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The First Isolation of *Brucella melitensis* from Bovine Aborted Fetus in Turkey

Esra Buyukcangaz*, and Aysin Sen
University of Uludag, Faculty of Veterinary Medicine, Animal Hospital, Department of Microbiology, 16059 Görükle / Bursa, TURKEY

ABSTRACT

Between 2004 and 2005 in Marmara region, Turkey, 41 abortion cases from cattle was investigated by standard isolation procedures for determinating possible role of *Brucella* spp. The isolates from 8 (19.5 %) of 41 bovine abortion cases were identified as *Brucella* spp. Biotyping revealed that, 7 (87.5 %) isolates were *B. abortus* biotype 3 and one (12.5 %) was *B. melitensis* biotype 3. Since the isolation of *B. melitensis* from an aborted bovine fetus was not substantially reported in Turkey until now, this report may be considered one of the indicative studies.

Key words: *B. abortus, B. melitensis*, bovine, abortion, homogenate, biotyping.

INTRODUCTION

Brucellosis is considered to be the most widespread zoonosis all around the world by FAO, WHO and OIE (Anonymous, 2007). Bovine brucellosis usually caused by *B. abortus*, less frequently by *B. melitensis,* is responsible for important economic losses in cattle farming and risk of human health. The disease is endemic particularly in Mediterranean and Middle East regions, parts of Africa, Europe and Latin America (Anonymous 1997; Corbel 1997). A study is carried out in different regions of Turkey has shown that seroprevalance of *B. abortus* is approximatelly 1.43 % (Iyisan et al 2000). Diagnosis of *Brucella* spp. infection is mainly based on the detection of antibodies in serum by serological tests (Nielsen 2002). Although serological tests are being used for monitoring the herds, bacteriological isolation is still the gold standard for either screening of the infection or preparing eradication programs (Bricker 2002).

The main purpose of this study was to determine the presence and biotypes of *Brucella* spp. in bovine abortion cases in Marmara region, Turkey.

MATERIALS AND METHODS

41 bovine abortion cases occurring in different herds in two birth seasons from Yenisehir, Inegol, Gonen, Mustafakemalpasa, Karacabey, Iznik, Manyas, Susurluk towns and İstanbul, Çanakkale, Balıkesir, Tekirdağ and Bursa city were evaluated. Samples were taken from several private farms having herd size of more than 50 animals. In this study, breeds and rearing systems of animals were not considered.

Aborted fetuses were necropsized and internal organs (lung, spleen, heart and liver) and their abomasum contents were taken for specimen. Abomasum contents were removed by searing an area of stomach wall with a heated spatula, plunging the tip of a sterile injector through the seared area and transferred some of the contents and inoculated to the Blood Agar Base No. 2 (Oxoid® CM 271) containing 7% defibrinated sheep blood and *Brucella* Medium Base (Oxoid®-CM0169) containing *Brucella* Selective Supplement (Oxoid® SR83). Pieces from internal tissues of aborted fetuses were collected with set of sterile forceps and scissors and flamed after plunged to ethanole. Tissues were dissolved in the sterile saline water at the same concentration and homogenized with ultraturrax (Miccra RT-D9 ®).

The polyclonal *Brucella* A, M, and A+M antisera, and reference strains; *B. abortus* biovar 1 (544-ATCC 23448), *B. abortus* biovar 2 (86/8/59-ATCC 23449), *B. abortus* biovar 3 (Tulya- ATCC 23450), *B. abortus* S19 (vaccine strain) and *B. melitensis* biovar 3 (Ether-ATCC 23458), were provided from the *Brucella* Laboratory of Pendik Veterinary Control and Research Institute, Istanbul, Turkey.

In smears of fetal membranes and abomasum content, the red stained organisms against blue background and gram negative coco-bacils were sought by Modified Ziehl-Neelsen method and Gram stain, respectively. Abomasum contents and homogenates removed for culture to the agar plates and incubated in 37 °C and 5 % CO_2 conditions, 3-8 days for growth. Colony morphology and opacity were detected by stereomicroscope (Olympus SZ61®). For distinguishing smooth and rough colony formation, a solution of neutral Acriflavine (Sigma® A 8126) was prepared at 1:1000 dilution freshly on the day, and a small amount of culture examined the slide under a low power stereomicroscope. The agglutination features of colonies were examined by polyclonal *Brucella* A and M antisera.

* Corresponding author: kocakaya@uludag.edu.tr

Serum Dextrose Agar (SDA) was used as base media for dye and antibiotic susceptibility tests. Suspansions were prepared by fresh culture in 1 ml of sterile saline. A sterile cotton swab was immersed in the bacterial suspension and four suspensions inoculated parallel each SDA plate. For determinating of susceptibility of phage lysis, 20 µl. of Tbilisi phage at $10^{4} \times$ RTD were dropped into inoculation zone. Growth in prescence of thionin (1/50.000), basic fuchsin (1/50.000), safranin-O (1/10.00), production of hydrogen sulphide, agglutination with polyclonal monospesific anti-A, anti-M sera were investigated for biotyping. Growth features in 37 °C and 5 % CO_2 conditions of 2.5 µg/ml concentration of penicillin and 1 µg/ml of i-erithritol were examined for differentiation between field strain and S19 (Alton et al 1988). Oxidase test was performed by the Kovaks' method. Urease activity of isolates were determined in Christensen's medium All plates were incubated at 37° C and %5 CO_2 conditions for requirement of CO_2, growth after 3-4 days (Alton et al 1988; Quinn et al 2002; Timoney et al 1988).

RESULTS

Brucella spp. were isolated from 8 (19.5 %) of 41 aborted fetuses. It was possible to isolate the agent in both the abomasum content and internal organ homogenates in all cases. The colony morphology of all 8 isolates was smooth and all were negative in the agglutination test with acriflavine and positive to catalase, oxidase, and urease. All isolated strains except one produced H_2S and required CO_2 for isolation. The lysis with *Tbilisi* phage was positive for 7 of 8 isolates and growth features in I-erithritol, penicillin and dyes were all positive. Seven of the 8 *Brucella* isolates were identified as *B. abortus*. All of seven *B. abortus* isolates were identified as *B. abortus biotype* 3 (87.5 %) and one isolate (Number: 5) was identified as *B. melitensis* biotype 3 (12.5 %) after biotyping. The biochemical identification characteristics and biotypes of isolates are shown in Table 1.

Table 1. Identification and Biotyping Tests of Bovine *Brucella* Isolates

Isolate Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Colony Morphology	S	S	S	S	S	S	S	S
Oxidase	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+
Urea	+	+	+	+	+	+	+	+
Agglutinatination with Acriflavine	-	-	-	-	-	-	-	-
H_2S	+	+	+	+	-	+	+	+
Lysis by *Tbilisi* phage	+	+	+	+	-	+	+	+
Penicilin	+	+	+	+	+	+	+	+
Basic Fuchcin	+	+	+	+	+	+	+	+
Thionin	+	+	+	+	+	+	+	+
Safranin- O	+	+	+	+	+	+	+	+
I-erithritol	+	+	+	+	+	+	+	+
Anti-A	+	+	+	+	+	+	+	+
Anti-M	-	-	-	-	+	-	-	-
Anti-A+M	+	+	+	+	+	+	+	+
Biovars	*B.abortus* biovar 3	*B.abortus* biovar 3	*B.abortus* biovar 3	*B.abortus* biovar 3	*B.melitensis* biovar 3	*B.abortus* biovar 3	*B.abortus* biovar 3	*B.abortus* biovar 3

S: Smooth

DISCUSSION

Bovine brucellosis caused by *B. abortus* has a major impact on human health, besides causing significant economical losses in animal husbandry. *B. abortus* is highly virulent biotype for humans (Dokuzoguz et al 2005). Bovine brucellosis–free European countries declared by EFSA (Anonymous 2008) are Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Luxemburg, The Netherlands, Slovakia, Sweden, United Kingdom, Norway and Switzerland. The disease is largely controlled by European Union supported eradication programs in Greece, Italy, Portugal, and Spain. Brucellosis is endemic and has high prevalence in Balkan Region (Taleski et al 2002), West Asia and Latin American countries (Anonymous 2008; Refai 2002). In Turkey, the occurrence of the disease is monitored by limited numbers of epidemiological studies (Atesoğlu 1998; Unver et al 2006).

There are seven biotypes of *B. abortus*, which are isolated in different countries. *B. abortus* biotype 3 is the most prevalent biotype in Turkey and other biovars determined were 1, 2, 4, and 6 for *B. abortus* (Refai 2002). In the study, seven (87.5 %) of eight isolates were found to be *B. abortus* biovar 3. This finding suggests that *B. abortus* biovar 3 plays an important role in bovine abortion cases in Marmara Region, Turkey.

Regarding the epidemiological aspects, it is important to emphasize the role of *B. abortus* S19 in abortion cases in countries like Turkey where cattle are vaccinated widely. *B.abortus* S19 isolation was reported in bovine aborted fetuses and milk secrets in various studies (Anonymous 2007a). Even the animals in this study were not monitored about vaccination in gestation period, none of the 7 *B. abortus* isolates was identified as a vaccine strain. This result shows us that *B. abortus* S19 was not responsible for bovine abortion cases in the study area.

Brucellosis in cattle is usually caused by biovars of *Brucella abortus*. In some countries, particularly in Southern Europe and Western Asia, where cattle are kept in close association with sheep and goats, infection can also be caused by *B. melitensis* (Anonymous 2007b). This picture will be a significant zoonotic problem for cattle and human health. Refai (2002), has reported that only *B. melitensis* biovar 2 was responsible of brucellosis abortion cases which were taken from sheep, goat, cattle and camel in Saudi Arabia, in ten years period. In our study, one of eight isolate was identified as *B. melitensis* biovar 3. Since the isolation of *B. melitensis* from an aborted bovine fetus was not substantially reported in Turkey until now, this report may be considered one of the indicative studies. Some investigators reported that *B. melitensis* was regularly isolated from cattle in Southern Europe and it was considered that *B. melitensis* has replaced of *B. abortus* in cattle (Godfoid and Käsbohrer 2002). The nature of infection and the immune response in cattle against *B. melitensis* is still unknown. The use of Rev 1 vaccine and its safety in cattle must be investigated clearly. Incidence of *B. melitensis* in cattle should be urgently substantiated by validation studies.

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