf. *Vet Pathol.* 2010;47(4):658-663.
- Stark R, Meyers G, Rumenapf T, Thiel HJ. Processing of pestivirus polyprotein: cleavage site between autoprotease and nucleocapsid protein of classical swine fever virus. *J Virol.* 1993;67(12):7088-7095.
- Vilcek S, Durkovic B, Kolesarova M, Paton DJ. Genetic diversity of BVDV: consequences for classification and molecular epidemiology. *Prev Vet Med.* 2005;72(1-2):31-35.
- Rumenapf T, Stark R, Heimann M, Thiel HJ. N-terminal protease of pestiviruses: identification of putative catalytic residues by site-directed mutagenesis. *J Virol.* 1998;72(3):2544-2547.
- Szymanski MR, Fiebach AR, Tratschin JD, et al. Zinc binding in pestivirus N (pro) is required for interferon regulatory factor 3 interaction and degradation. *J Mol Biol.* 2009;391(2):438-449.
- Schneider R, Unger G, Stark R, Schneider-Scherzer E, Thiel HJ. Identification of a structural glycoprotein of an RNA virus as a ribonuclease. *Science.* 1993;261(5125):1169-1171.

36. Ronecker S, Zimmer G, Herrler G, Greiser-Wilke I, Grummer B. Formation of bovine viral diarrhea virus E1-E2 heterodimers is essential for virus entry and depends on charged residues in the transmembrane domains. *J Gen Virol*. 2008;89(9):2114-2121.
37. Wang FI, Deng MC, Huang YL, Chang CY. Structures and functions of pestivirus glycoproteins: not simply surface matters. *Viruses*. 2015;7(7):3506-3529.
38. Lackner T, Müller A, Pankraz A, et al. Temporal modulation of an autoprotease is crucial for replication and pathogenicity of an RNA virus. *J Virol*. 2004;78(19):10765-10775.
39. Lamp B, Riedel C, Wentz E, Tortorici MA, Rümnapf T. Autocatalytic cleavage within classical swine fever virus NS3 leads to a functional separation of protease and helicase. *J Virol*. 2013;87(21):11872-11883.
40. Decaro N, Losurdo M, Lucente MS, et al. Persistent infection caused by Hobi-like pestivirus. *J Clin Microbiol*. 2013;51(4):1241-1243.
41. Meyers G, Ege A, Fetzer C, et al. Bovine viral diarrhea virus: prevention of persistent fetal infection by a combination of two mutations affecting Erns RNase and Npro protease. *J Virol*. 2007;81(7):3327-3338.
42. Schweizer M, Peterhans E. Pestiviruses. *Annu Rev Anim Biosci*. 2014;2(1):141-163.
43. Tautz N, Tews BA, Meyers G. The molecular biology of pestiviruses. *Adv Virus Res*. 2015;93:47-160.
44. Peterhans E, Schweizer M. BVDV: A pestivirus inducing tolerance of the innate immune response. *Biologicals*. 2013;41(1):39-51.
45. Blomström AL, Fossum C, Wallgren P, Berg M. Viral metagenomic analysis displays the co-infection situation in healthy and PMWS affected pigs. *PLoS One*. 2016;11(12):e0166863.
46. Zhang H, Wen W, Hao G, et al. Phylogenetic and genomic characterization of a novel atypical porcine pestivirus in China. *Transbound Emerg Dis*. 2018;65(1):e202-e204.
47. Gatto IRH, Harmon K, Bradner L, et al. Detection of atypical porcine pestivirus in Brazil in the central nervous system of suckling piglets with congenital tremor. *Transbound Emerg Dis*. 2018;65(2):375-380.

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