Diagnostic Approach to Brucellosis

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Editorial

Brucellosis is a worldwide zoonosis with a high degree of morbidity in humans. According to WHO data about 500,000 cases of this disease are registered in the world every year [1,2]. The presence of brucellosis in India was first established early in the previous century and since then has been reported from almost all states [3]. It is mainly transmitted from cattle, sheep, goats, pigs and camels through direct contact with blood, placenta, fetuses or uterine secretions, or through consumption of contaminated raw animal products (especially unpasteurized milk and soft cheese). Furthermore, brucellosis is the most common bacterial laboratory-acquired infection worldwide [4]. The disease primarily presents as fever of unknown origin with multiple clinical signs and symptoms. Patients regularly suffer serious focal complications such as spondylitis, neurobrucellosis or Brucella endocarditis [5]. The clinical picture is not specific and laboratory testing should support the diagnosis. Presumptive diagnosis of brucellosis can be made by the use of several serological tests to Brucella antibodies, but the “gold standard” remains isolation and identification of the bacterium. Despite the vigorous attempt for more than one century to come up with a definitive diagnostic technique for brucellosis, diagnosis still relies on the combination of several tests to avoid false negative results [6]. Certain newer molecular techniques have also been introduced for the diagnosis of brucellosis.

Among serological tests, Serum Agglutination Test (SAT) suffers from high false-negative rates in complicated and chronic cases. The Rose Bengal Plate Agglutination test (RBPT) is a rapid test which was designed originally for screening use in veterinary medicine, but is now often used for the diagnosis of human brucellosis [7]. Its high sensitivity, ease and speed of use, as well as its low cost, have made it very popular in hospital emergency departments for the diagnosis of febrile syndromes [8]. Lateral flow assay is simple and easy to perform. The sensitivity of this test calculated is 95% and specificity is 97%. Complement fixation test is a widely used confirmatory test for brucellosis. Coombs’ test may be more suitable for confirmation of brucellosis in relapsing patients or patients with persisting disease. The combination of positive rose Bengal test and Coombs’ test ≥ 1/320 was the best diagnostic criterion with 80% specificity and 100% sensitivity. The enzyme linked immunosorbent assay (ELISA) is a sensitive and rapid method for diagnosis of brucellosis. Detection of specific immunoglobulin by a single, simple and rapid test is a major advantage with ELISA [9]. Brucellacap is a single stage proprietary test kit and is a new form of the agglutination test to test for brucellosis antibodies with 95.1% sensitivity and 99% specificity. Brucellacap is more sensitive and usually shows higher titers than the Coombs test.

Culture from the blood of a patient provides definite proof of brucellosis [10]. There is a range of commercially available culture media for growing Brucella. The Castaneda two-phase system is the most convenient. Now semi-automated blood culture techniques like such as the BACTEC™ the time to detection has been significantly reduced. Brucellae can be detected in the blood of infected patients after four days of culture or less [11]. Molecular techniques such as Polymerase chain reaction (PCR) assays can be used to amplify and detect Brucella DNA in pure cultures and in clinical specimens. The QIAamp™ DNA Mini Kit and the UltraClean™ BloodSpin Kit are among the many commercial kits that have been successfully used to extract Brucella DNA from whole-blood, serum and tissue samples [12]. For the diagnosis of human brucellosis, a PCR assay with one pair of primers is developed, which amplifies the target genomic sequence of Brucella species. Studies showed that standard PCR appeared to be a more sensitive technique than microbiological methods, not only for the diagnosis of a first episode of infection, but also for the early detection of relapses. Real-time PCR is a valuable technique in quantification of nucleic acids in individual blood samples. It is highly reproducible, rapid, sensitive and specific [13].

G.Vrioni et al. [14] employed a simple Polymerase Chain Reaction-Enzyme Immunoassay (PCR-EIA) for the rapid laboratory diagnosis of human brucellosis directly from peripheral blood. Following the amplification of a 223 bp sequence of a gene that codes for the synthesis of an immunogenic membrane protein specific for the Brucella genus, the amplified product was detected in a microtiter plate by hybridization with specificity of 100% and sensitivity of 81.5% for whole blood specimens and 79% for serum specimens. Results suggest that PCR-EIA assay is a sensitive and specific method that could assist the rapid and accurate diagnosis of acute human brucellosis [15]. The Loop- Mediated Isothermal Amplification Assay (LAMP) assay is for the quantitative detection of Brucella spp. is highly sensitive and specific. A simple and inexpensive apparatus such as a water bath or heat block that provides a constant temperature of 63°C is sufficient for the assay, and, unlike PCR, the reactivity is directly observed with the naked eye neglecting the need for electrophoretic analysis. Moreover, the LAMP assay can be performed on site, as special equipment such as a thermal cycler is not required. Recently, a more rapid and inexpensive method based on the Lab on a chip technology has been proposed i.e. Multiple Locus VNTR Analysis (MLVA) Typing For Brucella Based On Microfluidics Technology. This miniaturized platform for electrophoresis applications is able to size and quantify PCR fragments, and was previously used for studying the genetic variability of Brucella spp. The strain and biovar typing of Brucella field samples isolated in outbreaks, detected by this method is useful for tracing back source of infection and may be crucial for discriminating naturally occurring outbreaks versus bioterrorist events, being Brucella a potential biological warfare agent [16].

Definitive diagnosis of brucellosis remains a difficult task. No single test is perfect, clinical history coupled with combination of two or more tests reduces diagnostic errors. Despite the vigorous attempt for more than one century to come up with a definitive diagnostic technique for brucellosis, diagnosis still relies on the combination of several tests to avoid false negative results. A lot of work needs to be done to approach the best diagnostic techniques in...
brucellosis diagnosis.

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