Establishment and Application of the Dual PCR Detection Method for Swine Respiratory and Reproductive Syndrome Virus (PRRSV) and Swine Pseudorabies Virus (PRV)

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Abstract

In recent years, there are more and more mixed infections or secondary infections caused by porcine reproductive respiratory syndrome virus and porcine rabies virus, which complicate the diagnosis, treatment and prevention for the rapid diagnosis of these two pathogens. Specific primer pairs were developed by sequence comparison and related literature based on the gene sequence related to the PRRSV and PRV viruses published on the official website of the National Center for Bioinformatics (NCBI). Using nucleic acids PRRSV and PRV as models, dual PCR assays were developed to rapidly identify PRRSV and PRV. The experimental results show that this method can obtain specific target fragments of 401 bp and 261 bp for amplified PRV and PRRSV and negative for amplified PEDV, CSFV and ASFV nucleic acids. Using the sensitivity experiments, the detection limits for PRV and PRRSV were 0.12 pg and 0.045 pg, respectively. The dual detection method established for PRRSV and PRV has a strong specificity and a good sensitivity, which can be used for the identification of these two pathogens.

Keywords
Pig respiratory and reproductive syndrome virus (PRRSV), pig pseudorabies virus (PRV), dual PCR detection application

1. Introduction

China is one of the first countries to domesticated wild boar into domestic pigs. After the reform and opening up, the further development of pig industry not only promoted the rise of feed industry, but also became the survival of many large-scale pig farmers and an important source of income for farmers.

In recent years, with the development of pig breeding, the occurrence and prevalence of pig diseases are increasingly harmful to pigs, and the mixed infection of several pathogens has become the main form of pig disease. Porcine respiratory Syndrome virus (PRRS) and porcine pseudorabies virus (PRV) are viruses that cause serious harm to pigs. Pig respiratory and reproductive syndrome was once known as mysterious swine disease, blue ear disease, late abortion, epidemic swine abortion and respiratory syndrome. It is caused by the PRRSV virus. PRRSV in the virus virus order, arteritis viridae, arteritis virus genus, most virus particles are spherical, all positive single-stranded RNA viruses. The disease was first found in the United States and was highly pathogenic. In 1992, the EC identified the disease as porcine reproductive and respiratory syndrome. Porcine pseudorabies is caused by PRV virus. The virus is a 150 KB linear double-stranded DNA virus. The genome contains a long (UL) and a short (US), which is extremely highly pathogenic. The emergence of these two diseases has caused huge economic losses to pig production [1].

At present, the detection methods for the above two viruses mainly include fluorescence quantitative PCR, RT-PCR...
2. A Literature Review

2.1 Porcine Respiratory and Reproductive Syndrome (PRRS)

The so-called “blue syndrome”, also known as “mystery disease” when the cause was unknown, is a disease caused by a highly capable RNA virus. The disease was first discovered in the southern United States, and then from the United States to Canada, to Germany in the 1990s, and a major outbreak in the Netherlands and throughout Western Europe. The disease first appeared in China in 1991 in Taiwan, and was also infected with the disease in Japan three years later, Chinese mainland also found PRRSV in piglets due to PPRS abortion. At present, the disease has been spread around the world, causing significant economic losses to the pig industry in various countries [2].

2.2 The PRRS pathogen

Most of the porcine Respiratory and Reproductive Syndrome Virus (PRRSV) virus particles are spherical, with a diameter of 50-65 nm and a core diameter of 30-35 nm. The icosahedral symmetry surrounds the single-stranded linear single-stranded positive RNA single-stranded [10] of the virus. There are two types of viral proteins: basic protein (N) and envelope protein (M and GP5). GP5 and M form dimers by disulfide bonds, which is required for infectivity and virus neutralization. With a genome size of 13000 – 15000 nucleotides, approximately 75% of the 5’ terminal insert is an RNA polymerase group, while the 3’ terminal poly-A contains genes encoding proteins that are infectious [3]. There are protrusions of about 5 nm on the surface, no hemagglutination activity or red agglutination in mammals or birds. They multiply in pig monocytes-macrophages (pig alveolar macrophages, peripheral monocytes, lung interstitial macrophages). Macrophages are mainly found in the tonsils, lung, and lymph nodes of the host cells. The virus proliferates in the nasal mucosa or upper respiratory system through blood circulation to invade other mononuclear macrophage systems. PPRSV is divided into North American type (amPRRSV) and European type (euPRRSV), according to genetic analysis, North American type is more likely to change than European type. At PH <5 or PH> 7, its infectious effect is reduced by more than 95% and can be preserved for 18 months at -70°C and inactivated by common drying, organic solvents and chemical disinfectants.

2.3 PRRS Epidemiology

PRRSV can infect many breeds of pigs with different growth periods and pigs of different directions, but with sows and births after pregnancy

Piglets that have grown for about a month are most likely to be infected, while the mortality rate of Suckling pigs can be 80-100% higher. PRRSV can be persistently infected, and the main source of infection is sick pigs and poisoned pigs. Once infected, the virus can spread rapidly, and can exist in the lungs, lymph nodes and serum of pigs for a long time. The pigs can be infected with contact to other susceptible pigs for 2 to 14 weeks. Adult pigs and pigs after infected may not show symptoms, but if infected pigs live in better environment, these pigs after infected will not show any symptoms, on the contrary, if living in poor environment there are other pathogens mixed infection environment, then infected pigs will show strong discomfort symptoms, there will be a lot of by dead, thus greatly accelerate the infection of the disease. If the breeding pig carries the virus, it is highly likely to be transmitted to the sow through body fluids. If the sow carries the virus, it will be transmitted to the pig at the birth. There may also be abortion in the early pregnancy and stillbirth in the middle trimester, which greatly reduces the birth rate of the pigs. After PRRSV infection, the immune function of pigs will decline, and then they will die by other viruses, resulting in the indirect cause of pig death. The infected strain has the possibility of another mutation in the circulating strain. There are two different types of PRRSV, one European and the other North American, and their differences are mainly in antigen, with little cross-reactivity. Viruses isolated from our country are also present. The mutant strain is missing, and PRRSV in our country is of the North American type [4].

2.4 Study on the diagnostic methods of PRRS

At present, the diagnostic methods for PRRS have appeared in large numbers, but most methods are only based on the symptoms shown by the sick pigs and some physiological changes.

Therefore, and to make the diagnosis more convincing, laboratory diagnostic methods play an important role in all diagnostic methods. At present, the commonly used laboratory diagnostic techniques include virus isolation and identification, serological experiment, indirect immunofluorescence test (IFA), indirect ELISA and serum neutralization test, molecular biology diagnosis (RT-PCR), etc [5].
2.4.1 Virus isolation is the most accurate method to diagnose PRRS, generally using susceptible cell isolation. At present, the cells used to isolate PRRSV are as follows: porcine primary alveolar macrophages (PAM) CL-2621 cells, and clonal strain MarC-145 of the MA-104 cell line. The um and lung tissue had the highest success rate. Vaccination inoculation with newly raised primary PAM or other passaged cell lines with characteristic CPE after passage indicated positive virus isolation. Because different isolates on cell culture growth and reproduction are different, so in the virus isolation, indirect fluorescence staining fluorescence reaction or the isolated virus into ultrathin section for electron microscopic observation, can also be used PCR method proliferation virus antigen, genetic gene identification, so as to make the results more reliable.

2.4.2 Serology is a diagnostic method widely used in laboratory, and four methods for detect PRRSV antibodies have been established at home and abroad. In 1991, the Netherlands Central General Medical Research Center Wensvoort et al. took the lead in establishing the immunoperoxidase monolayer cell test, USDA National Veterinary Service Laboratory Yoon et al. established the indirect fluorescence antibody technology (IFA) and serum neutralization test (SN). The ELISA method was established by preparing positive antigen with MarC-145 by Takirawa et al.

(1) Immunoperoxidase monolayer test (IPMA)

IPMA is the earliest serological test method for detecting PRRS antibodies, and it is still a commonly used test method in Europe. It has high sensitivity and specificity, and can detect PRRS antibodies from pigs 6 days after infection. The disadvantage is: IPMA results determine a certain subjectivity, cannot be automatically displayed, often rely on naked eye judgment, time-consuming, large-scale detection is too expensive, so it has not been widely used.

(2) Indirect fluorescent antibody test (IFA)

This method is widely used in the United States, and its specificity and sensitivity are similar to IPMA. It can detect PRRSV antibodies from animals 6 days after infection, and can be used for antigen detection, virus identification and serum antibody detection of PRRSV. IFA can be performed on PAMCL-2621, MA-104 and MarC-145 cultures, with serum antibody titer was positive at 1:20, with serum antibody titer> 1:64 as the marker of PRRSV activity. The disadvantage is that the experimental results are judged by naked eye observation, strong subjectivity is not conducive to automatic operation, not suitable for large-scale detection, so the use is limited to a certain extent.

(3) Serum neutralization test (SN)

This method has good specificity and can distinguish between different PRRS virus strains. However, microcell culture, which requires a large workload, is highly technical: time-consuming, laborious, subjective, and antibody appears late, so it is not suitable for early diagnosis and limited to laboratory research applications.

2.4.3 Molecular biology diagnosis

Molecular biology technology has become an indispensable disease diagnosis method in modern medicine. People began to use monoclonal antibody technology, polymerization, enzyme chain reaction (PCR) technology and nucleic acid probe technology to conduct molecular diagnosis and typing of PRRS, and to reveal the basis of antigen variation and the evolution process of virus strains from the molecular level [6].

2.5 Pseudorabies (PR)

PRV caused by pseudorabies is also known as swine herpesvirus type I, infectious oblongla palsy virus, pruritic virus, oyeyiki disease virus. It enables our ordinary domestic animals and other animals to have hot, itching (except pigs) and encephalomyelitis symptoms and have the ability to spread quickly, which has a strong ability to infect other animals. The origin of the disease is that the symptoms of infected animals are similar to rabies. So the disease is called "pseudorabies".

2.6 PR pathogen

Pseudorabies virus belongs to the herpesvirus family, the swine herpes virus type I of the alphaherpes subfamily, most virions are round or oval in shape, butSerotypes often have only one and one or somewhat different viruses, complete virions have a capsule, and the genome is linear dsDNA. Pigs have a good tolerance to PRV, so they are the main infectious source and a good host of PRV. PRV has a strong living ability in adversity and can be inactivated at three different temperatures and different time conditions, including inactivation for 50min at 55℃ and 3min, and can be inactivated instantly at 100℃. The best condition for the stable existence of viruses is low temperature, high humidity and PH in low temperature and humid environment. The capsule shell of PRV has a variety of proteins, among which gb, gc, ge, three proteins that can induce antibodies in pigs can be used as the basis for detection [7].

2.7 PR Epidemiology

The disease can occur throughout the year, especially prone to outbreaks during childbirth. PRV virus can be transmitted through the pig saliva, excreta, pigs can through direct or indirect contact with pigs and infection, but also can be eaten contaminated by PRV feed through the digestive system infection, the characteristics of pig pseudo rabies is a
latent phenomenon, detoxification of long time not only increase the chance of infection and will reduce the alert, which creates the conditions for the widespread spread of PRV [8]. PRV can also by pig breeding reproductive system infection, when pregnant sows were infected with the virus, likely through the placenta directly to belly pig, serious cases will appear miscarriage, pig in abdominal death, signs of very weak fetus even mummy, which will cause the decline of newborn pig quantity quality, directly cause serious economic losses.

2.8 Study of the diagnostic methods of PR

Pig pseudorabies is one of the common infectious diseases in pigs. It has a long incubation period and does not show specific clinical characteristics. During the onset of pig pseudorabies, its immune system is destroyed, and it will suffer from mixed infection by Escherichia coli and streptococcus, but mainly the pig pseudorabies virus will play a leading role [9]. At present, the commonly used diagnostic methods in the laboratory are pathogen virus isolation and identification, enzyme-linked immunosorbent test (ELISA), serum neutralization test (SNT), immunofluorescence technology (FA), latex agglutination test (LAT) and agar diffusion test, etc., molecular biology technology.

References


