METHODS OF MODERN LABORATORY DIAGNOSIS OF BRUCELLOSIS

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Successful control of brucellosis can only be achieved through the mobilization of scientific resources, practical healthcare, and veterinary services, as brucellosis is a zoonotic infection to which all species of domestic and wild animals are susceptible. In recent years, there has been a steady increase in human cases of brucellosis, especially in areas with developed livestock farming.

Keywords: brucellosis, domestic and wild animals, bacteriological method, laboratory methods;

It is known that the clinical presentation of brucellosis in humans is highly polymorphic. Among the significant variety of symptoms, the most common include radiculitis, arthritis, orchitis, musculoskeletal disorders, and spondylosis. In analyzing the available literature on brucellosis, we particularly noted the changing clinical course of the disease. It generally progresses benignly, but a chronic form often develops, leading to prolonged disability and serious socio-economic consequences.

Bacteriological method. Collection of clinical samples for testing, their packaging, and transportation is carried out by medical personnel in accordance with the existing sanitary rules for safety when working with microorganisms of pathogenicity groups I - II, regarding the accounting, storage, transfer, and transportation of microorganisms of pathogenicity groups I - IV, and methodological guidelines for the prevention and laboratory diagnosis of human brucellosis. Clinical materials for sampling, intended for further investigation of brucellosis from individuals suspected of having the disease, include: blood, bone marrow, cerebrospinal fluid, lymph node aspirates, urine, bile, and joint fluid (in cases of arthritis), pus (in cases of abscesses). Samples from patients suspected of brucellosis are taken upon admission before the onset of antibiotic therapy. For all forms of the disease, blood is collected in a volume of 10-15 ml, considering the need for bacteriological, serological studies, and polymerase chain reaction (PCR). At the

patient's bedside, 10 ml of blood is inoculated into two containers with a biphasic medium for the isolation of blood cultures, or 5 ml is introduced with a needle through a previously alcohol-treated rubber stopper into bottles with liquid nutrient medium for transporting the material and accumulating brucella, which allows combining the stages of transporting the material to the laboratory and culturing brucella. Blood is drawn from the cubital vein on an empty stomach in an amount of 5-10 ml, following aseptic rules, using a syringe or a vacuum system such as "Vakuette(R)" with a serum activator. The blood is transferred to a sterile tube. To obtain serum and prevent hemolysis, the tube with blood is left at room temperature in a tilted position until a clot forms. The obtained serum is then transferred to a plastic tube, securely closed, and sent to the laboratory for testing for specific antibodies to the causative agent of brucellosis. Bone marrow is obtained via puncture of the sternum with a syringe using a short and slightly blunted needle. The collected bone marrow (in a few drops) is inoculated into a tube with nutrient media. Cerebrospinal fluid is collected after lumbar puncture, suboccipital area, or from the brain ventricles in a volume of 0.1-0.3 ml and inoculated onto nutrient media. A sputum sample, obtained by deep coughing, is collected in a special sterile disposable container with a screw cap. When testing urine, the midstream portion (10-20 ml) is collected in a special disposable container with a screw cap. Bile samples (midstream) are collected during probing in the procedure room. Under the flame of a spirit lamp, the tube for material collection is opened, and the obtained bile (10-12 ml) is placed in a disposable sterile tube with a screw cap. When using a sterile glass tube closed with a gas-permeable cap, after filling the container, the neck and cap are sterilized in the flame of a spirit lamp, and the tube is closed. If using a tube with a gas-permeable cap, the sample is transported to the laboratory in a strictly vertical position to avoid soaking the cap with bile. The collected clinical material is inoculated onto nutrient media using the Castaneda method or on nutrient media for the accumulation of brucella. When examining patients who have undergone antibiotic treatment, cultures of blood, bone marrow aspirates, and lymph nodes are recommended to be taken one month and four to six months after the end of antibiotic therapy, as well as from patients with chronic brucellosis during exacerbations before starting treatment, on a special nutrient medium to isolate L-forms of brucella.

Molecular biological tests for Brucella detection. Considering that brucellae are slow-growing microorganisms, a final answer from bacteriological and biological testing methods can only be expected after 3-5 weeks. Therefore, a molecular biological method-the polymerase chain reaction (PCR)-has been developed and implemented in practice. The PCR method is based on the multiple copying (amplification) of a specific target DNA fragment, which serves as a marker for the species. PCR has high sensitivity, allowing for the detection of 100-1000 bacterial cells

in a sample. An important advantage over immunological tests for detecting brucella antigens is the high specificity of PCR (no cross-reactivity with E. coli, V. cholerae, F. tularensis, Y. enterocolitica 0-9, Y. pestis EV, S. typhimurium DNA). Sample preparation for testing (DNA isolation) and PCR execution is conducted using a special kit that includes all necessary ingredients. For diagnosing brucellosis in humans, DNA is determined in serum, cerebrospinal fluid, synovial fluid, and others. The material for PCR testing includes: blood, serum, lymph node aspirate, synovial fluid. The collection, transportation, and storage of biological material for PCR are carried out in accordance with current methodological guidelines for conducting PCR studies of materials infected with microorganisms of pathogenicity groups I - II. Samples are packaged according to the Recommendations for the Transport of Infectious Materials (2009-2010, WHO/HSE/EPR/2008.10), adhering to the principle of triple packaging. Materials are placed in a primary container (waterproof, airtight), which is packed with a sufficient amount of absorbent material to absorb any liquid in case of container damage. The secondary packaging (durable, waterproof, airtight) that encloses and protects the primary container (primary containers) packed in absorbent material. The secondary packaging is placed in an outer packaging for transport with enough cushioning material. The outer packaging, with minimum dimensions of at least 10x10 cm, is sealed and marked with the necessary orientation for the cargo, such as arrows or the inscription "top, handle with care." It is not permissible to place accompanying documents inside the container with samples.

Thus, the material is sent to a specialized laboratory via designated transport accompanied by medical personnel. For each clinical material sample sent to the laboratory, a referral is completed.

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