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LABORATORY DIAGNOSTICS OF BRUCELLOSIS

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Abstract
Brucellosis is a zoonotic infection transmitted from animals to humans by the ingestion of infected food products, direct contact with an infected animal or inhalation of aerosols. The socio-economic significance of the problem of brucellosis is the course of the disease with the development of chronic forms and disability. Therefore, early detection, treatment and follow-up observation of those who have recovered from the disease are of great epidemiological importance.

Keywords: Brucellosis, Brucellacaea, C-reactive protein, Coombs reaction, Wright reaction, enzyme immunoassay, polymerase chain reaction.

Introduction

Brucellosis is a systemic infectious disease characterized by a tendency to chronicity with long-term persistence of the pathogen and a high risk of disability, which determines the social significance of this infection. Brucellosis is transmitted from animals to humans through consumption of contaminated products, direct contact with infected animals, or inhalation of aerosols. The disease is widespread, especially in the Mediterranean basin, the Arabian Gulf, the Indian subcontinent, Mexico, Central and South America, East Asia, and Africa [1,3,8]. Humans are infected by 8 species (do not infect - B. ovis, B. neotomae). The most pathogenic are B. melitensis (the main hosts are goats and sheep), B. abortus (hosts are cattle), B. suis (hosts of different biotypes are pigs, hares, reindeer), B. canis (hosts are dogs). B. abortis causes a milder course of the disease, the primary chronic form develops more often, and the development of a primary latent form is possible. Infection with B. canis and B. melitensis is associated with severe disease progression, high rates of bacteremia, short incubation periods, and severe clinical manifestations [2,4,7].

In the pathogenesis of brucellosis infection, three stages are distinguished. In the first stage, the pathogen enters the host's body within 2 days after infection. In the second stage, the pathogen multiplies in various organs of the reticuloendothelial system from 2 days to 3 weeks, clinically corresponding to acute brucellosis. In the 3rd stage, known as chronic brucellosis, the pathogen

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forms pathology of various organs over a period of 6 months to 1 year or more [10,15]. Brucella persistence occurs in tissues of the mononuclear phagocytic system, including bone marrow, lymph nodes, liver and spleen. Brucella is found in bones, joints, male reproductive organs, and infects the placenta and fetus.

After brucellosis, short-term immunity is formed, with a maximum intensity of 10-12 months. The organism remains susceptible to re-infection, and when the pathogen enters again, severe allergic processes occur with organic irreversible damage to parenchymatous organs, connective tissue and the nervous system [9,12]. Recurrent cases are observed in 2-7% of patients. Brucellosis is diagnosed by collecting an epidemiological and clinical history, clinical examination, laboratory and instrumental examination methods and is aimed at determining the nosology and clinical form, severity of the condition, identifying complications and indications for therapy [5,13,16].

It is recommended to perform a general (clinical) blood test. In the general blood test of patients with brucellosis, anemia, leukopenia/leukocytosis, thrombocytopenia, pancytopenia, and an increase in ESR are noted. General blood test results allow us to judge the severity of the disease. In most cases, minor deviations are observed, but with a pronounced inflammatory reaction, leukocytosis and an increase in ESR are observed. Secondary hemophagocytic syndrome may develop (more common in children than in adults).

Biochemical blood test (AST, ALT, bilirubin, C-reactive protein, creatinine, blood urea). A biochemical blood test is performed for a comprehensive assessment of the severity of brucellosis in children and to identify damage to organs and systems. In brucellosis, deviations in acute phase inflammatory reactions (CRP), changes in the activity of liver enzymes (AST, ALT), and in rare cases, impaired renal function (increased levels of creatinine, urea) may be observed.

Brucellosis disease must be confirmed by specific laboratory tests, which include groups of methods: detection of the pathogen, soluble antigen, DNA; determination of specific antibodies; detection of sensitization of the body to brucellosis antigens. Clinical material intended for testing for brucellosis includes blood, bone marrow, cerebrospinal fluid, lymph node puncture, urine, bile, synovial fluid, and pus. Collection, transportation, storage of biological material, research on the isolation of the pathogen or its genome from the material are carried out in laboratories of especially dangerous infections that have a license to work with pathogens of pathogenicity groups I-II [6,17].

It is recommended to use several examination methods to confirm/exclude the diagnosis of brucellosis:

When conducting an epidemiological survey of the population in foci, the Heddleson reaction, RA, RNGA, ELISA, indirect immunofluorescence method, and Burne skin allergy test are used;

- To diagnose acute and subacute brucellosis, bacteriological tests, PCR, and serological tests (RA, RPGA, ELISA) are used. In cases of negative results, the Coombs test is performed.
- for the diagnosis of chronic brucellosis and for the follow-up observation of patients who have recovered from the disease, the Coombs reaction, ELISA and allergy tests are used.
- when examining the population before preventive vaccination, the Heddleson reaction, RNGA or ELISA and allergy tests (Burne skin allergy test or leukocyte lysis reaction) are carried out [5,6].

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The Heddleson agglutination test (on glass) is used in endemic areas to screen for acute and subacute forms of brucellosis. The Heddleson reaction is simple to perform and highly sensitive. It is more sensitive than the Wright reaction, since it occurs under conditions of contact between undiluted serum and antigen, and can detect specific agglutinins present in low concentrations. A negative reaction can be detected in newly infected individuals, in patients with chronic brucellosis (in whom the level of agglutinins has reached zero), and in individuals with impaired immunity.

The Wright agglutination test (a quantitative assay based on the agglutination of inactivated Brucella) should be performed first, as it is highly sensitive. Serological reactions are positive in almost 98% of cases. High antibody titers almost always indicate the presence of infection. The diagnostic value of the method is highest in the early stages of the disease (in the first 6 months). The advantage of agglutination reactions is the simplicity of the reaction, rapid results and sensitivity of the reaction. When performing the Wright agglutination reaction in non-endemic areas, a dilution of 160 or higher is considered positive, and in endemic areas - 320. In the Wright reaction, 92% of children with acute brucellosis have a titer of 1:320 or more.

There are limitations to the use of the Wright test: inability to diagnose B. canis and cross-reactions with other infectious diseases. A positive agglutination reaction with brucellosis antigen can be given by serums containing antibodies to microorganisms that have common antigenic determinants with brucellosis (Vibrio cholerae, Yersinia enterocolitica, Francisella tularensis, Salmonella typhimurium, Escherichia coli). Low antibody titers or their complete absence do not exclude the possibility of the disease.

For monitoring chronic brucellosis and dispensary observation of those who have recovered, the Wright reaction has no diagnostic value due to the absence of seroconversion after recovery for two or more years, which may be associated with the presence of blocking antibodies. In 29% of children, titers of 1:320 are maintained for 2 years after recovery.

For diagnostics and monitoring of the patient's condition, it is necessary to determine the titer of agglutinins in dynamics (a fourfold increase in the titer of antibodies in RPGA, RA or any quantitative test in paired sera taken at an interval of 2 weeks). As the duration of the disease increases (especially in chronic infections), when the percentage of positive serological reactions begins to fall, the detection of incomplete antibodies in the Coombs test becomes especially important. Enzyme immunoassay is the most effective serological method for diagnosing and monitoring all forms of brucellosis, diagnosing relapses, assessing the effectiveness of therapy, in epidemiological surveys of the population, and in selecting individuals for vaccination. The ELISA method is specific (95%) and sensitive (98%). Allows to determine separately the titer of IgG, IgA, IgM. Brucella IgM antibodies appear within the first week after infection, persist during the acute period, and decrease over several weeks during the subacute period. IgA and IgG antibodies increase during the second week after infection, persist for a long time, and increase during relapses. ELISA diagnostics are most informative for acute brucellosis, and to a lesser extent for diagnosing chronic forms and neurobrucellosis [1,2,14].

The Burnet skin allergy test and the leukocyte lysis reaction are performed to detect increased sensitization of the body to the brucellosis antigen. They are strictly specific. The Burnet test is detected in patients 28 later than antibodies, and persists for a very long time, sometimes for years, after the disappearance of clinical symptoms. A skin allergic reaction may be positive in cases of

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asymptomatic infection, in those vaccinated with a live brucellosis vaccine, and in individuals who have had prolonged contact with a specific antigen.

Isolation of pure Brucella culture is the “gold standard” for diagnosing brucellosis and is used to confirm the diagnosis and monitor the effectiveness of therapy. The material should be collected before and after the completion of a course of antibacterial therapy, as well as during an exacerbation in patients with a chronic form. It is recommended to carry out blood cultures, bone marrow aspirates and lymph nodes on a special nutrient medium to isolate L-forms of Brucella. The disadvantage of the method is the long period of time it takes to obtain a response (3-5 weeks), since brucellae are slow-growing microorganisms [11,17].

Molecular detection of Brucella DNA may be a sign of acute or chronic brucellosis, and may be detected in asymptomatic cases. The PCR method has advantages over the culture isolation method: it is easy and quick to perform and eliminates the risk of laboratory-acquired brucellosis. It has high sensitivity and specificity. It allows to determine the presence of a specific sequence of Brucella DNA and the species affiliation of the isolated strains within 24 hours. Allows to detect 100-1000 bacterial cells in a sample. The advantage of the PCR method over immunological tests is the high specificity of PCR (no cross-reactions with DNA of E. coli, V. cholerae, F. tularensis, Y. enterocolitica 0-9, Y. pestis EV, S. typhimurium). Real-time PCR (like culture isolation) is of limited value in diagnosing patients with suspected chronic brucellosis due to the possible absence of bacteremia. However, it is a valuable prognostic tool for early detection of relapse [3,10].

Quantitative PCR analysis is a fast and effective test for diagnosing and monitoring patients with brucellosis. It has 100% specificity. The bacterial load of Brucella DNA before treatment is 1.9-2.1×104 copies/μl, on the 4th week of treatment - 259 copies/μl, after 6 weeks - 14-38 copies/μl, 3 weeks after treatment - negative or up to 36 copies/μl. The average bacterial load in patients with relapses is 2×104 copies/μl.

In patients with chronic brucellosis, there is a decrease in the level of CD4+ lymphocytes and an increase in the level of CD8+ lymphocytes, an imbalance of Th1/Th2 towards Th2, which is manifested by suppression of the immune response and the macrophage system and is associated with long-term persistence of the pathogen. In patients with acute brucellosis, no significant changes in the immunogram are observed.

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