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Short Paper

Comparison of Dot-ELISA with microbial culture for detection of *Brucella* spp. in clinical specimens

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Summary

Definitive diagnosis of brucellosis is made by isolation of the causative agents, which is a time-consuming procedure. To evaluate the efficacy of Dot-ELISA for detecting brucellae in clinical samples, 94 different specimens taken from animal origin were cultured on brucella selective culture media and colonies were identified biochemically. The specimens were also examined after centrifugation by Dot-ELISA using a specific anti-brucella antibody, a suitable peroxidase conjugate and substrate. Of the 94 samples, 5 (5.31%) were positive in Dot-ELISA and 4 (4.25%) had positive cultures. In comparison with culture, the sensitivity and specificity of Dot-ELISA for detection of brucellae in the samples was 80 and 100%, respectively. There was 98.9% agreement between the two tests. The results indicated that Dot-ELISA is a good and rapid test with acceptable sensitivity and specificity for detection of *Brucella* spp. in aborted fetal stomach contents.

Key words: Brucellosis, *Brucella* spp., Dot-ELISA

Introduction

Brucellosis is a common zoonotic disease in some parts of the world. In humans, the disease may present with a broad spectrum of clinical manifestations. *Brucella melitensis* and *B. abortus* are the main causative agents of caprine, ovine and bovine brucellosis. Infection with these bacteria appears to be widespread worldwide except in some areas where are believed to be free from the disease agents. Clinically, the disease in animals is characterized by abortion, retained placenta, orchitis, epididymitis and rarely, arthritis (Radostits *et al*., 2000).

Definitive diagnosis of suspected cases of animal brucellosis may have an important effect on the success of the eradication programs. At present, a definitive diagnosis of brucellosis is made only by isolation of the causative agents, which is a time-consuming procedure. The objective of the present study was to evaluate the efficacy of Dot-ELISA for detecting brucella in clinical samples.

Materials and Methods

Ninety-four clinical samples including 30 aborted fetal stomach contents, 32 vaginal secretions and 32 milk samples of suspected animals were taken from cases referred to the Veterinary Hospital of Shahid Chamran University, Ahvaz, Iran.

Bacterial isolation trials from samples were made on blood agar base (Oxoid) supplemented with 5% defibrinated sheep erythrocytes and antibiotics (vancomycin, nalidixic acid, bacitracin, nystatin and cyclo-hexamide at the dose recommended in OIE manual 2000). Cultures were incubated for 10 days with and without 5% CO_2 at 37°C. Bacterial isolates were identified according to the conventional procedures (Quinn *et al*., 1994). Thereafter, a part of the original

samples was heated for 1 hr at 70°C and centrifuged at 4,000 g for 10 min. The precipitates were washed twice with phosphate buffered saline (PBS) and centrifuged. The precipitates were then used for Dot-ELISA. Dot-ELISA was done according to the general principals of immunoblotting (Harlow and Lane, 1988).

A hyper-immune anti-brucella serum for using in Dot-ELISA was prepared in sheep by four bi-weekly injections of 1 ml heat-inactivated *B. abortus* vaccine (S19), produced in Razi Vaccine and Serum Research Institute, Karadj, Iran.

Twenty µl of each of the samples and controls were applied to nitrocellulose membrane (Amersham). The membrane was blocked in 5% skim milk in PBS for 45 min.

Membrane was washed three times, 5 min each, with PBS-T (PBS containing 0.05% Tween 20), was placed in 1:100 dilution of anti-brucella serum in PBS-T and incubated at room temperature for 1 hr. The membranes were then incubated in conjugate rabbit anti-sheep peroxidase (Sigma) diluted 1:1000 in PBS-T for 1 hr. The same washing procedure was repeated after incubation with the anti-sheep peroxidase conjugate, followed by reaction with a chromogen-substrate solution consisting of 30 µl of 30% H_2O_2 in 50 ml of PBS mixed with 30 mg of 4-chloro-1-naphtol (Sigma) in 10 ml of cold methanol. The results were determined by observation of violet-stained spots on the membrane.

Results

B. melitensis was isolated from five (5.31%) of the 94 samples. The remaining 89 specimens had negative cultures. All positive samples were from abomasal content of aborted fetuses. Four (80%) of the five culture-positive samples had also positive Dot-ELISA, yielding a sensitivity of 80% and a specificity of 100% for the test. There was 98.9% agreement between the two tests.

All culture-negative samples were also negative with Dot-ELISA test. The negative control produced a non-specific faint violet-stained spot, which was readily distinguishable from the positive control.

Discussion

Several techniques including co-agglutination (Erganis *et al*., 2002), polymerase chain reaction (PCR) (Fekete *et al*., 1992; Matar *et al*., 1996; Gallien *et al*., 1998; Cetinkaya *et al*., 1999) and direct fluorescent antibody tests (Corbel, 1973) have been evaluated for detection of *Brucella* spp. in clinical samples. The sensitivity and specificity of co-agglutination test for the diagnosis of *Brucella*-induced abortions were reported 61 and 80%, respectively (Erganis *et al*., 2002). Several workers have applied PCR methods for detection of *Brucella* spp. in human blood (Matar *et al*., 1996), naturally infected adult cows (Gallien *et al*., 1998) and in abortion clinical samples taken from cow and sheep (Fekete *et al*., 1992; Cetinkaya *et al*., 1999). All of these studies indicated that PCR gives a more accurate result than culture for detecting brucellae.

Enzyme immunoassays have been evaluated for many years for their diagnostic performance to detect serum antibodies against *Brucella* spp. (Nielsen, 1995; Nielsen and Kwok, 1995; Sarav *et al*., 1995; Samrtinoa *et al*., 1999). To the best of our knowledge, so far, no one has applied these methods for direct detection of these bacteria in clinical specimens. Dot-ELISA as presented in this study, can be adapted for this application. According to our results, Dot-ELISA is able to detect *Brucella* antigens in fetal stomach content, with an acceptable specificity and sensitivity. Dot-ELISA is rapid and the results are obtained within three hrs. The test is easy and can be performed in the least equipped laboratories. Therefore, it may be useful as a presumptive test for *Brucella*-induced abortion, particularly where rapid and accurate diagnosis commanded.

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