Diagnosis and Prevention of *Glasserella parasuis*

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Abstract This paper introduced the characteristics of *Glasserella parasuis* from the aspects of pathogen, epidemiology, clinical symptoms and anatomical symptoms, and put forward its clinical and laboratory diagnosis methods. Moreover, the disease was differentiated from similar diseases such as mycoplasmal pneumonia of swine, swine flu infectious pleuropneumonia of swine, swine streptococcosis and porcine reproductive and respiratory syndrome. Finally, the prevention and treatment measures of the disease are proposed.

Keywords *Glasserella parasuis*; Pathogen; Epidemiology; Clinical symptom; Anatomical symptoms; Diagnosis method; Prevention and control measure

Glasserella parasuis (also known as Glasser's disease) is a bacterium causing pantropic infectious disease of pigs caused by *Haemophilus parasuis* (HPS). The main symptoms include fever, cough, dyspnea, wasting, abdominal distention, lameness, ataxia and coarse hair. With the development of pig industry, the disease has become one of the main bacterial diseases affecting the development of pig industry in the world, and the research on HPS is gradually deepening.

1 Pathogen

1.1 Classification and morphological characteristics HPS belongs to *Haemophilus*, Pasteuraceae. When viewed under a light microscope, a number of different forms can be seen, ranging from individual coccobacilli to long, elongated and filamentous bacilli. The bacterium usually has capsules but is susceptible when cultured *in vitro*. Strains with capsules are more inclined towards the morphology of coccobacillus, while those without detectable capsules have a variety of morphologies, ranging from brevibacterium to slender filamentous bacteria. The bacterium has the same filamentous cilia-like structure in chick embryo chorioallantoic membrane and allantoic membrane cultures.

1.2 Cultural traits HPS grows slowly and is particularly fussy about nutrients, sometimes requiring the addition of specific growth factors. Approximately 24–48 h of incubation at 37°C results in the growth of round, elevated, smooth-surfaced, neatly edged, grayish-white, small, semi-transparent anhemolytic colonies. It grows well on chocolate agar plates, and shows satellite growth after streaked and incubated on blood plates with *Staphylococcus* spp. for 24 h, with colonies growing well around the streak.

1.3 Biochemical and physicochemical properties HPS is not very resistant to the external environment, and dry environment can make it die, generally at 60 ℃ after 5–20 min. The commonly used medicinal pasteurizers can also kill the bacterium, and it can survive 7–10 d at 4 ℃. The bacterium can ferment glucose, sucrose, fructose, galactose, ribose, maltose, *etc*. The results of biochemical reaction are negative in urease test, negative in oxidase test and positive in contact enzyme test.

1.4 Pathogenesis HPS is a resident bacterial group in the upper respiratory tract of pigs, which does not show symptoms under normal conditions, but destroys the defense mechanism of the upper respiratory tract under a variety of stress factors and pathogenic conditions, causing a significant reduction in the activity of the cilia on the surface of the upper respiratory tract mucosa, damaging the ciliated epithelium, and causing purulent rhinitis, loss of cilia in the foci and acute swelling of nasal and bronchial mucosal cells. Mucosal damage may increase the chance of invasion of bacteria and viruses, causing systemic infection in sensitive pigs.

2 Epidemiology

2.1 Sources of infection and transmission routes Sick or infected pigs are the main source of infection of the disease. HPS is a frequent bacterium in the upper respiratory tract of pigs and can be isolated from the nasal passages, nasal secretions, trachea and tonsils of healthy animals. It can also be isolated from the lungs of pigs with pneumonia, but can not be isolated

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from the lungs of normal pigs. *G. parasuis* is spread mainly through the air, direct contact between pigs or contaminated excreta, and also through the digestive tract and other routes of infection.

2.2 Susceptible animals *G. parasuis* infects only pigs and can infect pigs of all ages, but primarily infects pigs before and after weaning and in the nursery. Unimmunized farms or particularly healthy herds are most susceptible to infestation. The incidence of the disease in swine herds is typically 10–15%, and in severe cases, the fatality rate can be as high as 50%. Within susceptible herds, mortality due to *G. parasuis* occurs primarily in piglets; it begins 1 week after weaning due to the lack of maternal antibody protection in piglets, but the peak of infection is usually 4–6 weeks after weaning.

2.3 Epidemic characteristics There is no obvious seasonality in the occurrence of this disease, and it is mostly endemic. It is often mixed with or secondary to other viruses and bacteria during winter/spring and fall/winter when the climate changes significantly and the feeding environment is poor. Recent reports suggest that HPS may be the primary cause of septic bronchopneumonia.

3 Clinical Symptoms

Mental status: depressed, open mouth breathing (Fig.1–1).

Skin: redness or cyanosis of the skin (Fig.1–2, Fig.1–3).

Eye: subcutaneous edema of the eyelid (Fig.1–4).

Joints: swollen joints, lameness, difficulty in standing or even paralysis (Fig.1–5).

Neurological symptoms: ataxia in some diseased pigs, lying on the side or with paddling limbs before death (Fig.1–6).

Farrowing: sows with the disease may abort.

4 Anatomical Symptoms

Whole body: sick and dead pig carcasses are emaciated, with hemorrhagic spots or purplish skin.

Abdominal cavity: seroperitoneum, hepatosplenomegaly and adhesion to the abdominal cavity (Fig.2–1, Fig.2–2).

Larynx, trachea: large amount of mucus in larynx.

Heart: pericardial effusion, thickening of the pericardial membrane, and a large amount of fibrin exuding from the surface of the myocardium (Fig.2–3, Fig.2–4, Fig.2–5).

Liver: enlarged with severe bleeding at the margins (Fig.2–6).

Spleen: enlarged with hemorrhages on the surface, with blood bubbles as large as rice grains bulging at the margins, and infarcts on some of the splenic margins (Fig.2–7, Fig.2–8).

Kidney: hemorrhagic spots in the kidneys, severe hemorrhage in the renal papillae, mucus and fibrinous film on the surface of some of the kidneys (Fig.2–9~Fig.2–12).

Lungs: pulmonary interstitial edema, fibrinous pneumonia in some sick pigs (Fig.2–13).

Joints: jelly–like materials in joint incision(Fig.2–14).

Lymph nodes: lymph nodes enlarged

Fig.1 Clinical symptoms of *Glasserella parasuis*

throughout the body with a consistent grayish-white color in section (Fig.2-15, Fig.2-16)

5 Diagnosis Methods

5.1 Integrated clinical diagnosis The disease is more likely to be prevalent in winter/spring and fall/winter; sick pigs often show high fever, loss of appetite, respiratory difficulties, cyanosis and nervous

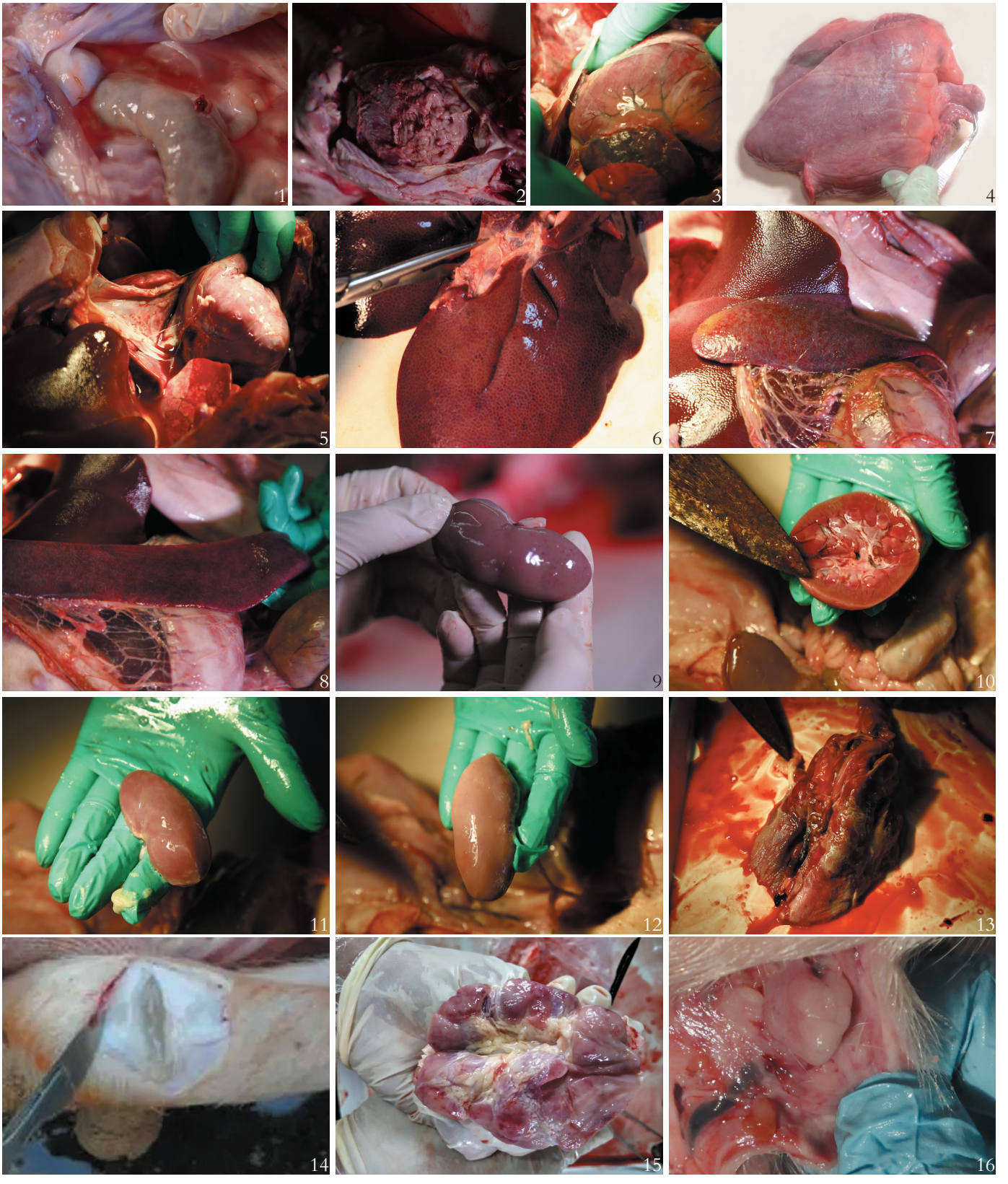

Fig.2 Pathological examination of *Glasserella parasuis*

disorders and other symptoms, while resistant pigs show loose hair, coughing, respiratory difficulties and growth stagnation; dissection of diseased pigs reveals serofibrinous pleurisy, pericarditis, peritonitis, meningitis, and polyarthritis. A preliminary diagnosis can be made by combining epidemiology, clinical symptoms, and pathological changes, and if the disease needs to be confirmed, laboratory diagnosis is also required.

5.2 Laboratory diagnosis Laboratory diagnosis mainly includes bacterial isolation and identification, indirect hemagglutination test, enzyme-linked immunosorbent assay, PCR rapid assay, real-time fluorescence quantitative PCR, and ring-mediated isothermal amplification technology. Among them, the isolation and identification of bacteria is simple, but time-consuming, mainly used for the isolation and identification of clinical materials. Indirect hemagglutination test is easy to operate and the test results are visible to the naked eye, but it is easy to be contaminated by external impurities and cause nonspecific agglutination. Enzyme-linked immunosorbent assay (ELISA) is highly sensitive, stable, easy to operate and rapid, and can be used for the detection of antigen or antibody samples in large quantities. PCR rapid assay is highly specific and sensitive, can avoid the limitation of serotypes, and is suitable for the rapid diagnosis and rapid differential diagnosis of the disease as well as epidemiological investigation. Real-time fluorescence quantitative PCR not only adheres to the advantages of traditional PCR such as reliability, sensitivity, specificity, rapidity and simplicity, but also has the advantages of real-time and contamination-free. Ring-mediated isothermal amplification technique has high sensitivity, short reaction time, no need for special instruments, simple operation, and is especially suitable for grassroots personnel, but it is easy to cause false-positive results due to aerosol contamination in practice.

6 Id entification of Similar Diseases

6.1 Mycoplasmal pneumonia of swine Similarities: both diseases mostly occur in piglets. Sick pigs have fever, swollen joints, lameness and respiratory distress. Dissection reveals fibrinous inflammation in the pleura, peritoneum and pericardium.

Differences: the shoulder, wrist, knee and hock joints of pigs with mycoplasma disease are severely affected, often showing body curling due to abdominal pain; redness and cyanosis of the surface skin are not seen. The synovial membrane is congested and swollen, and there is blood and serum in the synovial fluid. Microscopically, the plasma membrane surface produces purulent fibrinous exudate.

6.2 Swine flu Similarities: sick pigs all show elevated body temperature, with flushed skin all over the body in extremely sick pigs, extreme lethargy, anorexia or loss of appetite, respiratory distress, often lying on the ground.

Differences: it is usually the weaker pigs that are susceptible to HPS infection, whereas robust pigs show essentially no morbidity or the presence of insignificant morbidity; swine flu spreads rapidly within a herd, and often the presence of a sick pig will cause the entire herd to become sick. In addition, both can cause sick pigs to produce different types of snot, with those infected with influenza often producing clear, transparent snot, while those infected with HPS often produce cloudy, grayish-white or creamy-white snot. Swine flu occurs frequently in winter and spring, with acute onset, and will spread quickly in the herd once it occurs. Sick pigs show mental atrophy, coughing and sneezing symptoms; if there are no secondary onset and complications, there will be rare cyanosis symptoms on body surface and ears; the pigs will usually recover from the disease within a week, and there are almost no death cases.

6.3 Infectious pleuropneumonia of swine Similarities: sick pigs show elevated body temperature, reddened skin, depressed spirit, unwillingness to stand, refusal to eat, respiratory distress, coughing, sometimes open-mouth respiration, and cyanosis of the nose, ears, eyes and hindquarters skin.

Differences: infectious pleuropneumonia is characterized by wheezing, coughing, severe respiratory distress, often in the posture of canine sitting and open-mouthed respiration, and the mouth and nose can discharge blood-like secretions; the coughing sound of pigs with HPS is mild, with only 2–3 short coughs each time, and there is no blood-like secretion coming out of the mouth and nose. Lesions caused by HPS infection are mainly characterized by pericarditis, pleurisy, peritonitis, arthritis, meningitis and other multiple inflammations, especially pericarditis is the most common; in contrast, infectious pleuropneumonia causes lesions characterized primarily by fibrinous, hemorrhagic, necrotizing pleurisy and pneumonia, and is confined to the thoracic cavity rather than the abdominal cavity. In mixed infections, it is difficult to distinguish by dissection alone and must be identified by laboratory test.

6.4 Swine streptococcosis Similarities: both tend to cause pigs to show signs of markedly elevated body temperature, arthritis and meningitis.

Differences: HPS easily occurs in parturient sows, weaned piglets and elder ones, while it is essentially absent in lactating piglets; streptococcal arthritis is usually more likely to occur in lactating and weaned piglets after 10 days of age, but not in nursery pigs weighing more than 30 kg, and it can only be spread in a small area or in single case. In addition, swine with HPS will have essentially no swelling of the joints, but the entire diseased limb or hoof is swollen or there is no swollen joint disease but lameness occurs; whereas swine with streptococcal disease tend to have significant swelling of the joints.

6.5 Porcine reproductive and respiratory syndrome Similarities: sick pigs show elevated body temperature, flushed skin, cyanotic ears and respiratory distress.

Differences: pigs with porcine reproductive and respiratory syndrome develop conjunctivitis after weaning, with cyanosis of the skin on the dorsal margins of both ears, the tail, and the legs, and a transient dark greenish-purple coloration; dissection reveals mildly enlarged kidneys with a dark red appearance, diffuse dark red petechiae, and diffuse streaks of hemorrhage on the cut surface. Sow employs ventral respiration, with cyanosis of ears, teats, vulva, abdomen, tail and legs in a few sows, most commonly at the ear tips. Sows are cyanotic at the margins of the ears, vulva and abdomen, with formation of discoloration, edema and necrosis in the dermis. Dissection of the sow reveals pulmonary edema, pyelonephritis and cystitis, and inflammatory and degenerative lesions in the placent.

7 Prevention and Control Measures

7.1 Prevention measures

(1) Enhancing nutrient supply. The feeding management of the herd should be strengthened, and the whole herd of pigs is offered with the water containing immune interferon or Jintaosu (70% astragalus polysaccharide, levamisole, immune proteins, *etc.*) plus Jinmoxilin (70% amoxicillin, slow-release synergist) for 5–7 d, in order to enhance the resistance of the body and reduce the stress response.

(2) Improving sanitation. The pig house is cleaned up thoroughly; the floor and wall of the pig pen is sprayed with 2% sodium hydroxide solution, rinsed with water after 2 h, and then disinfected with Shuangyoudian (povidone iodine, enhancer) for consecutive 3–5 d.

(3) Vaccine inoculation. Sow inoculation can produce protective immunity to piglets up to 4 weeks of age; first-farrowing sows are immunized for the first time at 40 d prior to farrowing and for the second time at 20 d prior to farrowing; one immunization 30 d before farrowing is sufficient for multiparous sows; in pig farms seriously threatened by the disease, piglets should also be immunized, with first immunization at 10 days of age and booster immunization after 15 d.

(4) Drug prevention. Generally, a preventive dose of antibiotics, such as 50–100 mg/kg tiamulin, 200 mg/kg lincomycin, 150 mg/kg ciprofloxacin, *etc.*, is added to sow, suckling and nursery feed 1 week before farrowing and 1 week after farrowing.

7.2 Control measures

(1) Florfenicol injection is injected intramuscularly at the dose of 0.1 mL/kg (double for severely sick pigs) once a day for consecutive 3 d.

(2) Tilmicosin is mixed in the feed at the dose of 2 mg/kg for consecutive 7–10 d.

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