Naturally occurring *Brucella melitensis* infection in cattle in Iran

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Summary: Brucella melitensis infection in cattle was investigated between 1971 and 1984 at the Razi Institute in Iran. A total of 1,407 fetuses and 21,196 milk samples were examined bacteriologically and B. melitensis was isolated from 48 (3.4%) and 52 (0.24%) cases, respectively. The prevalence of this infection in cattle in Iran was increasing. Since brucellosis is a zoonosis, reduction of its incidence in animals would result in fewer human cases.


INTRODUCTION

*Brucella melitensis*, a causative agent of abortion in sheep and goats, was isolated in Iran for the first time in Isfahan in 1950 (6) from the milk and fetus of a goat and subsequently from fetuses, milk and milk products of sheep and goats from different parts of the country.

In Iran, brucellosis in cattle is chiefly caused by *Brucella abortus*, but sometimes *B. melitensis* may infect cattle even under normal conditions (2).

Infection of cattle with *B. melitensis* has been reported in several countries in conjunction with infection in sheep and goats (5, 7).

The first reported isolation of *B. melitensis* from cattle in Iran, as a cause of abortion, goes back to 1964, following a survey of some dairy farms around Tehran. Entessar and Ardalan (unpublished data) reported the isolation of the organism from an aborted fetus in 1955, and from the milk of a cow in 1963.

Since typing of *Brucella* strains commenced at the Razi Institute in 1971, *B. melitensis* has been isolated on numerous occasions from fetuses and milk of cattle.

Cattle, sheep and goats are the principal farm animals in Iran, and the prevalence of *B. melitensis* in sheep and goats would be expected to lead to the infection of cattle.

The organism can become localized in the uterus, with occasional abortion, or in the supramammary lymph nodes and the udder, with excretion in the milk. On the other hand, it may remain in tissues of infected cattle for a long time, at least a month and possibly for years, without the animal showing any clinical signs. The chronic stage may well be accompanied by a waning of antibody titre to serological

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tests, often to below or around the diagnostic titre. Therefore bacterial examination is important for diagnosis.

This report provides particulars of the *B. melitensis* cases seen in cattle in Iran between 1971 and 1984.

**MATERIALS AND METHODS**

**Samples**

A total of 1,407 fetuses and 21,196 milk samples from cattle were examined. Milk samples positive to the milk ring test (MRT) were cultured directly and all other samples were inoculated onto serum dextrose agar antibiotic plates (1, 3).

**MRT antigen**

Haematoxylin-stained antigen for the milk ring test was prepared and standardized by using *B. abortus* strain 19 or 99, according to the method recommended by Alton *et al.* (1).

**Brucella antisera**

Monospecific serum against *B. abortus* (anti-A) and *B. melitensis* (anti-M) was prepared and tested by inoculating rabbits with *B. abortus* strain 544 or *B. melitensis* strain 16M (1).

**Milk ring test**

To 1 ml of a milk sample in a narrow tube, one drop of antigen was added. These were gently mixed and incubated at 37°C. The test was read and results recorded after 1-3 hours of incubation (1, 3).

**Cultures**

Milk samples positive to the ring test and fetal samples were each inoculated on 3-5 plates and incubated at 37°C in an ordinary incubator. They were examined 3 to 5, and sometimes 6 to 7 days later for *Brucella*-like colonies. The plates were discarded if no growth was evident after 7 days of incubation. *Brucella*-like colonies were emulsified in sterile normal saline and acriflavine, and tested for agglutinability using *B. melitensis* and *B. abortus* monospecific antiserum and *Brucella* negative serum. Subcultures of colonies which agglutinated in *B. melitensis* antiserum were prepared on brucella agar slopes incubated at 37°C for 2 to 3 days. The production of hydrogen sulphide was evaluated by using lead acetate paper in tubes containing the brucella agar medium. The subcultures, after being checked for purity and agglutinability, were typed by the procedure recommended by Alton *et al.* (1) and Corbel *et al.* (4).

**RESULTS**

Identifications of *B. melitensis* infection in cattle in Iran from 1971 to 1984 are shown in Table I. From a total of 22,603 fetuses and milk samples, *B. melitensis* was isolated and identified in 100 (0.44%). The rate of positive culture for fetuses
TABLE I
The results of tests for *Brucella melitensis* infection in cattle in Iran from 1971 to 1984

<table>
<thead>
<tr>
<th>Samples</th>
<th>No. of samples tested</th>
<th>No. positive for <em>B. melitensis</em> (%</th>
<th>Biotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetus</td>
<td>1,407</td>
<td>48</td>
<td>3.4</td>
</tr>
<tr>
<td>Milk</td>
<td>21,196</td>
<td>52</td>
<td>0.24</td>
</tr>
<tr>
<td>Total</td>
<td>22,603</td>
<td>100</td>
<td>0.44</td>
</tr>
</tbody>
</table>

was 3.4% (48/1,407) and for milk samples, 0.24% (52/21,196). All isolates were classified by biotype as previously reported (8).

**DISCUSSION**

Since 1950, when *B. melitensis* was reported from goat milk in Iran, this organism has been isolated sporadically in different parts of the country. In an area where ovine and caprine brucellosis is endemic, cattle could be expected to carry *B. melitensis*. This organism was first isolated from a bovine fetus in 1955, and from cow milk in 1963 (Entessar and Ardalan, unpublished data). In the present investigations, *B. melitensis* biotypes 1 and 2 were isolated from 52 samples of raw milk and 48 fetuses.

The results suggest an increasing trend in the prevalence of *B. melitensis* infection in cattle. If such cases become more common, the health problems created by brucellosis will obviously become more important in the areas where ovine and caprine brucellosis is serious. The extension of brucellosis among sheep and goats may not only be a source of infection to other animals, such as cattle, but to human beings as well. The following proposals are made to prevent the spread of infection.

The regional distribution of infection can be indicated by differential tests, and the movement of animals should be controlled by appropriate regulations. Infected animals must be slaughtered and, once a year, all calves should be vaccinated with strain 19 and all lambs with Rev. 1 vaccine.

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**INFECTION NATURELLE À *BRUCELLA MELITENSIS* CHEZ LES BOVINS EN IRAN. — E. Zowghi et A. Ebadi.**

Résumé : L'Institut Razi, en Iran, a étudié de 1971 à 1984 l'infection due à *Brucella melitensis* chez les bovins. Au total, 1,407 fœtus avortés et
21.196 échantillons de lait ont été soumis à des analyses bactériologiques, et B. melitensis a été isolé dans 48 (3,4%) des premiers et 52 (0,24%) des seconds. On a constaté une prévalence grandissante de cette infection chez les bovins. La brucellose étant une zoonose, la réduction de son incidence chez les animaux permettrait de diminuer le nombre de cas de la maladie chez l'homme.


INFECCIÓN NATURAL POR BRUCELLA MELOTENISIS DE LOS VACUNOS EN IRÁN. — E. Zowghi y A. Ebadi.

Resumen : El Instituto Razi de Irán estuvo estudiando de 1971 a 1984 la infección debida a Brucella melitensis en los vacunos. En total, se hicieron análisis bacteriológicos de 1.407 fetos abortados y 21.196 muestras de leche, aislando B. melitensis en 48 (3,4%) de los primeros y 52 (0,24%) de los segundos. Se comprobó una prevalencia en aumento de la infección en los vacunos. La brucelosis, al ser una zoonosis, si se disminuye su incidencia en los animales, se podrá reducir el número de casos de la enfermedad en el hombre.


REFERENCES