Isolation of *Brucella* organisms from the milk of seronegative cows

E. ZOWGHI, A. EBADI and B. MOHSENI

**Summary:** During an investigation of bovine brucellosis in Iran, conducted 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 bacteriological 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* - Brucellosis - Cattle diseases - Diagnostic techniques - Iran - Milk.

**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 *Brucella*-like colonies. Subcultures 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).

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RESULTS

Of the 6,472 serum samples tested, 1,056 were positive for Brucella infection.

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

<table>
<thead>
<tr>
<th>B. abortus biotype</th>
<th>Seronegative cows</th>
<th>Seropositive cows</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>103</td>
<td>253</td>
<td>356</td>
</tr>
<tr>
<td>9</td>
<td>16</td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>278</td>
<td>397</td>
</tr>
</tbody>
</table>

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 intracellularly, often in the supramammary 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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Résumé : Lors d'une étude conduite en Iran par l'Institut Razi sur la brucellose bovine, sur une période de douze mois, des échantillons de sérum et de lait ont été recueillis simultanément chez 6 472 vaches de huit élevages infectés, aux fins de contrôles sérologiques et bactériologiques. Au total, 1 056 vaches étaient séropositives et, sur les 6 472 échantillons de lait, 1 632 étaient positifs à l'épreuve de l'anneau. La mise en culture des échantillons de lait positifs a permis d'isoler 397 souches de *Brucella* dont 119 provenaient des 5 686 vaches séronégatives. Ces souches appartenaient à *Brucella abortus*, types 2 (1 souche), 3 (356 souches) et 9 (40 souches).

MOTS-CLÉS : Brucella abortus - Brucellose - Iran - Lait - Maladies des bovins - Techniques de diagnostic.

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RESUMEN: En ocasión de un estudio sobre brucelosis bovina llevado a cabo por el Instituto Razi en Irán, durante doce meses, se recogieron muestras de suero y de leche simultáneamente en 6472 vacas de ocho rebaños infectados, para control serológico y bacteriológico. Resultaron seropositivas 1056 vacas del total, y sobre los 6472 muestras de leche, 1632 se mostraron positivos tras la prueba del anillo. El cultivo de los muestras de leche positivos permitió aislar 397 cepas de *Brucella*, 119 de las cuales provenían de 5686 vacas seronegativas. Las cepas eran de *B. abortus*, tipos 2 (una cepa), 3 (356 cepas) y 9 (40 cepas).

PALABRAS CLAVE: Brucella abortus - Brucelosis - Enfermedades de los bovinos - Irán - Leche - Técnicas de diagnostico.

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REFERENCES


