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Diagnostic techniques for Glässer's disease

格拉瑟病诊断技术

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1 Scope

This document describes the clinical diagnosis of Glässer's disease and the laboratory diagnostic methods of sample collection, handling and storage, bacterial isolation and identification, nested PCR detection, real-time fluorescent PCR detection and indirect ELISA.

This document applies to the diagnosis and the monitoring of Glässer's disease.

4 Abbreviations

The following abbreviations apply to this document. bp, base pair; Ct value, cycle threshold; DNA, deoxyribonucleic acid; dNTPs, deoxyribonucleoside triphosphates; ELISA, enzyme linked immunosorbent assay; IgG, immunoglobulin G; MGB, minor groove binder; NAD, nicotinamide adenine dinucleotide; PBS, phosphate buffer solution; PCR, polymerase chain reaction; Taq enzyme, Taq DNA polymerase; TSA, tryptic soy agar; TSB, tryptic soy broth.

5 Clinical diagnosis

Epidemiology. Glässer's disease is a bacterial infectious disease caused by infection with Glaesserella parasuis. The organism infects only pigs, and sick pigs and carrier pigs are the main source of infection. Pigs of every age, sex and breed are susceptible, and the disease occurs mainly in pigs around weaning and in the nursery stage. It can occur in any season of the year, and is most frequent in early spring and late autumn. Infection is mainly by the respiratory route, and may be acute or chronic, chronic infection being the more common.

Acute cases. Fever, with the body temperature rising to 40.5 degrees Celsius to 42 degrees Celsius, dullness and reduced appetite. Laboured breathing, with mucous and serous discharge from the nostrils. Reddening of the skin early in the infection and cyanosis of the margins of the ears later. Lameness, trembling and nervous signs.

Chronic cases. Falling appetite, wasting and weakness, a rough coat and poor growth. Recurrent fever, coughing and laboured breathing. Swollen joints, weakness of the limbs or lameness.

Pathological changes. Inflammation of the pericardium, the pleura, the peritoneum and the joint synovium is characterised by serous and fibrinous exudate. The joints are swollen and contain serous or fibrinous exudate or a jelly-like material. The thoracic cavity contains a large quantity of pale yellow or slightly reddish fluid together with clots of fibrinous exudate. There is pericardial effusion, the pericardium is thickened and often contains caseous exudate adhering to the heart so as to form a villous heart; there are haemorrhagic spots in the myocardium and abundant fibrinous exudate on its surface. The lungs are congested and oedematous, covered on the surface with a fibrin film and adherent to the chest wall. There is serous or fibrinous peritonitis, with ascites or adhesion of the internal organs. The lymph nodes throughout the body are enlarged, and the liver, the spleen and the kidney show congestion and focal haemorrhage.

Interpretation. A case that meets 5.1 and meets three or more of the signs in 5.2, or that meets one or more of the pathological indicators in 5.3, or both, is judged a suspect case of Glässer's disease; confirmation calls for laboratory diagnosis.

6 Sample collection, handling and storage

Samples for microscopy. Fresh tissue such as lung, heart and brain, thoracic, abdominal or pericardial effusion or joint fluid, and anticoagulated blood are collected aseptically from

pigs that have died of suspected Glaesserella parasuis infection.

Serum samples. Blood is taken aseptically from the ear vein or the anterior vena cava, not less than 5 mL from each animal; the serum is separated aseptically into a 2 mL centrifuge tube, numbered, and used for ELISA detection.

Tissue samples. Fresh tissue samples with obvious lesions, such as lung, heart and brain, are taken. About 0.5 g of the sample to be tested is cut out with sterile scissors and forceps into a tissue homogeniser or a mortar and thoroughly homogenised or ground; 0.3 mL to 0.5 mL of PBS is added and mixed, and the tissue suspension is transferred into a sterile centrifuge tube, numbered and set aside.

Effusion. Between 2 mL and 3 mL of thoracic or abdominal effusion or of joint fluid is collected with a sterile syringe, transferred into a sterile centrifuge tube, numbered and set aside.

Anticoagulated blood. Between 2 mL and 3 mL of anticoagulant, that is Alsever's solution, is first drawn into a sterile syringe; an equal volume of blood is taken from the ear vein or the anterior vena cava, mixed quickly and transferred into a sterile centrifuge tube, numbered and set aside.

Enrichment culture. 500 microlitres of the enrichment culture is taken with a sterile pipette tip into a sterile centrifuge tube, numbered and set aside.

Storage. After collection the samples are placed in an insulated box with pre-cooled ice packs and sealed, and should be sent to the laboratory within 24 h. Samples kept at 2 degrees Celsius to 8 degrees Celsius shall be held for not more than 24 h. Where they have to be kept for a long time they shall be placed at minus 70 degrees Celsius.

7 Bacterial isolation and identification

Main apparatus. Biological safety cabinet; constant-temperature incubator; optical microscope; automated microbial identification system; refrigerator at 2 degrees Celsius to 8 degrees Celsius.

Main reagents and materials. TSA solid medium and TSB liquid medium prepared in accordance with A.1 and A.2 of Annex A. NAD stock solution prepared in accordance with A.3 and kept at 2 degrees Celsius to 8 degrees Celsius. Gram staining reagents, comprising crystal violet with ammonium oxalate, Lugol's iodine, 95 % alcohol and safranin, kept at room temperature. Sheep blood plates without NAD, and micro biochemical identification tubes for glucose, sucrose, fructose, lactose, galactose and xylose, kept at 2 degrees Celsius to 8 degrees Celsius. Nitrate reduction test reagents and 0.1 % dimethyl-para-phenylenediamine dihydrochloride solution prepared in accordance with A.4 and A.5 and kept at room temperature.

Isolation culture. In the biological safety cabinet the sample is picked up aseptically with an

inoculating loop and streaked onto TSA solid medium, then incubated at 37 degrees Celsius for 24 h to 48 h so that colonies form for identification. Pinpoint colonies of about 1 mm to 2 mm in diameter, round, raised, smooth and moist, colourless, transparent and with an even edge are picked from that culture and streaked onto TSA solid medium for pure culture; after 24 h to 48 h at 37 degrees Celsius the same small colonies appear, as shown in Figure B.1 of Annex B.

Smear, staining and microscopy. One or two drops of sterile distilled water are placed on a slide, a colony of the pure culture is picked into it, mixed and spread as a thin film of bacteria. The smear is left to dry naturally in air. The dried slide is held film upward and fixed by passing it five or six times through the flame of a spirit lamp. It is stained by the Gram method, the operation following the instructions of the Gram staining kit, and examined under an optical microscope at 1 000 times magnification, that is 10 by 100. Glaesserella parasuis appears as Gram-negative short rods or coccobacilli of uneven size, about 0.5 micrometres by 1.5 micrometres to 2.0 micrometres, mostly slender rods, without flagella and not forming spores, as shown in Figure B.2.

Satellite growth test. In the biological safety cabinet a single colony from the purified culture is picked aseptically and streaked in a line on a sheep blood plate without NAD, and Staphylococcus aureus is then streaked at right angles to that line. After 24 h to 48 h at 37 degrees Celsius the growth of the colonies is examined; Glaesserella parasuis does not haemolyse and shows the satellite growth phenomenon, that is the colonies of Glaesserella parasuis close to the staphylococcus are larger and those further away from it are smaller.

Enrichment culture. In the biological safety cabinet a single non-haemolytic colony showing the satellite growth phenomenon is picked aseptically and purified once more on TSA solid medium; a single colony is then inoculated into 5 mL of TSB liquid medium and incubated at 37 degrees Celsius for 24 h to 48 h. If the liquid becomes turbid the enrichment culture is judged successful.

Biochemical identification, sugar fermentation test. In the biological safety cabinet the biochemical identification tubes are opened aseptically and NAD is added to each tube to a final concentration of 10 micrograms per millilitre; with an inoculating needle the enriched culture is inoculated aseptically into micro biochemical identification tubes of glucose, lactose, galactose, mannitol and xylose, which are incubated at 37 degrees Celsius for 24 h and then read. Fermentation of glucose and galactose without fermentation of lactose, mannitol or xylose is positive; otherwise the result is negative.

Catalase test. One drop of the enriched culture is taken onto a clean slide, one drop of 3 % hydrogen peroxide is added and mixed, and the reaction is read within 30 s; abundant bubbles appearing at once are judged positive and no bubbles negative. Nitrate reduction test. 0.2 mL each of 0.8 % sulfanilic acid in glacial acetic acid and 0.5 % alpha-naphthylamine in ethanol are mixed, and 0.1 mL of the mixed reagent is added to the enriched culture; the result is read at once, a red colour being judged positive and no red