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Isolation of Brucella Organisms From the Milk of Seronegative Cows

E. ZOGHI, A. EBADI and B. MOHSENI

Summary: During an investigation of bovine brucellosis in Iran, coducted by the Razi Institute over a twelve-month period, samples of serum and milk were collected simultaneously from 6,472 cows in eight infected herds for serological and bacterilogical testing. A total of 1,056 cows were serologically positive and 1,632 of 6,472 milk samples were positive to the milk ring test (MRT). Culture of the positive milk samples yielded 397 isolates of Brucella, 119 of which came from the 5, 686 seronegative cows. The isolates belonged to Brucella abortus biotypes 2 (one isolate), 3 (356 isolates) and 9 (40 isolates).

Keywords: *Brucella abortus / Cows / Milk hygiene.*

Introduction

Control of brucellosis in cattle in Iran is based mainly on detecting infected animals, followed by the slaughter of these animals. Since it is not feasible to isolate the causative organism from infected cases, serological testing is important in routine diagnosis of the disease. Other reports on bovine brucellosis refer to the isolation of *Brucella* organism from the milk of seronegative cows (2,5,6,7). Therefore, in conjunction with a scheme for controlling brucellosis in cattle, the culture of milk samples was carried out in addition to serological tests.

Materials and Methods

For one year, serum and milk samples were taken simultaneously from 6,472 cows in eight infected herds. All serum samples were tested and interpreted by RBPT, SAT, CFT and 2-MET tests according to the methods recommended by the FAO and WHO (1,3,8). Positive milk ring test (MRT) samples were inoculated onto serum dextrose agar antibiotic plates (1,3,9). All plates were incubated at 37°C in a carbon dioxide incubator suitable for *Brucella* organisms. Cultures were examined three to five, and sometimes six to seven, days later for evidence of *Brucells*-like colonies. Subculatures of colonies, after being checked for purity and agglutinability with mono-specific sera, were biotyped using the techniques recommended by the FAO and WHO (1,4,10).

Results

Of the 6,472 serum samples tested, 1,956 were positive for *Brucells* infection.

At the same time, 1,632 of the 6,472 milk samples reacted in the MRT. ALL MRT-positive samples were cultured. *Brucells* cultures were obtained from 397 samples; 119 of the cultures came from the 5,686 seronegative cows (2.09%). The isolates belonged to *Brucells abortus* biotypes 2, 3 and 9, as shown in Table I.

Table 1. Biotypes of B. abortus obtained from 397 milk sampes

B. abnortus Biotype	Seronegative cows	Seropositive cows	Total
2	--	1	1
3	103	253	356
9	16	24	40
Total	119	278	397

Discussion

A number of circumstances complicate the diagnosis of bovine brucellosis. Since the introduction of the *Brucella* organism into the body is followed by the appearance of antibodies in the blood, a combination of serological tests can be used to detect infected animals. However, these tests have limitations, particularly after the disease has entered the chronic stage, when the organism is harboured interacellularly, often in the suprâmammary lymph nodes and the udder. In this situation, antibody titres may decline or remain around the diagnostic threshold. Some such animals may shed *Brucella* organisms in the milk (2,5,6). Therefore, in relation to brucellosis eradication, it is advisable to test milk samples bacteriologically in addition to serological tests.

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