Analysis of human brucellosis sera using western blot assay

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=ABSTRACT=

The objective of the current study was to evaluate the efficiency of western blot (WB) assay for detecting Brucella specific IgG antibodies in the sera of brucellosis patients in Korea. A total of 87 human sera were tested by indirect enzyme-linked immunosorbent assay(ELISA), the tube agglutination test(TAT) and 2-mercaptoethanol agglutination test(2-MAT) for measuring Brucella specific antibody response. On the basis of optical density(OD) value of iELISA and patient’s treatment history, 17 sera were selected for WB assay. Sera were divided into five groups: Brucella positive sera(n=5), positive sera obtained from patients treated with antibiotics(n=7), equivocal sera(n=2), equivocal sera of patients treated with antibiotics(n=2) and negative serum(n=1). All groups of sera were analyzed by SDS-PAGE and WB assay using sonicated soluble antigen of Brucella abortus strain 2308. In WB assay, positive sera showed B. abortus specific strong bands at the molecular weight of 58 kD, 56 kD, 52 kD, 45 kD, 43 kD, 36 kD, 32 kD and 21 kD. Six antibiotic treated positive sera(PA group) did not show B. abortus specific bands. In equivocal sera, one serum showed strong band at 58 kD, 56 kD, 45 kD, 43 kD, 36 kD and 32kD. Strong reacting protein bands at 58 kD and 56 kD were appeared in one serum of antibiotic treated equivocal patient. It was concluded that WB assay could be a promising diagnostic tool for proper diagnosis of human patients in Korea infected with B. abortus.

Key words : human brucellosis, western blot assay, ELISA, Korea

Introduction

Brucellosis is an important zoonotic disease with worldwide distribution[1, 16]. It is caused by several species of the genus Brucella that have their natural reservoir in domestic animals[1, 16]. Brucellosis has emerged as a serious animal and public health issue in Korea[12, 19] despite animal control

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and eradication program. The first human case of brucellosis was reported in 2002 in Korea [18] since then more than 200 cases of brucellosis were recorded in farmers, veterinarians and artificial breeding technicians. Infection in both humans and cattle in Korea is commonly caused by B. abortus biotype 1 [2, 19]. In human this disease cause high clinical morbidity and protean clinical manifestations as any organ can be affected [7]. The disease is transmitted to humans through direct contact of infected animal and consumption of contaminated dairy products [17].

A key issue in the control of human and animal brucellosis is the availability of rapid, sensitive and specific diagnostic tests [13, 14]. It should be capable of differentiating between acute, chronic and subclinical disease and be specific enough to distinguish between Brucella and other cross-reacting organisms [5]. Currently the ELISA, TAT and 2-MAT are widely used for diagnosis of brucellosis but they have problems in distinguishing between false negative and positive results [3]. Microbiologic culture remains the gold standard but it is not always effective and may identify less than 65% of the cases that is why supplementary serologic tests are indicated [4]. The objective of the current study was to evaluate the efficiency of WB assay for the diagnosis of human brucellosis in Korea.

Materials and Methods

1. Study area and population

In our previous study [10] a total of 87 serum samples were collected from the residents of Chungnam and Chonbuk Provinces. In the present study 17 sera out of 87 sera were selected for WB analysis. On the basis of patient’s treatment history and ELISA OD values 17 sera were divided into five groups: Brucella positive (P group) sera(n=5), positive sera (n=7) of patients treated with antibiotics (PA group), equivocal (E group) sera(n=2), equivocal sera(n=2) of patients treated with antibiotics (EA group) and negative (N group) serum(n=1). Sera of PA and EA groups obtained from patients treated with antibiotics over a period of 6 months prior to WB analysis.

2. Serological tests

All serum samples were screened for measuring Brucella specific antibody titer by means of the LPS-based IgG iELISA, TAT and 2-MAT. The iELISA was performed according to the manufacturer’s protocol using Panbio diagnostic (USA). Screening of sera by TAT and 2-MAT was performed by using previously described routine protocols [15, 24, 25]. Serum IgG iELISA OD value of ≥ 1.1 and TAT and 2-MAT antibody titers of ≥ 1 : 160 and ≥ 1 : 140, respectively, were considered to be positive for Brucella. Serum IgG ELISA OD value of 0.9 ≤ 1.1 and TAT and 2-MAT antibody titers of ≥ 1 : 20 ≤ 1 : 80 and ≥ 1 : 20 ≤ 1 : 80 respectively, were considered as equivocal. Serum IgG ELISA OD value of ≤ 0.9 and TAT and 2-MAT antibody titers value of 0 were considered as negative to brucellosis.

3. Preparation of sonicated soluble antigen of B. abortus 2308

B. abortus 2308 obtained from Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Virginia 24061, USA. The bacteria were grown in bulk in Roux flasks at 37°C for 5 to 7 days under 5% CO₂. The bacterial culture was harvested and washed three times with sterile phosphate buffered saline (PBS) at 10,000 g for 15 minutes. The pellet was treated with 60% methanol for 1 week at room temperature and washed three times with sterile PBS by centrifugation at 10,000 g for 5 minutes at 4°C. The washed pellet was finally adjusted to a 10% suspension using 0.01 mol/L PBS at pH 7.4. The cell suspension was sonicated at melting ice temperature, applying 6 cycles at 100 W, each cycle was of 1 min duration. The sonicated cell lysate was centrifuged at 1,200 g for 20 min at 5°C. The supernatant was collected in 2 mL aliquots and stored at −20°C until tested.