ISSN 2616-5910 Vol. 4, Issue 1: 14-19, DOI: 10.25236/AJALS.2023.040103

Diagnosis and Treatment of a Case of Porcine Asthma

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Abstract: *Porcine asthma is a highly contagious respiratory infectious disease that poses significant challenges in disease control. It spreads rapidly within pig herds and can contaminate the surrounding environment, posing a threat to the health of other animals. Clinical manifestations of porcine asthma include rapid breathing, nasal discharge, wheezing, and coughing, with severe cases potentially leading to pig mortality. Furthermore, the virus can potentially transmit between humans and pigs, posing a latent threat to human health. Porcine asthma inhibits pig growth, reduces meat and other livestock product yields, resulting in economic losses in the livestock industry. Effective diagnosis and treatment are of utmost importance to mitigate economic losses in the swine industry. This paper aims to provide insights into the incidence and epidemiological characteristics of porcine asthma, analyzing and summarizing clinical symptoms and pathological changes. Drawing upon previous research experiences, laboratory diagnostic methods and treatment protocols for porcine asthma are introduced, along with a brief overview of their current research status, advantages, and limitations. This study aims to offer assistance to future diagnosis and treatment of porcine asthma in the swine industry.*

Keywords: *Porcine asthma, Clinical symptoms, Diagnostic methods, Treatment, Prevention*

1. Introduction

In recent years, the scale of pig farming industry has been expanding rapidly. However, the emergence of various diseases has led to severe economic losses in pig farming, and porcine asthma is one such disease. Porcine asthma, also known as Mycoplasma pneumonia in pigs, is a highly prevalent and contagious chronic respiratory infectious disease caused by infection with porcine Mycoplasma pneumonia [1]. Porcine asthma exhibits a global distribution and can occur throughout the year. It can infect pigs of different ages, breeds, and genders. Although the disease has a relatively low mortality rate, it significantly impacts the growth and development of pigs, leading to decreased feed conversion efficiency and productivity. The primary mode of transmission is through respiratory infection, with the pathogen being released into the external environment through sneezing, breathing, and coughing. It spreads through aerosols and droplets, with infected and carrier pigs serving as the main sources of transmission. Improper husbandry practices and lax quarantine and vaccination measures can also contribute to the spread of the disease. Additionally, infection can occur through the gastrointestinal tract, conjunctiva, and placenta[2]. Research has found that porcine asthma is prone to secondary mixed infections, such as porcine reproductive and respiratory syndrome virus (PRRSV), streptococcus, Pasteurella multocida, and circovirus, leading to more complex clinical symptoms in pigs and a significant increase in mortality rates [3]. The disease primarily affects the lungs and trachea of pigs, severely impairing the respiratory system. The main clinical symptoms include dyspnea or abdominal breathing, wheezing accompanied by coughing, lethargy, and reduced appetite. During post-mortem examination, affected pigs typically exhibit a "cooked shrimp" appearance in their lungs, which aids in preliminary diagnosis. In order to promote the better development of the pig farming industry, effective diagnostic methods and scientifically-based treatments are crucial tasks in today's swine industry. Based on the analysis of the pathogen, epidemiological characteristics, and clinical features of porcine asthma, this study draws upon previous research experiences and summarizes relevant information. By doing so, we have developed rapid and widely applicable laboratory diagnostic methods and scientific treatment measures for porcine asthma. The aim is to provide assistance to the pig farming industry in China, reducing losses and maximizing the benefits of pig farming.

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2. Mechanism and Etiology of Porcine Asthma Pathogenesis

The pathogen responsible for porcine asthma is Mycoplasma hyopneumoniae (Mhp), also known as a pleomorphic microorganism [4]. Mhp possesses unique external structures and lacks a cell wall. It is a facultative anaerobic bacterium composed of DNA, ribosomes, and cell wall components. Mhp exhibits diverse external morphologies, commonly appearing as spherical, rod-shaped, or filamentous forms, with occasional pear-shaped, ring-shaped, and helical structures observed. Gram staining of this bacterium yields a negative result, although its visualization is less effective compared to Giemsa or Wright staining [5]. When pigs become infected with Mycoplasma hyopneumoniae, the pathogen initially enters the respiratory tract by adhering to the mucous membranes of the oral cavity. The pathogen primarily attaches to the cilia and subsequently invades and reproduces, leading to the destruction of ciliary tissue. This disruption impairs the clearance of foreign substances, facilitating further penetration of the pathogen into the mucosal layer and triggering systemic infection.

1) The inadequate awareness of biosecurity measures is observed in certain pig farms. Some farms fail to conduct timely quarantine inspections upon acquiring new pigs and mix them with existing herds, or neglect regular check-ups. Moreover, certain personnel involved in quarantine procedures evade their biosecurity responsibilities through various means. These factors contribute to the introduction of pathogens into pig populations.

2) Some pig farms only administer vaccines for other contagious diseases to their herds, neglecting the vaccination against Mycoplasma hyopneumoniae, making the pigs more susceptible to the disease. In the case of free-range pig farming households, some lack awareness of epidemic prevention knowledge. Due to limitations in manpower, financial resources, and other factors, epidemic prevention measures are not implemented properly, resulting in cross-infection within the pig population and the spread of pathogens.

3) Improper farm management practices and inadequate handling of deceased pigs contribute to the spread of pathogens. Factors such as poor sanitation conditions in pig pens, inappropriate temperature and humidity levels, inadequate ventilation, poor air quality, and substandard or moldy feed can lead to decreased resistance in pig populations, providing opportunities for pathogenic microorganisms to invade. Furthermore, the improper disposal of dead pigs, such as indiscriminately discarding them into rivers, instead of implementing appropriate disposal methods, exacerbates the transmission of pathogens.

3. Diagnostic Methods for Porcine Asthma

3.1 Epidemiological Diagnosis

Porcine asthma infections are not limited to specific pig breeds, ages, or genders. However, higher incidence rates are observed among neonatal and post-weaning piglets, as well as among pregnant and lactating sows. Porcine asthma can occur throughout the year, with higher prevalence observed during the winter and spring seasons due to lower temperatures affecting the environmental conditions of pig pens [6]. The disease exhibits an acute course and presents as an outbreak in newly affected areas, while in long-established regions, it manifests as a chronic or latent infection. Porcine asthma is a global respiratory infectious disease in swine, and China is one of the world's largest producers and consumers of pork. As a result, porcine asthma has had a significant impact on China's livestock industry. Since 2019, China has experienced multiple porcine asthma outbreaks, resulting in widespread infections and fatalities, as well as triggering issues such as increased pork prices. Porcine asthma is prevalent worldwide, with outbreaks reported in countries such as Europe, the United States, Japan, and South Korea. In Southeast Asia, porcine asthma has a higher incidence, with more severe outbreaks documented in countries like the Philippines and Vietnam. In North America, Canada and the United States have also faced porcine asthma outbreaks, although timely and effective measures have helped control the situation.

3.2 Clinical Symptom Diagnosis

Porcine asthma can be classified into acute and chronic forms based on the duration of the disease course. This classification distinguishes between two distinct patterns of disease progression. Acute forms of porcine asthma primarily occur in newly established pig farms, with a typical disease duration of approximately 1 to 2 weeks. Affected pigs exhibit a rapid onset of symptoms, including lethargy and increased respiratory rate. Generally, there is no significant increase in body temperature, although secondary infections may result in elevated body temperatures [7]. The acute form of porcine asthma is associated with a higher mortality rate.

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Chronic forms of porcine asthma predominantly occur in long-established endemic areas and among older age groups of pigs. This type of pneumonia also manifests with respiratory symptoms, with coughing progressively worsening as the disease progresses, eventually presenting as persistent spasmodic coughing. Some pigs may exhibit difficulties in standing or a hunched back posture. In the absence of secondary infections, body temperature does not show a significant increase. However, chronic pneumonia can adversely affect the growth and development of pigs [8].

3.3 Pathological Diagnosis

Pathological diagnosis plays a critical role in confirming the presence of porcine asthma and understanding the underlying pathological changes. This paragraph provides an overview of the key components and methods involved in pathological diagnosis.

Gross Pathology: Gross pathology involves the macroscopic examination of organs and tissues during post-mortem examinations. In cases of porcine asthma, affected lungs may exhibit characteristic gross pathological changes. These changes include areas of consolidation, discoloration, and increased consistency. Additionally, the presence of excess mucus, fibrin, and inflammatory exudates in the airways and bronchioles may be observed.

Histopathology: Histopathological examination involves the microscopic evaluation of tissue samples collected during necropsies. In porcine asthma, characteristic histopathological findings include inflammatory cell infiltration, primarily lymphocytes and plasma cells, within the bronchial walls and alveolar septa. These infiltrates may lead to epithelial hyperplasia, destruction of cilia, and glandular hypertrophy. Other notable features may include peribronchial and perivascular lymphoid follicle formation and the presence of inflammatory exudates.

Immunohistochemistry (IHC): Immunohistochemistry is a valuable tool in the pathological diagnosis of porcine asthma. It involves the detection of specific antigens using labeled antibodies. Immunohistochemical staining techniques can identify the presence of Mycoplasma hyopneumoniae antigens within lung tissues, providing confirmatory evidence for the diagnosis of porcine asthma.

3.4 Serological Diagnosis

Serological diagnosis plays a vital role in the identification and confirmation of diseases through the detection of specific antibodies or antigens in serum samples. This paragraph provides an overview of the key components and methods involved in serological diagnosis.

Indirect Hemagglutination Assay (IHA) is a commonly used diagnostic method in the laboratory, known for its good specificity and ease of operation. Donwdle et al. were the first to utilize this assay to detect antibodies against Mycoplasma hyopneumoniae (Mhp) and observed hemolysis of sheep red blood cells induced by serum from affected pigs. However, the use of pig red blood cells instead of sheep red blood cells in this indirect detection method results in lower titers compared to those obtained using sheep red blood cells, leading to discrepancies between the test results and the actual infection status. As a result, the clinical application of this method is limited [9]. Zhang Shunfeng [10] employed an IHA test using sensitized sheep red blood cells with ultrasonic disruption antigens at a protein concentration of 50 μg/ml to evaluate the serum samples of 36 healthy pigs and 48 pigs affected by MPS. The results showed that all healthy pigs tested negative, while the detection rate for MPS-infected pigs was 97.7%. Thus, the IHA assay demonstrates a high level of sensitivity.

Fluorescent Antibody Test (FAT), first reported as early as 1970, has been utilized for the detection of Mycoplasma hyopneumoniae (Mhp). In 1971, Earlier employed the direct fluorescent antibody test to examine rabbit meat broth inoculated with Mhp. Subsequently, both the direct and indirect fluorescent antibody tests were modified and improved. Amanfu et al. utilized the modified FAT technique to detect Mhp in pigs experimentally infected with the pathogen. The results revealed the presence of Mhp in the bronchioles and bronchial epithelium of affected pigs from 2 to 12 weeks post-inoculation, with the strongest fluorescence observed at 4 to 6 weeks, gradually weakening from 8 to 12 weeks. The application of the indirect immunofluorescence technique in frozen section detection has been implemented in Danish specific pathogen-free pig herds [11]. The FAT technique is particularly suitable for the detection of Mhp acute infections when a significant amount of the pathogen is present in the lungs of affected pigs. However, when the acute form transitions to the chronic or latent form, false negatives may occur due to insufficient levels of the pathogen in the serum.

Complement Fixation Test (CFT) is a commonly used serological diagnostic method for detecting

Mycoplasma hyopneumoniae (Mhp) antibodies and is known for its high sensitivity. In the field of pig farming, it is frequently employed. When conducting CFT testing on affected pigs, it is necessary to combine clinical symptoms and pathological changes to make more accurate and effective judgments. Although experimental results have shown a good correlation between the positivity rate in affected pigs and lung lesions when using CFT for testing, inevitable false-positive or false-negative results may still occur [12]. Therefore, the CFT test has certain limitations in terms of sensitivity and specificity.

Immune Enzyme Assay (IEA), Due to the limitations of fluorescent antibody techniques in detecting acute Mhp infections and the inability to preserve fresh frozen tissues, fluorescence microscopes, and samples required for the tests, researchers have found that immune enzyme assay technology can overcome these drawbacks. When using this method to examine tissue smears of affected organs, irregularly shaped particles with a light reddish-brown color, indicative of the presence of Mhp, can be observed [13]. Doster et al. fixed and embedded the pig lung lesions in formalin and paraffin, respectively, for long-term sample preservation. The use of IEA enables clear visualization of the Mhp structures under a microscope [14] [22]. This method can be employed when fresh samples of affected pig tissues are unavailable. IEA detection technology is more sensitive than fluorescent antibody techniques and can be used to detect Mhp infections in cases of chronic infection.

3.5 Biological diagnosis

In Situ Hybridization Technique (ISHH), was first employed by Gall and Pardue at Yale University. They utilized ribosomal gene probes from clawed frogs and hybridized them with ribosomal genes in oocytes, revealing the gene's location in the nucleolus and establishing the ISHH technique. Kwon et al. utilized ISHH technology by using a 520 bp repetitive DNA sequence amplified by PCR as a DNA probe. They conducted in situ hybridization with non-radioactively labeled DNA probes on tissues from 20 pigs affected by Mhp-induced pneumonia, successfully detecting Mhp [15]. Clear hybridization signals were observed in the bronchial and bronchiolar epithelial cells of these 20 affected pig tissues. This detection technique not only identifies Mhp in naturally infected pig tissues but also enables the localization of vaccine strains after immunization. ISHH technology offers high sensitivity and specificity. However, this method requires post-mortem samples from diseased pigs, is time-consuming, and is not suitable for rapid diagnosis of Mhp-induced pneumonia.

Loop-mediated isothermal amplification (LAMP) detection was invented by Japanese researchers, including Notomi, in 2000 as a constant temperature nucleic acid amplification technique. Guo Panpan et al. designed six specific primers targeting the conserved gene 16S rRNA of Mycoplasma hyopneumoniae and established the LAMP detection method, which could detect the pathogen in just 30 minutes under isothermal conditions at 63 ℃. To confirm its sensitivity and accuracy, Guo Panpan et al. used LAMP detection technology to detect recombinant plasmids containing the target gene fragments, and all 127 clinical nasal swab samples showed positive results [16]. The experimental results demonstrated that LAMP technology surpasses PCR diagnostic techniques in terms of detection speed, sensitivity, specificity, accuracy, and ease of operation, without requiring specialized laboratory equipment. It is particularly suitable for clinical diagnosis in livestock farms. This method can also be utilized for the detection of bacteria, viruses, and parasites.

TaqMan assay, In order to reduce the difficulty and time-consuming nature of laboratory detection for Mycoplasma hyopneumoniae (Mhp) cultures, Stemke et al. cloned randomly cut DNA fragments of Mhp using restriction enzymes into the M13mp19 vector in 1989. The resulting recombinant product was used as a nucleic acid probe capable of detecting approximately 104 bacterial bodies in 10 pg of DNA content. However, this nucleic acid probe could only detect Mhp DNA after pathogen cultivation. To assess the specificity of TaqMan, Ahrens et al. labeled a 1.3 kb DNA fragment from the Mhp gene library with a radioactive isotope and used dot blot hybridization to detect Mhp in pigs and cattle. The results showed a specific reaction only with Mhp [17]. Although TaqMan exhibits high sensitivity and specificity, this method requires strict experimental conditions and is less convenient to perform compared to PCR. Currently, TaqMan is primarily used for gene cloning and clone product identification purposes.

4. Therapeutic method and precautionary measures

4.1. Western medicine therapy

In the treatment of swine asthma with Western medicine, antibiotic therapy can be used. Zhang

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Chunhong [18] treated several cases of swine asthma in the Jinning District of Kunming City between 2016 and 2017, utilizing Western medicine treatment. The treatment plan, tailored based on the weight and condition of the affected pigs, is as follows:

(1) Feed method: Mix 1 kg of streptomycin and 1 kg of tilmicosin into 1000 kg of feed, and administer continuously for 7 days.

(2) Intramuscular injection:

① Administer intramuscular injections of colistin sulfate based on the weight of the affected pigs, twice daily, for 3-5 days.

② Administer intramuscular injections of lincomycin and tilmicosin to the affected pigs according to the dosage instructions, once or twice daily, for 4-5 days.

③ Administer intramuscular injections of lincomycin hydrochloride at a dosage of 0.3-0.5 mL/kg to the affected pigs once daily for 4-5 days.

In addition, a combination treatment of tylosin and procaine, kanamycin, tiamulin, flunixin, and other medications can also effectively treat swine asthma. Among them, tilmicosin is one of the most commonly used methods in the treatment of swine asthma and has a significant effect on acute cases. Lincomycin also shows excellent therapeutic effects in treating affected pigs, but long-term use should be avoided to prevent the development of drug resistance [19].

4.2. Combined Traditional Chinese Medicine and Western Medicine therapy:

When treating pigs with swine asthma, both traditional Chinese medicine (TCM) and Western medicine approaches have shown good therapeutic efficacy. However, combining TCM and Western medicine during the treatment process can lead to even better results, achieving a comprehensive treatment approach that addresses both the symptoms and root causes of the disease [20]. Care should be taken regarding medication dosage. The following medication formula can be considered:

(1) For severely affected pigs, administer 0.1mL/kg of flunixin meglumine and doxycycline hydrochloride via injection, with a dosage of 15mL of Shuanghuanglian Injection per pig administered intramuscularly in the morning and afternoon.

(2) Add 2kg/t of Maxing Shigan Powder and 1kg/t of Qibangqing Granules to the feed, along with 0.2kg/t of 80% berberine hydrochloride and 2kg/t of 10% doxycycline hydrochloride. Administer this treatment formula during the first week.

(3) Add 2kg/t of licorice granules to the feed, along with 1kg/t of 10% thiamphenicol premix and glucose oxidase separately, for the second week of treatment.

After two weeks of treatment, the affected pigs show significant recovery with normalized eating habits and improved mental state, and no recurrence is observed. During the treatment process, it is important to maintain normal drinking water supply, and the addition of vitamins to the water can enhance the pigs' immune response. The combined use of TCM and Western medicine not only enhances the treatment efficacy but also shortens the treatment duration.

4.3. Precautionary measure

Immunization is the most effective preventive measure against swine pneumonia caused by Mycoplasma hyopneumoniae. Establishing a scientifically designed immunization program not only reduces the chances of infection but also minimizes the economic losses associated with treatment and prevention. Currently, vaccines for preventing swine asthma include attenuated vaccines, inactivated vaccines, and freeze-dried vaccines, all of which can be administered via intramuscular injection [21]. Among them, attenuated vaccines are the most commonly used in clinical practice. Although thoracic cavity injection may yield better results for attenuated vaccines, it carries certain side effects. Therefore, intramuscular injection is recommended for the initial immunization. In high-risk breeding farms without active infection, freeze-dried vaccines are commonly used for emergency immunization. It is important to note that during the vaccine injection process, needle replacement should be done promptly to prevent cross-contamination. Antibody testing should be performed after vaccination to ensure the effectiveness of immunization.

ISSN 2616-5910 Vol. 4, Issue 1: 14-19, DOI: 10.25236/AJALS.2023.040103

5. Conclusion

Effective diagnostic methods and scientific treatments are important aspects of today's swine farming industry. In this study, relevant literature on swine pneumonia was reviewed, and an analysis was conducted based on the etiology, epidemiological characteristics, and clinical features of the disease. Drawing upon previous research experiences and summarizing the findings, rapid and widely applicable laboratory diagnostic methods for swine pneumonia were developed. Additionally, scientific treatment measures were established. These advancements have further enhanced the pork production capacity and industry profitability in the swine farming sector.

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