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Serological and molecular diagnosis of human brucellosis in Najran, Southwestern Saudi Arabia

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Summary This study aimed to investigate the prevalence of human brucellosis in Najran, southwestern Saudi Arabia, and to assess the performances of ELISA and PCR as diagnostic tools for brucellosis with respect to conventional methods. The study included 340 patients with clinical characteristics of brucellosis. Blood samples from cases and controls were subjected to culture, standard tube agglutination test (SAT), ELISA for IgM and IgG, and *brucella* PCR. The diagnosis of brucellosis was confirmed in 54 (15.9%) of the 340 provisionally diagnosed brucellosis patients. Blood culture identified only 14 (25.9%) cases. The SAT was positive for 50 (92.6%) cases, whereas ELISA IgM, IgG and PCR were found positive in 46, 52 and 38 cases respectively. The sensitivities of ELISA IgM and IgG were 85.2% and 96.3% respectively and the specificity was 100% for each. For PCR, the sensitivity and specificity were 70.4% and 100% respectively. In conclusion, ELISA offers a significant advantage over conventional serological methods in the diagnosis of brucellosis in endemic areas. The PCR test results can be particularly important in patients with clinical signs and symptoms, and negative serological results, allowing the early and rapid confirmation of the brucellosis.

Introduction

Brucellosis is a systemic disease caused by bacteria of the genus *Brucella* that affects humans and numerous animal species. The transmission to humans occurs by the ingestion of raw or unpasteurized milk and other dairy products, by direct contact with infected animal tissues, or by the accidental ingestion, inhalation, or injection of *Brucella* [1].

According to the World Health Organization, half a million of new human cases are reported each year, but these numbers greatly underestimate the true incidence of the disease in humans [2]. Although the disease incidence has decreased

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markedly in industrialized countries, brucellosis remains a major public health problem in many developing countries. In Saudi Arabia, brucellosis is hyper-endemic, with an incidence of 5.4 per 1000 per year. Its prevalence varies in different regions of the country, with values of 8.8—8% having been reported [3]. According to Memish and Mah [4], more than 8000 cases are reported per year to public health authorities.

In humans, brucellosis behaves as a systemic infection with a very heterogeneous clinical spectrum [5]. The infection is characterized by protean manifestations and prolonged recurrent febrile episodes. The features of acute disease are varied and may be insidious, whereas the features of chronic disease, which may persist or recur for years, are often vague [6]. The disease, therefore, cannot be diagnosed on a clinical basis alone, and microbiological confirmation is required through the isolation of *Brucella* spp. from blood cultures or the detection of specific antibodies through the use of serological tests. However, the established methods for laboratory diagnosis are often unreliable in several respects.

Culturing is a time-consuming procedure. Furthermore, failure to detect the pathogen is a frequent occurrence, and *Brucella* spp. are class III pathogens, posing considerable risk to laboratory personnel [7]. The common serological tests include the Rose Bengal plate agglutination test (RBPT), the standard tube agglutination test (SAT), the Coombs test and ELISA. Conventional serological methods also have important limitations: their sensitivity is poor during the early stage of the disease, and their specificity is reduced in areas where the disease is highly endemic and frequent relapses of the disease occur [8]. The development of specific PCR assays is a recent advance in the diagnosis of human brucellosis, but information concerning the use of this diagnostic tool is scarce.

This study aimed to investigate the prevalence of human brucellosis in Najran, southwestern Saudi Arabia, and to assess the performances of ELISA and PCR as diagnostic tools for brucellosis with respect to conventional methods.

Materials and methods

From April 2010 to September 2011, a total of 340 patients seen at the Infectious Diseases Clinic of King Khalid Hospital in Najran and presenting with clinical characteristics of brucellosis were included in this study. Based on the duration of their symptoms, the patients were classified into 3 groups: the acute group, with symptoms for <2 months ($N = 180$); the sub-acute group, with symptoms for 2—12 months ($N = 110$); and the chronic group, with symptoms for >1 year ($N = 50$). One hundred healthy personnel who were blood donors at the hospital's blood bank were enrolled as the controls; the median age of controls was 34.6 years (range, 18—57); and 74 were male, and 36 were female. Eight milliliters of blood (5 ml for culture and serology and 3 ml mixed with EDTA for PCR) was obtained from each patient and control. A diagnosis of brucellosis was made according to one of the following criteria: (i) the isolation of *Brucella* spp. in blood culture or (ii) the presence of a compatible clinical assessment together with a positive result by either SAT (at $\geq$1/160) or ELISA [8] or both.

Bacteriological and serological techniques

The blood cultures were processed inside a class III biological safety cabinet [7] using biphasic blood culture medium (BioMerieux, France) and were incubated at 37 °C in an atmosphere of 5—10% carbon dioxide for 30 days; sub-culturing was performed weekly. Suspected colonies were identified according to standard techniques [9].

For serology, all of the sera from the patients and controls were tested using SAT and ELISA for IgM and IgG antibodies against *Brucella* species. In the SAT, the serum samples were serially diluted in 0.5% saline, and equal volumes of the dilutions (from 1:10 to 1:1280) and of *B. abortus* and *B. melitensis* antigens (Omega Diagnostic Ltd, UK) were mixed in test tubes and incubated in an incubator at 37 °C for 24 h. Known negative and positive control sera were included. A titer of 1/160 was considered positive [8]. The ELISA testing for IgM and IgG against *Brucella* spp. was performed using commercial reagents (Genzyme Virotech, Germany). The absorbance values obtained were converted into Virotech units (VE) using the following formula, according to the manufacturer's instructions: patient sample (mean) absorbance $\times$ 10/mean absorbance value of cut-off controls (>11 VE was considered positive). Borderline results were re-tested and confirmed as either positive or negative.

Brucella PCR

The detection of a 223-bp target sequence within the gene coding for the production of a 31-kDa membrane protein specific to the genus *Brucella* was performed by PCR using specific primers (Qiagen, USA), as previously reported [10]. The sequences of these primers were forward