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Serodiagnosis of *Brucella* Infection in Aborted Cattle by an Indirect Enzyme-Linked Immunosorbent Assay in some Dairy Herds in Mashhad, Iran

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Abstract

Brucellosis is an infectious disease caused by different species of *Brucella*. These microorganisms cause disease in many different vertebrates. Pigs, cattle, goats, sheep and many kinds of wild animals are susceptible to *Brucella* infection. In Iran, brucellosis is a notifiable disease. While bacteriological isolation is the most specific diagnostic test, the frequency of isolation is usually low, and results are not available immediately. For this reason, many serological tests have been developed for the rapid diagnosis of brucellosis. Agglutination tests, Complement Fixation Tests and ELISA's are most widely used for screening or confirmation.

In the present study, one hundred and four blood samples were collected from aborted cows in different trimester of pregnancy from different industrial dairy herds in Mashhad, Iran. The blood samples were centrifuged at 2000 × g at room temperature for five minutes to separate sera. Serum samples were stored at -20ºC until used. Serum antibodies were tested by indirect ELISA.

The results of antibody detection by indirect ELISA showed that out of 104 serum samples, 8 samples were positive. These cows had been vaccinated against brucellosis between 3-6 months of age. From these positive samples, 5 (% 4.80) and 3 (% 2.88) samples were related to the second and third trimester of pregnancy, respectively. From 8 seropositivesamples in aborted cows, 1 (% 0.98) sample was positive of *Brucella abortus* in culture.

Key words: Brucellosis, Serum, Indirect ELISA, Cattle.

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Introduction

Brucellosis is an infectious disease caused by different species of *Brucella*. These microorganisms cause disease in many different vertebrates. Pigs, cattle, goats, sheep and many kinds of wild animals are susceptible to *Brucella* infection [1]. The disease is a widespread zoonosis which is transmitted to human through direct contact with infected animals or through consumption of contaminated raw animal products, especially unpasteurized milk and soft cheese [2]. Among the genus, *Brucella abortus* (*B. abortus)* and *Brucella melitensis (B. melitensis)* are the leading cause of brucellosis in livestock. They are also the most important causal agent of brucellosis in humans. Infections in animals that are caused with *Brucella* spp. frequently result in abortions and diminished level of milk production. From clinical point of view, *Brucella abortus* is one of the most prevalent *Brucella* spp [3, 4]. Infection rates vary greatly from one country to another, within a country and production systems [5]. Brucellosis is prevalent on the southern and eastern edges of Mediterranean basin, particularly in Tunisia, Libya, Egypt, Syria, and in the Arabian Peninsula and Iran [6]. In Iran, brucellosis is a notifiable disease and despite the existing data about the disease, little has been documented about the prevalence of *Brucella* in dairy cattle in Iran [7].

Diagnosis of brucellosis in cattle is difficult to establish because of the variable time of incubation and the absence of clinical signs other than abortion [8]. *Brucella* diagnosis is a challenge, as no reliable ante mortem gold standard exists for determining infection status. While bacterial culture is considered 100% specific for brucellosis, it requires specific media and specialized incubation conditions, and Brucella's slow growth rate often leads to overgrowth of non target bacteria on the culture plates [9]. The rate of isolation of *B. abortus* from blood or tissue cultures generally presents low sensitivity and the results are not available immediately because this procedure may be time consuming and processing a large number of samples is cumbersome, the diagnosis is based mainly on serological methods [10, 11]. Although the Rose Bengal test (RBT) and complement fixation test (CFT) are commonly used for the routine serological diagnosis of ovine, caprine and bovine brucellosis [12,13], many serological tests, such as serum agglutination test (SAT), milk ring test (MRT) and enzyme-linked immunosorbent assay being used in the diagnosis of animal brucellosis [14, 15, 16]. These tests are mainly based on the detection of antibodies directed against the lipopolysaccharide (LPS) portion of the cell membrane which induces a strong antibody response [17, 18, 19]. The classical serological techniques rely mainly on the detection of antibodies to this antigen fraction [11].

ELISAs have been evaluated for diagnostic performance to detect serum antibodies to *B. abortus* in cattle, and such techniques offer several advantages over other tests, including that sera do not need to be heat inactivated as for CFT or pretreated with 2-mercaptoethanol (2ME) as for SAT/2ME; the reactivity is measured objectively, reducing subjective errors; and they are particularly advantageous in mass testing programs [20, 21].

The purpose of this study was to detect the rate of *Brucella* infection in aborted cows in different trimester of pregnancy from

different industrial dairy herds in Mashhad by indirect enzyme-linked immunosorbent assay (ELISA) for the diagnosis of bovine brucellosis.

Material and Methods

1- Sample Collection. One hundred and four blood samples were collected from aborted cows in different trimester of pregnancy from different industrial dairy herds in Mashhad, Iran. Sixty samples were related to third and 44 samples were related to second trimester, respectively. The blood samples were centrifuged at 2000 × g at room temperature for five minutes to separate sera. Serum samples were stored at -20ºC until used.

2- Medium culture

Brucella abortus was cultured on *Brucella* agar medium. The cultures were incubated for 72 hours at 37 ºC.

3- Indirect ELISA assay. Serum samples were tested for the presence of antibody against *Brucella abortus* using SERELISA *Brucella* OCB kit (Synbiotics Europe, France), in a 96-well micro titration plates. Tests were carried out in duplicate. According to the manual, serum samples were diluted (1:1) by wash solution and 100 µl of diluted sera was loaded into wells and incubated for 2 hours at 37 ºC. Positive and negative control sera were used as indicated in the kit. The wells were washed five times with 300 µl of wash solution. Following the final washing, the plates slapped vigorously, well down on a bench top which covered with paper towels. Then, 100 µl of Horseradish Peroxidase (HRP) conjugated was loaded into all the wells and incubated for one hour at room temperature. The plates were washed as described above to remove the excess conjugate. For color development, 100 µl of 3, 3', 5, 5'-Tetramethyl Benzedrine (TMB) substrate solution (TMB/H2O2 solution) was added to each well and incubated at room temperature for 10 minutes in the dark. The reaction was terminated by the addition of 100 µl of stop solution to each well. The absorbance at 450 nm was monitored in ELISA reader.

4- Test validation

According to SERELISA *Brucella* OCB kit (Synbiotics Europe, France), any sample presenting an index ≥ 0 is considered as positive and any sample presenting an index < 0 is considered as negative. According to manual, samples are calculated as follow:

Sample index= 0.50 × (sample OD – 0.6 ×OD P)

5- Statistical analysis

The statistical analysis was performed using SPSS package ver.16. The time of abortion and *Brucella* infection was analyzed with Chi-squared test. *P* value ≤ 0.05 was considered as significant.

Results

The presence of antibody against *Brucella abortus* in sera from cattle with history of abortion was investigated by indirect ELISA. The results of antibody detection by indirect ELISA showed that out of 104 serum samples, 8 (%7.7) samples were positive according to manual. These cows had been vaccinated against brucellosis between 3-6 months of age. From these positive samples, 5 (% 4.80) and 3 (% 2.88) samples were related to the second and

third trimester of pregnancy, respectively. From 8 seropositive samples in aborted cows, 1 (% 0.98) sample was positive of *Brucella abortus* in culture. There was no significant relationship between time of abortion and *Brucella* infection (P= 0.24)

Discussion

Brucellosis is one of the main diseases causing serious economic losses in milk production. In the specific case of *Brucella* testing, the difficulties with culture have resulted in the lack of a true ‘‘gold standard’’ to detect infection (8). Serology provides the best opportunity for successful and accurate diagnosis of *B. abortus* infections [8]. In many countries, RBT is considered as a screening test, followed by a confirmatory test such as the CFT, to detect brucellosis in an infected flock [15]. When the complement fixation test (CFT) was considered as a gold standard test, indirect ELISA was the most sensitive test (98%), followed by serum agglutination test (SAT) (94%) and Rose Bengal test (RBT) (91%), respectively [16]. The serological test results are usually considered as an indication of brucellosis and provide presumptive identification [22, 23]. Bovine brucellosis has been controlled by programs that are based on *B. abortus* S19 vaccination using different schemes followed by serological diagnosis and elimination of seropositive animals [24]. Thus, diagnostic procedures for brucellosis are required to differentiate antibody responses after vaccination from natural infection, and they should be specific, sensitive, and able to detect all stages of infection. Currently, no such test exists. ELISAs have been evaluated as alternative techniques for this purpose [19].

Our results showed that the prevalence of *Brucella abortus* in aborted dairy cattle by an indirect ELISA in Mashhad area was % 7.70. In Iran, the first *B. melitensis* isolation from an aborted sheep fetus was reported in 1950 [25, 26]. Thereafter it has been widely isolated in different parts of the country mainly from sheep and goats but also occasionally from cattle, camel, and sheepdogs [27]. Hamali and Jafari Joozan (2011) were reported that Six out of 76 dams (7.8 percent) were seropositive to the *Brucella* spp. They indicated that ELISA and PCR protocols have equal value for diagnosis of abortions caused by *Brucella* spp. [28]. Moshkelani *et al* (2011) were reported that in total of the 276 specimens, 40 (14.4%) and 25 (9.0%) were identified positive for *Brucella* spp. and *Leptospira* spp., respectively [29]. A total number of 851 aborted sheep and goat fetuses were cultured microbiologically and 265 *Brucella* were isolated. Biotyping of these 265 isolates showed that 246 (92.8%) were *B. melitensis* biovar 1. Eighteen isolates (6.8%) were identified as *B. melitensis* biovar 2; and interestingly, one isolate (0.4%), which was obtained from Mazandaran province, was determined as *B. abortus* biovar 3. *B. melitensis* biovar 3 was isolated in none of the six provinces during the study period, and *B. melitensis* biovar 2 was only isolated in two provinces which are Khorasan Razavi and Kerman belonging to intermediate and low prevalence categories, respectively [30].

Sabbaghian (1975) in Isfahan province of Iran isolated *B. melitensis* from 56 of 677 cheese samples and 1 of 160 cream samples. After an epidemiological study on human brucellosis, they also reported that raw dairy product consumption is the most probable source of *Brucella* infection in

urban area [31]. The prevalence rate of *Brucella* in the examined cheese of Sarab city, which is located in East Azarbayjan province, was reported in spring and summer 2.5 and 2.62% respectively. Overall 2.2% of examined cheese samples were contaminated with *B. abortus* and *B. melitensis*. This result revealed an important and serious public health problem [7]. In another study, in Khoy city, nearby the Urmia, in West Azerbayjan province, the prevalence of brucellosis has been reported to be 26.66% [positive milk ring test (MRT)] in spring, 2008. In contrast, Maadi *et al.* (2011) reported that the prevalence of brucellosis in cattle was 1.22% in spring in Urmia [32]. A study which was carried out in Iran showed that, consumption of unpasteurized dairy products is significant risk factor for human brucellosis [33].

In Turkey, a total of 626 serum samples of cattle obtained from 27 herds with a history of abortions was examined for *Brucella* antibodies by RBPT, SAT and ELISA. Of the cattle sera analyzed, 221 (35.30%) and 206 (32.92%) and 247 (39.45%) were found to be positive by RBPT, SAT and ELISA, respectively [34]. Otlu *et al* (2008) reported that a total of 407 serum samples of cattle from 27 herds having history of abortions were examined for *Brucella* antibodies by RBPT and SAT. Of the cattle sera analyzed, 134 (32.92%) and 141 (34.64%) were determined as positive by RBPT and SAT, respectively [35]. In another study in Turkey, the antibodies against *B. abortus* were detected in serum samples of aborted dairy cattle as 68.1, 65.6, 58.9 and 55.2% by the Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA), Complement Fixation Test (CFT), Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT), respectively [36]. The true prevalence of antibodies against *Brucella* in Jordan in cows and cattle herds was 6.5% and 23%, respectively. The seroprevalence of brucellosis in cows older than 4 years of age was significantly higher than that in the younger cows [37]. Despite its control in many developed countries the disease remains endemic in Saudi Arabia where the national seroprevalence of the disease is 15% [38]. It has been reported an overall herd seroprevalence rate of brucellosis of 3.14% in cattle herds and 2.94% in small ruminant flock in Syria [39].

Bovine brucellosis has recently been reported from different countries in Africa including Ethiopia, Zambia and Zimbabwe, indicating that the disease is present throughout the continent [40, 41, 42, 43]. It has been reported that bovine brucellosis is widely distributed in indigenous cattle breeds of the Western Zone of Tigray, Ethiopia [5]. It has been reported a seroprevalence of brucellosis ranging from 5.3% to 6.2% for trade cattle slaughtered between 2004 and 2006 at the Bodija Municipal Abattoir in Ibadan in southwestern Nigeria [44]. Bovine brucellosis was also reported from two of Nigeria's neighbor countries, Chad and Cameroon [45, 46]**.** In another study in Egypt, the true proportions of brucellosis were estimated as 0.79%, 0.13, 1.16% and 0.44% among cattle, buffaloes, sheep and goats respectively [47].

In conclusion, our study showed that the rate of infection in aborted cows was higher in third trimester than second trimester which was detected by indirect ELISA in our region.

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