CHAPTER 07

PATHOGENESIS AND PREVENTION OF PORCINE CONTAGIOUS PLEUROPNEUMONIA

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INTRODUCTION

Porcine infectious pleuropneumonia has brought significant economic losses to the pig industry, so it has been listed as one of the important diseases endangering the pig industry in the world. After the pigs are infected with APP, they can be divided into acute and chronic diseases according to their clinical course. It mainly exists in the lungs and tonsils of diseased pigs, and pigs are the only host. All breeds and day-old pigs can be infected with the disease. Three to five months old pigs are susceptible to the disease, and adult pigs often show recessive process. The disease can occur all year round, such as winter and spring cold season or climate change season. The disease can be easily induced by high density of pigs, poor ventilation of piggery, poor sanitary conditions, or by transport, mixing, long-distance transportation, weather mutation and other stress (Blackall et al. 2002). The disease spread mainly at the respiratory level but can also spread by direct contact. Infected pigs will spread APP into the air when they cough or exhale and infect other healthy pigs through air droplets. In the acute stage, pig secretions contain a lot of pathogens, which can be carried by pig farm staff through clothes, shoes or tools.

The disease usually occurs in sporadic or small-scale outbreaks. In the acute outbreak stage, the disease can spread by "jump" in fat pig herds, with high incidence in large-scale intensive pig farms. The morbidity and mortality were different in different pig farms and different strains. The morbidity and mortality were higher in pigs with the first disease and tended to moderate after a period of time. The morbidity was generally 8.5-100% and the mortality was about 0.4-100%. Acute pleuropneumonia is characterized of dyspnea, frequent standing or sitting posture, foam-like secretions on the nose and mouth, blue-purple skin on the ears, nose and extremities, temperature up to 41.5℃, and high mortality (Rossic et al. 2013). Cellulosic necrotizing tinea plantar pneumonia is a typical symptom of chronic pleuropneumonia. Pigs with chronic pleuropneumonia may experience slow growth, but the mortality rate is low.

Characteristics of *Actinobacteria pleuropneumoniae*

Actinobacteria pleuropneumoniae (APP) is typically a rod shape, with capsule or slender small bacteria, polymorphism. The bacteria in the material can be bipolar color, facultatively anaerobic, with no spore formation and no motility. APP currently has a total of 15 serotypes, which can be divided into biological type I and biological type II based on the growth dependence on NAD (Nicotinamide Adenine Dinucleotide, nicotinamide adenine dinucleotide, also known as v factor). Except for serotypes 13 and 14 belonging to organism II with NAD-independent growth, the remaining 12 serotypes were NAD-dependent organism I and all associated with porcine pathogenesis.

Biological Characteristics

The main pathogenic factors of APP include Apx exotoxin, ApxI, Apx, capsular polysaccharide, lipopolysaccharide, outer membrane proteins, and transiron-binding proteins.

Exotoxin

At present, there are four kinds of Apx exotoxins found in APP: ApxI, ApxII, ApxIII and ApxIV, which all belong to the family of cell pore-forming proteins, with leukocytes to toxicity and hemolysis, which can damage the host defense mechanism and cause damage to host cells and tissues. Apx high concentration of exotoxin can perforate the cell membrane of phagocytes and other cells, which can effectively escape the relevant defense mechanisms and cause cell swelling and death due to altered osmotic pressure.

The major virulence factor of APP pathogenesis is its extracellular exotoxin-Apx toxin. Apx is a substance that acts on alveolar macrophages and suppresses the phagocytic activity of alveolar macrophages, which is thought to be one of the major causes of infection and severe damage to the lung tissue. Apx toxins, include ApxI, ApxII, ApxIII and ApxIV (Bode et al. 2003). ApxI can cause the disease to develop typical lung lesions, which is also the strongest virulence factor causing infectious pleural pneumonia in pigs. Apx is secreted by all serotype APP, and the toxin has so weak virulence that Apx expression is only induced by APP in animals, but not on any other medium. This is the only gene found in APP which can be expressed *in vivo*. Apx has strong specificity and does not cross-react in species genera closely associated with *Actinobacter pleuropneumoniae*. Once the pig is infected with APP, any serotype can respond to the expressed Apx. If

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biologically active Apx toxin, four genes CABD operon are required in certain order. C gene encodes toxin structural protein, A gene encodes toxin-activated protein, translational protein products of B gene and D gene produce transmembrane channels on the cell wall, responsible for the toxin from cell to cell (Qian et al. 2002).

Characteristics of ApxI

Apx I, expressed and secreted by serotypes 1,5,9,10 and 11, has strong hemolytic activity, and strong cytotoxic effects on alveolar macrophages and neutrophils, and is one of the potent protective antigens for APP (Meyns et al. 2007). The production, activation, and secretion activity of ApxI is regulated by an operon that includes the above four complete CABD genes: ApxIC, ApxIA, ApxIB and ApxID, a typical operon of RTX toxin, and the product of ApxI is responsible for secretion to extracellular (Nkando et al. 2012; Li et al. 2022). Serotypes I, 5a, 5b, 9, 10 and 11 are clinically APP outbreak serotypes with severe lung injury and high mortality, and strong hemolytic and cytotoxic ApxI because they have intact ApxI, while serotypes 2,4,6,7,8 and 12, although not producing ApxI, have a truncated ApxI retaining the original promoter whose expressed products have secretory functional (Nielsen R et al. 2000). It can be seen that ApxI toxin plays a dominant role in most serotypes. But the diagnostic method of ApxI is poorly specific and it is difficult to detect all serotypes of APP and it cross-reacts with other *Actinobacterium* (Liu et al. 2009). For example, the homology of ApxI with *Escherichia coli* HlyA and *Pasteurella multocida* was 60% and 44% (Wei et al. 2012) Therefore, the accuracy of the diagnosis and treatment of the disease is very difficult. In the early 20th century, the genetically engineered expression product of ApxI was applied to subunit vaccines, and many researchers studied its protective pyrogenicity, believing that the expression product is not only protected against isoforms, but also resistant to some extent. It has been shown that the expression of ApxI protein N-terminal by *E. coli* can basically achieve the immune effect of ApxI, laying a foundation for the study of subunit vaccine (Fang Xie et al. 2010.).

Characteristics of ApxⅣ

Apx was first identified in 1999 and was only expressed *in vivo*. In 1997, Anderson identified CM5, a sequence similar to the meningitis FrpA and FrpC genes, appearing downstream of the serum type 1 LacZ gene, suggesting the possibility of a fourth Apx toxin gene in the APP (Shin et al. 2011). A suspected fourth Apx toxin was identified by Sealler in 2014, both by gene sequencing and by gene expression, and it was named as ApxIV (Liu J et al., 2009.). Unlike ApxI, ApxII, and ApxIII, which have weak virulence and are not detected *in vitro* culture (Rossic et al. 2013), ApxIV is the only gene which was only expressed *in vivo* in APP. Because the operon structure is different from the first three toxins of Apx, the ORFI gene is immediately upstream of Apx A, but its expression products are functionally similar to Apx C and are also responsible for the activating of Apx (Nkandoi et al. 2012). The structure of the Apx has the similar characteristic of the RTX toxin (Bradford 2005.). Thus, Apx is secreted by all serotype APP, and no other strains in the Actinobacter genus secrete Apx, demonstrating that other *C. elegans* other than pleuropneumoniae lack of the Apx gene and have strong interspecies specificity (Frey et al. 2003.) but not by ApxI, ApxII, and ApxIII. Due to the specificity of Apx, the high specificity and conservation of the 442bP fragments are used as the target genes to detect APP. Domestic APP vaccines are mostly toxin-free inactivated seedlings, combined with Apx toxin expression characteristics only in the host, can distinguish between immune animals and wild virus infection (Shin M et al., 2011). Other researchers reported the establishment of the Apx-ELISA method, so that the Apx antibody cannot be detected in the APP carriers without infection symptoms (Rossic et al. 2013). This is also in line with the study showing that no serum cross-reactive was present between antibodies to the recombinant Apx A protein and other Apx toxins, and no Apx antibody was detected from both the other standard positive serum and whole APP inactivated seedlings, but Apx antibody was detected from the serum of animals infected with live APP (Bode et al. 2003; Li et al. 2022). The ELISA method established with recombinant Apx protein in Previous reports have suggested that APP could detect antibodies to Apx in animals, but not in animals immunized with inactivated seedlings (Christensen 1982). This indicates that Apx is not only species-specific and can only be used for the detection of APP infection, but also can be applied to differential diagnose of whole-inactivated vaccine or subunit vaccine from naturally infected pigs, so this protein is the best candidate antigen for the diagnosis of APP infection. However, Apx alone is difficult for the differential diagnosis of infectious pleural pneumonia in pigs. Apx antibodies can be detected in recovered pigs naturally infected with each serotype, and pigs recovered after infection can resist all toxins. Of course, Apx is also an important part of them. Some studies have shown that Apx can pass through thorns.

Persular Polysaccharide

Capsular polysaccharide (CP) is the main component of the extracellular membrane of the bacterial cell wall, with CP in all APP serotypes, mainly composed of sugars. Persular polysaccharide is one of the necessary virulence factors of porcine infectious *C. thyuropneumoniae*, and its virulence is determined by the type (Bradford 2005). It protects the bacterium from phagocytosis, and its specific antibodies can opsonize on Actinobacteria of *C. pleuropneumoniae*. The number and type of capsular polysaccharide and the secretion mechanism are directly related to APP pathogenicity, which shows that capsular polysaccharide is the factor affecting APP virulence.

Lipopolysaccharide

Lipopolysaccharide (LPS) is a surface antigen substance and a receptor for many phages, which is toxic to the host. As one of the virulence factors of Actinobacter, purified LPS can activate some blood clotting factors, which can induce blood agglutination and fibrinolysis, leading to tissue necrosis. Meanwhile, LPS acts together with the exotoxin to strengthen the toxic effect of Apx exotoxin on phagocytes, and LPS is also associated with APP adhesion to the trachea and pathological changes in the lungs. LPS is immunogenically weak. LPS is only resistant to disease and is not disease-resistant, so LPS only provides protection against APP attacks.

Exterior membrane Protein

External membrane protein is a very special but very important protein. Outer Membrane Proteins (OMP) is the main

structure of the outer membrane, is the direct permeability of cell membrane and efflux pump system, and plays an important role in ensuring material transport (Kamp et al. 2012). At the same time, OMP has stable structure of bacterial external membrane, which can adapt to the intracellular environment and resist tracellular sterilization and other important role, and is closely related to bacterial virulence. It can regulate phagocyte function, activate cellular actin production, and was first identified in the immunogenic and antigen-protective in the 1980s.The main antigen involved in immunity is 17 KD peptidoglycan-related lipoprotein, and 32 and 42KD proteins, which expressed an outer membrane protein PalA. It is immunogenic. But later studies showed that antibodies produced by this protein have negative effects on some serotype antibodies and therefore cannot be used as a vaccine component (Bagdasarian et al. 1998). It was believed that this may be one of the reasons for the unstable quality of some bacterial virulence.

Transgenic Iron-binding Protein

Transferring binding proteins (Tbp) is a transmembrane glycoprotein on the surface of most bacteria. However, iron is also a necessary material for bacterial growth. Under normal circumstances, all the iron ions required for cell metabolism have Tbp transport (Leiner et al. 1999). It has been shown that a part of bacteria in the Actinobacteria population have a mechanism to obtain iron. That is, transiron binding protein exists on its surface. When iron is absent, its receptor is expressed, thus helping bacteria to absorb iron from the animal body to meet the needs of bacterial growth and reproduction. The specific transiron binding protein located in the vitro membrane of APP bacteria is mainly composed of binding proteins A (TbpA) and B (TbpB). The bacteria can uptake iron ions, and Batles et al. confirmed that these two genes are virulence factors (Nielsen et al. 2000). In addition, Wang Fang used molecular biotechnology to amplify TbpB from five lines of APP, and cloned straight *E. coli*, providing technical help for the development of a new vaccine for infectious pleural pneumonia in pigs.

This bacterium is a facultative anaerobe. The optimum growth temperature is 37℃. It does not grow in the common medium, and V factor is needed to be added for the bacterium to grow. Under the condition of 10% CO2, myxoid colonies could be formed and cultured on chocolate AGAR for 24-48h to form opaque pale gray colonies with a diameter of length around 2mm. Two types of colonies can be formed. One is round, hard "waxy", and sticky. The other is a flat, soft, shining colony. Strains with pods can form rainbow colonies on AGAR plates. A-β-hemolytic ring is usually produced on AGAR plates of bovine or sheep blood. The hemolysin produced by *Staphylococcus aureus* had a synergistic effect with the β-toxin of *S. aureus*. That is, *S. aureus* could enhance the hemolysis of *S. aureus*, and CAMP reaction was positive.

The bacteria are not strong resistant to the outside world. It is sensitive to common disinfectants and temperature. General disinfectant can easily kill it, at 60℃ 5-20min. It usually survives for 7-10 days at 4℃. Pathogens that are not resistant to desiccation and are released into the environment are very weak, whereas pathogens in mucus and organic matter can survive for several days. It has certain resistance to crystal violet, bacitracin, lincomycin and spectacular mycin. It is more sensitive to tetracycline antibiotics such as oxytetracycline, penicillin, tylosinin, sulfadiazine, cephalosporin and other drugs.

Clinical Diagnosis and Symptoms

The natural infection incubation period of this disease is 1-2 days, and the clinical manifestations of pigs vary with the immune status, environment and management of pigs (Klausen et al. 2007). According to the length of the course of the disease, it is usually divided into four types: (1) the most acute, (2) acute, (3) subacute, and (4) chronic.

Regarding the most acute type, the following symptoms are specified. The body temperature of the pig rose to 41-42℃. The patient animals showed depression, loss of appetite, short-term diarrhea and vomiting. The patient animals also show cyanosis of skin of nose, ears, legs and sides, later severe dyspnea, mouth breathing, spasmodic cough, and sitting-dog position. There was a large amount of blood-colored foam discharge from the nose and mouth prior to death. Death usually occurs within 1-2 days. Some cases have asymptomatic and sudden death. The case fatality rate is up to 80-100%.

Regarding acute type, the following symptoms are specified. The sick pig spirit is listless, has appetite waste, temperature up to 40.5-41℃, cough, painful mouth and tongue, and abdominal breathing. Because of the different breeding and management conditions, the length of the disease is different, which can be subacute or chronic type.

Regarding subacute type and chronic type, the symptoms are no fever or mild fever, lack of energy, reduced feed intake, abnormal breathing, cough or intermittent cough, slow growth. The course of disease can last from a few days to two weeks (Christensen 1982). If the environment is good and there are no other complications, it can survive, but the disease has a certain impact on weight gain, and if other diseases are secondary, death is inevitable.

Pathological Changes

The Most Acute Type

Main pathological changes occur in respiratory tract, with bloody nose and outflow liquid, and tracheal and bronchial hemorrhagic secretions of foam samples. Pneumonia is more bilateral. The lesion area mainly includes the leaves, and part of the diaphragmatic leaves. There are clear boundaries between lesions and surrounding healthy tissue. There are lung congestion, hemorrhage, edema, a dark red or purple area, with the quality of the material to be solid. There is pink foam sample liquid when cut out (Fig. 1 & 2).

Acute Type

There is light red fluid in the chest, cellulose exudate on the lung and pleura surface, some adhesion of cases of lung and pleura, lung congestion, bleeding, and edema. The lung has solid texture, clear outline, purplish red lesions, obvious surrounding fibrosis and sections like liver sections. There is the trachea and a fibrinous exudate with blood-colored foam in the bronchus (Fig. 3).

Subacute or Chronic Type

The changes include lung serosal membrane and chest wall uneven thickening, a large number of small nodules protruding on the lung surface and internal. The lung surface has cellulose attached, lung serosal membrane and chest wall and pleura adhesion.

Fig. 1: Lung congestion.

Fig. 2: Bloody nasal discharge.

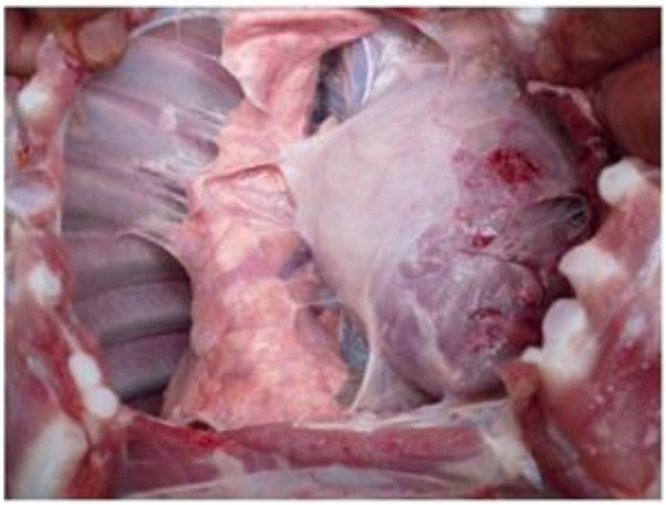

Fig. 3: The lungs are fused with the ribs.

Autopsy is difficult to separate. There are numerous purulent small nodules in the diaphragm surrounded by thick connective tissue. Arthritis, endocarditis, meningoencephalitis and other parts of the abscess can also be seen in varying degrees. This type of disease has a long course of the disease. Pigs have yellow-green spots on the pleura.

Laboratory Inspection

The diagnosis of the disease can be based on traditional methods of epidemiological investigation, clinical symptoms and pathological changes at autopsy, combined with laboratory diagnosis. Common laboratory diagnosis methods include the following five methods:

(1) Bacterial isolation and identification. Lung disease, heart, peritoneal effusion and nasal secretions of diseased and dead pigs were inoculated with lipid plate or chocolate plate and cultured overnight at 37°C. Waxy colonies were observed. *Actinobacillus pleuropneumoniae* inoculated in *Staphylococcus aureus* culture showed satellite appearance and typical β hemolysis. Combined with related biochemical examination, it was confirmed as pleuropneumoniae.

(2) Complement binding test uses the immune hemolysis mechanism as an indicator system to detect antigens or antibodies of another reaction system. In 1971, Nicolet established the complement binding test (CFT) method, which was improved by Lombin. In 1982, this method was internationally recognized as the standard method for APP detection. In 1990, Zhu Shisheng et al. made it convenient for the diagnosis of pleural pneumonia in pigs by freeze-drying multiple serotypes of antigens mixed with full-cell antigens in a certain proportion (Lara et al. 2008).

(3) Indirect hemagglutination test. It is to wrap antigens (or antibodies) on the surface of red blood cells to become sensitized carriers and then combine with corresponding antibodies (or antigens) to make red blood cells together and produce visible agglutination reaction. Mittal established indirect hemagglutination test (IHA) for APP detection and serological typing, which is fast and sensitive (Meyns et al. 2007). The method for APP detection can effectively distinguish serum type 4 from serum type 7.

(4) Agglutination test. Agglutination test (AA) is a simple and rapid method for APP detection. The direct antigen and corresponding antibody combine to agglutinate, which can be used for serotyping. The test includes tube agglutination, glass agglutination, co-agglutination, etc.

(5) Other diagnostic methods. Latex agglutination test (LAT) is a simple method. Immunodiffusion assay (IDT) is a classical immunological method with strong specificity (Liu et al. 2009).

Treatment

There are many causes of infectious porcine pleuropneumonia, so comprehensive prevention and treatment are appropriate. First of all, we should improve the level of feeding management, maintain piggery hygiene, good ventilation, regular disinfection, and achieve reasonable feeding density, in the process of feeding, and should also minimize various stress factors. Secondly, regular injection of vaccines, including inactivated vaccine, subunit vaccine and attenuated vaccine, can prevent APP infection to varying degrees, reduce the mortality rate of infected pigs and have certain protective effect, but it cannot reduce morbidity and chronic infection rate. Adhere to self-breeding, and try to reduce the introduction of diseases. In addition, standardized introduction procedures should be adopted. After the purchase of pigs from other places, some work should be conducted including isolation, and inspection, to ensure that no disease can enter the feed. For attenuated vaccine, although it has partial toxicity, which can easily cause disease and potential toxicity in inoculated animals, and the

accurate usage of attenuated vaccine cannot be controlled, attenuated vaccine has a good market prospect at present. Attenuated vaccine can be continuously screened to minimize virulence and have good immunity (Nielsen et al. 2000). Data show that attenuated vaccine nasal drip immunization can achieve good immune effect, and animals can also be inoculated by inhaling aerosol with APP through airtight fogging device, so as to achieve better infection effect (Huang et al. 2005). In addition, pigs of different age should be raised in different groups, and all pigs should be brought in, and all pigs should be brought out to reduce the probability of disease infection. At the same time, active drug prevention should be carried out to achieve better results as far as possible. Tallosin, cephalosporins and tetracycline antibiotics are more sensitive to *Actinobacillus pleuropneumoniae*, and appropriate drugs can be added to drinking water and feed for early prevention of pigs without disease, so as to effectively control the occurrence of the disease (Wei et al. 2012). Practice has proved that the use of such drugs has a certain degree of resistance, so in the process of use, it is necessary to frequently use different drugs, and do drug sensitivity test in advance, in order to conduct the prevention and control work as efficiently as possible.

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