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Pathological Mechanism and Prevention and Control Measures of Porcine Epidemic Diarrhea

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Abstract:

This article elaborateshow the porcine epidemic diarrhea virus pathogenic mechanism and harm in pigs. Porcine epidemic diarrhea is a contagious disease with different therapeutic effects on pigs of different ages. It shares common characteristics with coronaviruses, like suppressing the production and function of interferon, replicating extensively in infected pigs. In addition, there are similarities between the symptoms of porcine epidemic diarrhea virus and transmissible gastroenteritis virus, scientists devote to find the characteristics of PEDV to help farmers distinguish between the two diseases and treat the infected pigs in time to reduce loss. Porcine epidemic diarrhea is more common at the transition from winter to spring, when most piglets are born at this time. The infected pigs with various clinical symptoms including watery diarrhea, vomiting and dehydration. These symptoms lead to rapid and irreversible dehydration and death of piglets, and it has had a serious negative impact on the development of the pig farming industry. This means that the prevention and control of PEDV is necessary. Scientists collect data on the targeted structure of the spike protein in PEDV to facilitate the development of more broadly effective vaccines by focusing on studying the mechanisms of action, pathological features and prevention methods in multiple aspects. These prevention and control measures provide practical guidance for the scientific handling of the epidemic, and explore its research value in etiology and epidemiology, which is of great significance for ensuring the healthy development of the pig farming industry.

Keywords: PEDV; IFN; vaccines; reproductive functions.

1. Introduction

Porcine epidemic diarrhea virus (PEDV) is highly contagious and can affect pigs of all breeds and age groups easily. The primary clinical symptoms of porcine epidemic diarrhea (PED) include acute watery diarrhea, vomiting, and dehydration, with high lethality in neonatal and weaned piglets [1]. But the effects of this disease on pigs of different age groups show significant differences. For instance, almost all piglets die from dehydration caused by diarrhea and infections of other diseases, while adult pigs show almost no symptoms of illness, although infection can cause irreversible damage to some reproductive functions of adult pigs, especially for pregnant sows. PEDV can cause giving birth to mummified piglets or weak piglets, and even leading to miscarriage. PEDV is a kind of coronavirus that primarily infects porcine intestinal epithelial cells. Its genome consists of a single-stranded positive-sense RNA enveloped by a lipid membrane. This special genome makes it relatively prone to mutation. PEDV primarily targets and infects porcine intestinal epithelial cells. By destroying the villus structure in the intestine, it leads to malabsorption of nutrients and severe dysfunction of the digestive system. The main structural proteins of PEDV are spike (S) proteins, which contribute to the reproduction of the virus within host cells. S proteins can mediate the contact between viruses and host cells, assist in suppressing the immune response of host cells, so it can be one of the most important proteins in the whole virus while other proteins also play a role in multiplying PEDV. Other structural proteins such as the membrane (M) protein and envelope (E) protein participate in virus assembly, and nucleocapsid (N) protein binds to viral RNA to maintain genome stability. At present, PEDV is mainly detected through the specific recognition of the S proteins, which have two different parts. They are called S2 and S1.S1 is regarded as the main pathogenic site. Currently, this disease is widespread globally. The incidence of PED in major economically important pig categories (piglets, growing pigs, and finishing pigs) can reach nearly 100%, while the incidence rate of adult pigs is usually less than 30%. incidence rate difference of different groupscausing significant economic losses to the global swine industry. It also increased veterinary costs, and animal mortality cause massive economic losses to the global swine industry. Therefore, preventing and treating PED is an urgent priority in pig farming. The common treatment method is supplying nutrients, but it cannot be cured completely. Thus, the most effective method to prevent PED is vaccination, along with other prophylactic measures that can be carried out. Existing control methods constitute a comprehensive system of control strategies primarily involving symptomatic treatment, bio-security measures, and vaccine immunization, with vaccination being the most cost-effective approach for effectively reducing morbidity and mortality rates.

2. Structure and Mechanism of Action of Porcine Epidemic Diarrhea Virus

Porcine epidemic diarrhea virus is composed of four different structural proteins, including the S protein, E protein, M protein and N protein [2]. To begin with, the E protein and M protein are primarily involved in viral assembly and budding. The M protein can induce the production of interferons in the host. Besides, the N protein is mainly involved in the encapsidation of viral RNA and the release of viral particles. In contrast, the S protein is a glycoprotein located on the viral surface, serves a more direct role in initiating infection by mediating viral entry through its recognition and binding to host cells. Due to the key effects of S protein in inducing immune response and in recognizing and binding to host cells, S protein has become a primary target for PEDV. Scientists used this primary target to distinguish PEDV from other viruses, and use it for vaccine development. But the structure of S protein (glycoprotein) is highly mutable, which reduces the efficacy of existing vaccines and increases the complexity of PEDV control. S protein is a membrane glycoprotein located on the viral surface, which is composed of 1,383-1,387 amino acids (aa) that exhibits high genetic diversity. It forms trimers that appear as rod-shaped spikes approximately 20 nm in length on the viral surface. These spikes exhibit a characteristic crown-like appearance under electron microscopy, forming the distinctive feature of coronaviruses. Fig 1 illustrates this structure of S protein. Notably, there are two regions in S protein named S1 and S2 [3]. The S2 subunit mediates the fusion between the viral envelope and the host cell membrane, and the S1 subunit handles the initial step of infection by recognizing and binding to receptors on host cells [4]. And because the S1 subunit is the principal domain responsible for triggering the host's production of neutralizing antibodies, it had become a central focus for research in both virology and immunology [5].

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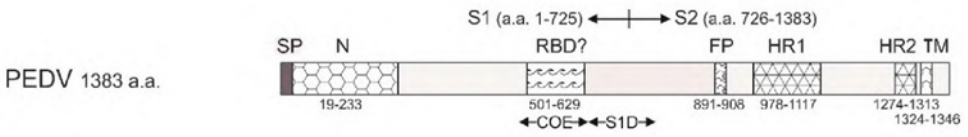

Fig. 1 Structural schematic of the S protein [2]

There are several major host factors for S protein. The first one is porcine aminopeptidase N (pAPN). pAPN is a transmembrane glycoprotein that is highly expressed in intestinal epithelial villous cells and constitutes approximately 8% of their total membrane protein [6]. The functional epitopes of pAPN can bind directly to the S protein, this will lead to the infection of PEDV. The second one is named alic acid. Sialic acid facilitates with a specific binding site on the N-terminal domain of S1 subunit, which enables PEDV to bind with hostal cells and lead to infection [7]. The last one is heparan sulfate (HS). HS is a complex and ubiquitously expressed polysaccharide found on cell surfaces and within the extracellular matrix, also serves as a significant attachment factor for the virus. Collective research focusing on these multiple receptor interactions consistently indicates that the intestinal tract and other associated digestive system organs of pigs serve as the primary in vivo targets for PEDV infection and pathogenesis.

2.1 Porcine Epidemic Diarrhea Virus mechanism of infection action

Interferon (IFN) plays a critical role in innate antiviral immunity. After a virus infects the host, the host will rapidly produce IFN to establish an antiviral response in the infected cells and the adjacent healthy cells. These interferons are classified into three types based on their protein structure. Type I, Type II and Type III [8]. Among these, both type I and type III can activate specific signal transduction. They will activate multiple interferon receptors by using transcriptional activators that bind to the interferon receptor, leading to the phosphorylation of Signal Transducer and Activator of Transcription 1 (STAT1) and Signal Transducer and Activator of Transcription 2 (STAT2) transcription factors. The phosphorylated STAT1/STAT2 dimer then associates with IRF9 to form the interferon-stimulated gene factor 3 (ISGF3) complex [9]. The main factor exerting antiviral immunity in the intestinal tract of pigs is IFN-III. Because IFN-III is the main target of PEDV, so the small intestine in pigs will be infected quickly. This infection will lead to watery diarrhea, vomiting, and dehydration, because the virus causes heavy damage to the absorbing ability of the small intestines. Another ability of PEDV is it can promote autolysis of infected cells. When the intestinal epithelial cells are infected, the cytolysin will make host cells die quickly, including the atrophy of intestinal villus and the damage of barrier function in the intestinal tract. Although host cells can produce IFN, PEDV and other coronaviruses have the ability to resist immune response by carrying redundant mechanisms. This ability can inhibit the production of interferon after infection and evade the host's immune response.

At least ten viral proteins in PEDV are IFN-I antagonists. In the CDS of PEDV (coding sequence is the DNA sequence corresponding one-to-one with the protein sequence. And not contain sequences that do not correspond to this protein in the middle, and without considering sequence changes during mRNA processing and other processes.) is expressed as nsp1, nsp3, nsp7, nsp14, nsp15, nsp16, E, M, N and ORF3. In addition, the nsp1 protein has the strongest antagonistic effect on host cells. Here are some examples. The nsp1 can directly bind to CREB-binding protein (CBP) in the cell nucleus to degrade the IFN, thereby preventing the interaction between CBP and interferon regulatory factor 3 (IRF3). By doing this, the form of IFN enhancer will be prevented and then the production of IFN will be stopped. Other CDS such as nsp3 can encode papain-like proteases (PLpro) of many kinds of coronaviruses to restrain the expression of IFN and interferon-stimulated genes (ISGs). These CDS play an important role in helping the virus attack host cells and reproduce new virus. Besides inhibiting the expression of IFN, PEDV also inhibits downstream JAK-STAT (Janus kinase-signal transducer and activator of transcription) signaling and IFN signal transduction, thereby counteracting the antiviral state induced by IFN and facilitating the establishment of infection.

3. The pathological feature of Porcine Epidemic Diarrhea Virus

The incubation period of PED is usually 5 to 8 days, and then the symptom appearance started. During this period, the porcine digestive system of pig will suffer from a series of symptoms caused by the targeted destruction of virus. The symptoms include a structural damage to the intestinal barrier and a decline in its nutrient absorption function of infected pigs. Typical pathological changes include the small intestine being filled with light yellow

fluid, the pyloric region exhibits necrotic lesions and petechial hemorrhages are observed in the kidneys. These will lead to include dehydration, vomiting, watery diarrhea, or diarrhea accompanied by vomiting, with vomiting often occurring after eating or nursing [10]. In addition, the PED shows significant age-related characteristics. For example, the suckling piglets (particularly newborns within their first week) rapidly develop profound dehydration and frequently succumb to circulatory failure within 1-3 days without prompt fluid intervention. While adult and fattening pigs generally experience a self-limiting diarrheal episode lasting approximately one week, recovered animals often exhibit subsequent growth retardation and persistently diminished feed conversion efficiency. Clinical signs include severe vomiting, watery diarrhea (the infected feces are yellowish or grayish-white with a sour odor), severe dehydration, depression, anorexia, dry skin, and sunken eyes. Infected piglets often die from dehydration and electrolyte imbalance within 3-7 days, and surviving piglets often exhibit stunted growth. In contrast, adult sows infected with PEDV show mild or even asymptomatic infections. Some infected sows may present with mild diarrhea and loss of appetite. However, infection can impact reproductive performance, leading to outcomes such as abortion, stillbirth, and mummified fetuses, as well as the birth of weak piglets and reduced milk production.

3.1 Diagnosis and main prevention and treatment measures of PEDV

Porcine epidemic diarrhea (PED) occurs predominantly in the cold and humid winter and spring seasons, typically concentrated between November and March of the following year. However, cases are frequently reported in other seasons as well. This indicates it has a trend toward non-seasonal prevalence [11]. In practice, however, outbreaks mainly occur during the climatically variable winter and spring, as the cold and damp conditions during these periods favor viral survival and transmission. In China, the high-incidence period for PED spans from December each year to April of the following year. During this interval, the number of susceptible pigs increases, and the disease tends to be more severe. PEDV can spread rapidly through pigs' contact, air, water, and other propagation modes. Once the PEDV is introduced into a pig farm, the virus can disseminate rapidly throughout the facility, leading to large-scale infection within the herd that may affect animals across different production stages. For the detection of PEDV and other swine viruses, PCR-based methods are widely used in the rapid diagnosis of livestock infectious diseases due to their speed, specificity, sensitivity, and accuracy [12]. Laboratory diagnostics for the disease encompass techniques broadly categorized as pathogen detection, immunological detection, and molecular biological detection [13]. For example, the way of pathogen detection include virus isolation and cultivation are suitable for strain identification, direct microscopic examination belongs to etiological way to observe the morphological characteristics of pathogens and colloid golden spot is a effective molecular biological way to detect target molecules. In addition, immunochromatographic-based rapid test strips (such as colloidal gold test strips) offer a simple and fast detection method, suitable for on-site diagnosis. Clinically, advanced molecular techniques such as multiplex RT-PCR are also routinely utilized for simultaneous detection of PEDV and other relevant pathogens [14]. Preventive measures are equally critical as diagnostic testing, and the prevent and control strategies are generally international standards. The prevention and control of PEDV should strictly adhere to three principles of infectious disease management. Controlling the source of infection, blocking transmission routes and protecting susceptible animals. To do this, the pig farms must implement robust biosecurity measures. Firstly, the management of human and vehicle movement should be strict. All the access points should be equipped with disinfection channels featuring ultraviolet or spray-based systems for personnel and vehicles. Secondly, the site should be built in a remote area to keep away from main roads and residential areas to hinder pathogen spread. The farm must source forage from non-epidemic areas, and any raw materials potentially contaminated with the virus must be excluded. Last but not least, the necessary supplies like veterinary drugs, vaccines, and routine tools must be disinfected before entering into production zones to prevent pathogen introduction. Furthermore, regular and comprehensive disinfection of the farm environment should be conducted to ensure effective interruption of transmission pathways. These measures can make the best of a bad job. To dispose of the infected pigs, there are several things to do. Once the sick pigs are found in the pig houses, the sick pigs should be immediately removed from the large hog lot and all suspected cases should also be strictly isolated and raised in dedicated isolation sheds. The farm management should immediately seal off the affected pigsty and prohibit the movement of people and vehicles through the main gate to prevent the spread of the disease. After that, the employees should disinfect themselves and the entrance to the safe pig houses, then forbidden to all suspected cases. During the lockdown period, the manure and waste generated must be treated in a dedicated area to eliminate the risk of pathogen transmission. The diseased pig houses and their surrounding environments should be disinfected at least once a day. The dead pigs should be

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buried or incinerated in accordance with the „Four Prohibitions and one disposal“ principle (no slaughtering, no consumption, no sale, no transportation, and harmless disposal) [15].

As no specific therapeutic agents are currently available for porcine epidemic diarrhea (PED), control strategies primarily focus on prevention rather than treatment. A variation of PEDV can influence the effects of existing vaccines. For example, pig farms can give the first vaccination of sows with the bivalent inactivated vaccines for porcine transmissible gastroenteritis and porcine epidemic diarrhea about 40 days before giving birth, and the second vaccination is 20 days later, which can increase the antibody levels in the serum and colostrum of sows, enabling piglets to obtain maternal antibodies through lactation and enhance their resistance to the virus. In addition, providing corresponding emergency vaccinations to 3-day-old and 10-day-old piglets can also effectively reduce the incidence and mortality rates. Vaccines against classic strains have poor cross-immune protection against variant strains, and there are no specific drugs to treat this disease. Because PED is a devastating and frequently occurring disease in the world, it is also a common disease with high morbidity, disability and death rates. Nowadays, common prophylactic vaccines include inactivated vaccines and live attenuated vaccines. Inactivated vaccines are prepared through physical or chemical inactivation of the virus, capable of stimulating the pig‘s immune system to produce specific antibodies against PEDV. However, they generally exhibit weaker immunogenicity and provide relatively short-lived protection. Live attenuated vaccines, developed by attenuating the virulent virus, allow controlled replication in the host to elicit robust immune responses. While demonstrating high safety profiles and strong protective efficacy, they carry potential risks including reversion to virulence and possible contribution to variant emergence. Despite these limitations, vaccination is still the most effective measure for preventing and controlling PED [16].

However, swine operations should ensure all the pigs have enough nutrition in all growth stages. When the supply of proteins, essential amino acids, vitamins, and other critical nutrients enhances is sufficiently large, the pigs can overcome disease easily. Concurrently, implementing properly timed vaccination protocols maximizes vaccine efficacy and provides cost-effective protection against PEDV. Therefore, understanding the mechanisms of PEDV and developing methods for its early diagnosis and management are critical for effective prevention and control of the disease. Timely and accurate diagnostic methods are of significant importance for the prevention and control of PEDV [17]. Currently, PED has become a major disease jeopardizing piglet production and impacting the economic efficiency of swine operations, making its control a top priority for the pig industry [18].

4. Conclusion

All in all, PEDV is a kind of coronavirus and it belongs to the RNA virus. Due to the genome being a single stranded sense RNA (lacking correction function), PEDV can mutate into different variants easily, which means people must pay more attention to find out different vaccines to prevent infection. With the development of science and technology, the new PEDV detection technology is becoming faster, more accurate and more convenient. Therefore, we are now better able to monitor the virus’s mutations. Apart from immunization and isolation methods, people can use antibiotics, vitamins and fluid replacement to help reduce mortality in the prevention and control of PED. However, the core of the treatment is to address dehydration and electrolyte imbalance, maintain the stability of the internal environment, prevent secondary infections, and promote the recovery of pigs, though treatment for PEDV cannot completely eliminate its effects (such as growth retardation) and side effects farmers know it would be difficult to undo the damage that had been done. So, conducting genetic diversity analysis of PEDV can enhance our understanding of its variation patterns is necessary. This understanding is highly significant for developing targeted prevention and control measures against PEDV, thereby effectively supporting the sustainable development of China‘s swine industry in the future. Due to the high transmissibility and high fatality rate of PEDV, PED has a strong impact on the development of the swine industry.

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