

Mini Review

An alternative strategy for studying emerging atypical porcine pestivirus

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Abstract

Atypical Porcine Pestivirus (APPV) is an emerging agent that belongs to the genus *Pestivirus* of the family *Flaviviridae* and causes Congenital Tremor (CT) in newborn piglets. Piglets with CT are mainly characterized by rhythmic tremor in the limbs and head, complicated by ataxia. Affected animals often die due to insufficient suckling with a mortality rate of 10%-30%. Histopathological findings of such piglets are mainly characterized by increased vacuoles in the white matter of the cerebellum, hypomyelination of the spinal cord, and microglial proliferation. APPV has been widely spread around the world since it was first reported in 2015, bringing huge economic losses to the pig industry. However, as a newly discovered virus, no vaccine is currently available to prevent and control APPV infection. In addition, the difficulties in APPV isolation and its high genetic variability severely hamper the development of APPV vaccines. Here, we propose an alternative strategy, the reverse genetics system, may be a prospective platform to address the issues with APPV vaccine design.

Introduction

Congenital Tremor (CT) of piglets, also known as “shaker pig disease” or “dancing pig disease”, is a neurogenic disease that occurs in newborn piglets. At present, CT has been classified into type A and type B according to whether the Central Nervous System (CNS) has histopathological lesions. CT type A is mainly manifested by vacuolization and hypomyelination in the cerebellar and spinal cord, while CT type B has no significant pathological changes in the CNS. Based on the distinct causative factors, CT type A is further categorized into five subtypes (types A I-V). Recent studies have shown that the newly emerging virus, Atypical Porcine Pestivirus (APPV), may be associated with CT type A-II [1,2].

Currently, accumulating evidence indicates that APPV-infected CT type A-II has developed into a global disease, thus posing great challenges to pig production. Although numerous

studies have been carried out on APPV in the past few years [3-7], including epidemiology and genetic evolution, there are relatively few reports on its pathogenesis, immune response, transmission route, and particularly vaccine design, the most important aspects of disease prevention. Additionally, the virus is difficult to isolate and passage in cell culture, which significantly hinders APPV research, and leads to a need of finding new and alternative approaches to study the virus. Thus, a comprehensive summary of the latest findings on APPV will contribute to further research on APPV and provide new strategies for the design of APPV vaccines.

Obstacles to APPV vaccine development

Vaccination is an effective measure to prevent infectious diseases. However, there is currently no commercial vaccine for the prevention and control of APPV infection. Since the discovery of APPV, numerous efforts have been made to isolate

and culture the virus on multiple cell lines (MDBK, MDCK, PK-15, and Vero, etc.), but all have failed [3]. Notably, recent studies have shown that APPV from positive serum samples could be amplified on several cell lines, even though the virus titers were low [4,8], bringing strong prospects for APPV isolation. The inability to effectively propagate the virus in cell cultures substantially hampers the development of APPV vaccines. However, novel vaccine designs provide new strategies for the prevention and control of APPV. A subunit vaccine based on APPV E2 glycoprotein has been developed in our laboratory [9], and the results showed that the subunit vaccine could induce a Th2-type immune response in mice. The immunogenicity of the subunit vaccine must be further evaluated in swine herds.

Reverse genetics system may be a promising platform for APPV research

Reverse genetics is a methodology opposite to classical genetics, which studies the structure and function of biological genes by conducting site-directed mutation, insertion, deletion, and substitution on the basis of obtaining biological gene information. Since the successful rescue of the first RNA virus Q β phage in 1978 [10], great progress has been made in molecular biology research of various RNA viruses. In 1981, the first full length cDNA clone of a eukaryotic positive stranded RNA virus, the poliovirus, was constructed successfully [11], which opened the new era of reverse genetics research. The pestiviruses genome is a positive-sense single-stranded

RNA, which can be directly used as messenger RNA (mRNA) to produce enzymes required for RNA replication. Thus, it is easy to manipulate the viral genome at the level of cDNA. Construction of infectious clones of RNA viruses provides robust reverse genetic tools for pestiviruses (Figure 1), and may be a feasible solution for further research on APPV.

Based on the infectious clone, the deletion, site-directed mutation, and substitution of the viral genome can reveal the roles of various regulatory sequences and viral proteins in the viral life cycle and pathogenesis [12]. In addition, the infectious clone can also serve as a useful tool for antiviral assays via the insertion of exogenous sequences [13]. The establishment of the reverse genetics system provides a new approach for the development of pestivirus vaccines, including live attenuated vaccines and chimeric vaccines [14,15]. Moreover, infectious clones can be used as seeds for existing live attenuated vaccines to reduce production batch differences and ensure vaccine stability and safety.

Conclusions and perspectives

Pestiviruses are one of the most important pathogens of infectious diseases in the breeding industry, resulting in considerable economic losses worldwide. The high genetic variability of pestiviruses is the key to triggering epidemics. Although extensive research has been conducted on APPV, in view of the wide distribution and genetic variability of

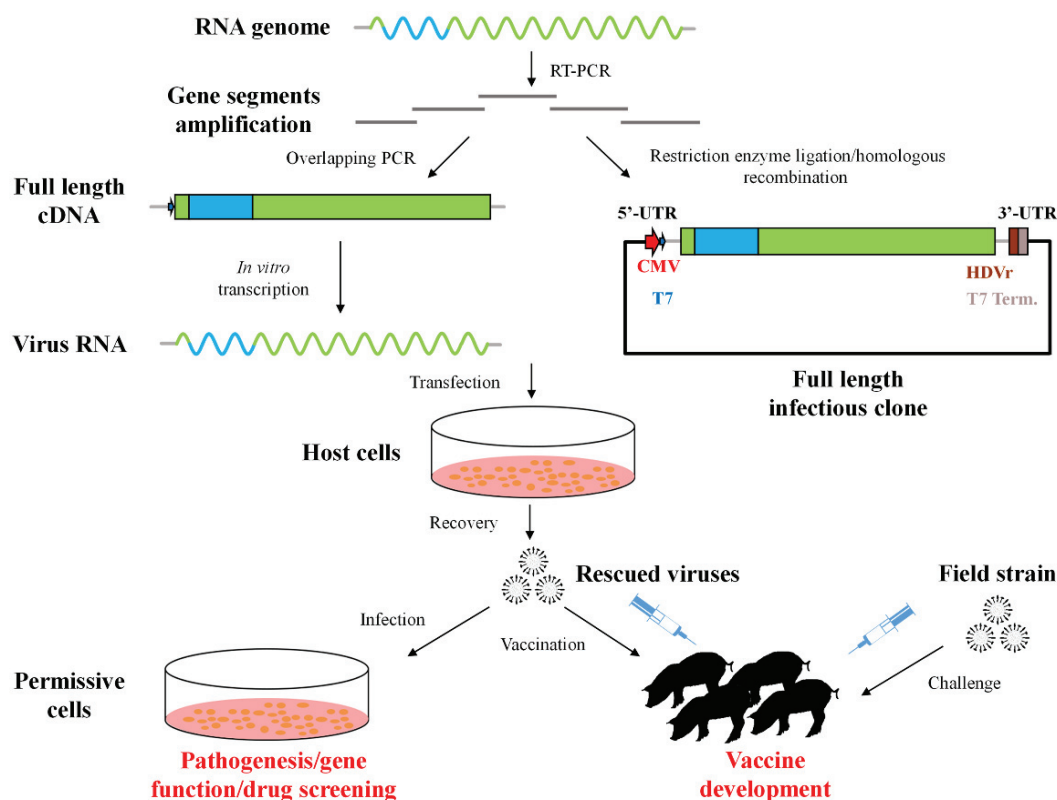


Figure 1: General strategy for the construction of reverse genetics system of pestivirus and its application. The full length cDNA or infectious clone of pestivirus is constructed via overlapping PCR and restriction enzyme ligation or homologous recombination. After purification of the constructs, infectious viruses are rescued either via transfection of virus RNA obtained by *in vitro* transcription or via direct transfection of infectious clone when using a bacteriophage promoter or a eukaryotic promoter. Rescued viruses can be used for further research, including pathogenesis, gene function, drug screening, and vaccine development, etc.

APPV in different countries, it is necessary to conduct an in-depth study on its etiology, epidemiology, transmission routes, pathogenesis, diagnosis, and preventive strategies. In particular, an epidemiological surveillance system for APPV should be established to explore the genetic and evolutionary relationships of the virus.

Previous studies have reported that APPV can be found in semen from commercial boar studs [16], hence, the crucial role of semen in the dissemination of APPV requires further investigation. In addition, recent studies have revealed the presence of APPV in wild boars, suggesting that the wild boars may be a critical virus reservoir for APPV [17–19]. Further investigation is required to elucidate the role of wild boars in the epidemiology of APPV. So far, few data are available regarding the immune response against APPV. Although IFN- α was detected in naturally infected piglets [20] and the humoral immune response against APPV has been described [21] in two independent studies, the important role of the innate and adaptive immune response to APPV still needs further study.

As a newly discovered virus, no commercial vaccine is currently available against APPV infection. Furthermore, the virus is difficult to isolate and passage in cell cultures, resulting in the deficiency of live virus materials, which severely hampers the intensive study of APPV. The reverse genetics system is an effective tool for molecular biology research and development of new vaccines for RNA viruses. Multiple reverse genetics systems of pestiviruses have been developed to study their pathogenesis, gene function, and vaccine design, which may become a prospective strategy to address the issues with APPV vaccine design. Although a reverse genetics system for APPV has been successfully established in a recent study [22], the titer of the rescued virus is low, and the biological characteristics including virulence and pathogenicity of the rescued virus have not been investigated in depth. Similar to other flaviviruses, the cDNA clone of APPV is unstable in host bacteria, which makes its genome prone to mutation. Several approaches could be employed to overcome this problem, including *in vitro* ligation / transcription [22,23], intron insertion [24], reduction of bacterial promoter activity [25,26], and use of low-copy-number plasmids [27,28]. Furthermore, greater efforts are required to ensure vaccine stability and safety, and to improve the efficiency of the reverse genetics system for APPV, to offer a solid foundation for studying the replication, assembly, invasion mechanism, and pathogenesis of APPV. Meanwhile, animal models of APPV infection can be constructed by using rescued virus or APPV-positive serum to further facilitate the development of APPV vaccines, such as E2 protein-based subunit vaccines. Continued surveillance of epidemiology for APPV and intensive vaccine development will help eradicate the disease and maintain a healthy pig production environment.

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