Serological investigations on brucellosis in cattle, sheep and goats in Iran

E. ZOWGHI and A. EBADI*

Summary: The prevalence of brucellosis in cattle, sheep and goats in Iran was investigated serologically during the years 1970-1984. The number of serum samples tested in this period was 175,676 from cattle and 110,817 from sheep and goats. The percentages of seropositive samples were 17.6% for cattle and 14.7% for sheep and goats. The results obtained demonstrate the value of the simultaneous use of various serological tests to provide accurate interpretation.

KEY-WORDS: Brucellosis - Cattle - Epidemiological surveys - Goats - Iran - Serological techniques - Sheep.

INTRODUCTION

Eradication of brucellosis is based mainly on detecting infected animals, followed by the slaughter of such animals. Since it is not feasible to isolate the causative organism from infected cases, serological tests, namely the Rose Bengal plate test (RBPT), serum agglutination test (SAT) and complement fixation test (CFT) are important in routine diagnosis of the disease.

The RBPT is accepted as an efficient method for use in cattle, sheep and goats, but diversity in quality of antigens produced at different laboratories or in the test procedure may give rise to variation in its sensitivity. In cattle, in areas where there is little or no infection and particularly where there has been much strain 19 vaccination, the RBPT positive sera have to be subjected to confirmatory tests.

In heavily-infected herds, it may prove economical to remove all animals positive to this test, since many such animals, although negative to confirmatory tests, may be in the early stages of infection and likely to become dangerous in spreading brucellosis later (6, 3).

The SAT has, so far, been the principal and the most useful serological method used for bovine, ovine and caprine brucellosis, even in cattle vaccinated with strain 19 vaccine, provided they were vaccinated as calves (9, 2).

The CFT is considered by many workers to be the most accurate serological test for brucellosis in cattle, sheep and goats, and is now widely used as a supplementary test on samples that have a doubtful SAT titre, or as a confirmatory test on sera positive to RBPT (5, 2, 4).

In conjunction with a preliminary scheme for eradicating brucellosis in Iran, the prevalence of the disease in cattle, as well as in sheep and goats, has been investigated serologically for 15 years ending in 1984. A combination of serological tests was applied and the results are presented in this paper.

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MATERIALS AND METHODS

Serum samples

Samples from 175,676 cattle and 110,817 sheep and goats from different parts of Iran were provided by the Brucellosis Eradication Unit of the Veterinary Organisation. Samples were stored at 4°C and tested within a week from the time of collection.

Antigens

The antigens routinely used in all the tests were prepared from Brucella abortus strain 99 or 19. Antigen for SAT was standardised against the International Standard Anti-Br. abortus serum so as to give 50% (++) agglutination at a final dilution of 1 in 500 of this standard serum (1, 2). The antigen used for the CFT was standardised to give 50% haemolysis at a dilution of 1 in 100 of the International Standard Anti-Br. abortus serum, using overnight fixation at 4°C (9).

The RBPT antigen was prepared and standardised according to the method described by Alton et al. in 1975. This antigen consisted of Brucella cells stained with Rose Bengal and suspended in buffer at pH 3.6 and preserved at 4°C.

Serological tests

The presence of Brucella antibodies was detected by RBPT, SAT and CFT according to methods recommended by the FAO/WHO (1, 2).

Interpretation

RBPT results were interpreted as negative or positive.

SAT: in vaccinated cattle aged 20 months or over, a ++++ reaction at 1:80 (212 IU/ml) or above was judged to be positive, and ++++ reactions at 1:40 (106 IU/ml) to 1:80 were doubtful.

In unvaccinated cattle a ++ reaction at 1:40 (80 IU/ml) or above was judged to be positive, a ++ reaction at 1:40 to 1:20 (40 IU/ml) was doubtful, and titres less than 1:20 were negative.

However, in sheep and goats a ++ reaction at 1:20 (40 IU/ml) or above was judged to be positive, and a ++ reaction at 1:10 (20 IU/ml) doubtful (8).

CFT: a titre of 1:40 was considered to be positive, and 1:20 doubtful in cattle and goats, while 1:20 was considered to be positive in sheep, and 1:10 doubtful (8, 7).

RESULTS

Results of the serological tests are shown in Tables I and II. Out of 175,676 serum samples from cattle, 31,078 (17.6%) were positive and 14,689 (8.4%) were doubtful. Positive sera from sheep and goats numbered 16,281 (14.7%) out of the 110,817 samples tested.
TABLE I
Serological prevalence of brucellosis in cattle in Iran during the years 1970-1984

<table>
<thead>
<tr>
<th>Years</th>
<th>No. tested</th>
<th>Positive</th>
<th>Doubtful</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1970-74</td>
<td>27 873</td>
<td>3 864 (13.8)</td>
<td>1 759 (6.3)</td>
<td>22 250 (79.9)</td>
</tr>
<tr>
<td>1975-79</td>
<td>79 317</td>
<td>12 052 (15.2)</td>
<td>3 710 (4.7)</td>
<td>63 555 (80.1)</td>
</tr>
<tr>
<td>1980-84</td>
<td>68 486</td>
<td>15 162 (22.1)</td>
<td>9 220 (13.5)</td>
<td>44 104 (64.4)</td>
</tr>
<tr>
<td>Total</td>
<td>175 676</td>
<td>31 078 (17.6)</td>
<td>14 689 (8.4)</td>
<td>129 909 (74)</td>
</tr>
</tbody>
</table>

TABLE II
Serological prevalence of brucellosis in sheep and goats in Iran during the years 1970-1984

<table>
<thead>
<tr>
<th>Years</th>
<th>No. tested</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1970-74</td>
<td>58 520</td>
<td>10 443 (17.8)</td>
<td>48 077 (82.2)</td>
</tr>
<tr>
<td>1975-79</td>
<td>23 800</td>
<td>3 113 (13.1)</td>
<td>20 687 (86.9)</td>
</tr>
<tr>
<td>1980-84</td>
<td>28 497</td>
<td>2 725 (9.5)</td>
<td>25 772 (90.5)</td>
</tr>
<tr>
<td>Total</td>
<td>110 817</td>
<td>16 281 (14.7)</td>
<td>94 536 (85.3)</td>
</tr>
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</table>

DISCUSSION

Animal brucellosis presents a number of circumstances which complicate diagnosis. The disease often has a long incubation period, varying from a few weeks to eight months or even more. After the initial exposure, abortion and retained placenta are the sole clinical features of the disease, but most animals abort only once. Since the introduction of brucella into the body is followed by the appearance of circulating antibodies, these can be used for the diagnosis of infected animals. However, this method has its own restriction and shortcomings, and a single serological test is unreliable. During the incubation period, the results of one or other serological test may be negative, even though such animals may abort soon afterwards (3).

After the disease has entered the symptomless chronic carrier stage, the organism is harboured intracellularly, often in the supramammary lymph nodes and the udder. During the chronic stage, the antibody titres may wane and fall below or remain around the diagnostic threshold. Some such animals may excrete brucella in the milk (3). The use of vaccines, particularly strain 19 vaccine, leads to the production of antibodies, the persistence of which depends mainly on the age of the animal at the time of vaccination. Although it is generally accepted that not every Brucella-infected cattle will invariably show a diagnostically significant titre, the limitations of the use of SAT alone, even in the absence of any complication arising from vaccination, are specifically described by Nicoletti (6) in cattle, Unel et al. (8) in sheep and Brinley Morgan et al. (3) in cattle, sheep and goats.
In sheep, a proportion of bacteriologically positive animals fail to react to SAT, even in repeated tests. This was confirmed by Unel et al. (8), who made the point that it had not been possible to eradicate sheep brucellosis from state farms in Turkey by using SAT alone, with removal of reactors, because many infected animals failed to give a positive reaction to the SAT. Our own results (unpublished data) indicate that Brucella organisms were sometimes recovered from sheep and goats with a doubtful or even a negative SAT titre. Thus it seems advisable that the SAT positive animals in unvaccinated flocks should be considered as reactors and removed from the farms, while those with negative SAT should be subjected to another confirmatory test such as CFT.

The CFT has proved to be an extremely reliable test for the diagnosis of brucellosis in animals, especially in those cases where the results of SAT have been negative or inconclusive, as may happen in the incubation period or in the late chronic stage. It is also helpful in differentiating antibodies induced by vaccination from those following infection (5, 4).

Notwithstanding reports by Nicoletti and Fincher (6) and Sutherland and Le Crass (7) on the use of RBPT, our experiments suggest that this could be useful only as a screening test.

Consequently, in countries where payment of compensation for slaughtering is not available, or where there are other restrictions, eradication schemes should be based on a combination of serological tests rather than on a single one.

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RECHERCHES SÉROLOGIQUES SUR LA BRUCELLOSE CHEZ LES BOVINS, OVINS ET CAPRINS EN IRAN. — E. Zowghi et A. Ebadi.

Résumé : La prévalence de la brucellose chez les bovins, ovin et caprins en Iran a fait l'objet d'une enquête sérologique au cours des années 1970 à 1984. Les nombres de prélèvements sanguins examinés se sont élevés à 175 676 chez les bovins et 110 817 chez les ovin et les caprins. Les pourcentages d'échantillons séropositifs étaient de 17,6 % chez les bovins et de 14,7% chez les ovin et les caprins. Les résultats ont démontré l'intérêt d'utiliser simultanément diverses épreuves sérologiques en vue d'une interprétation précise.

MOTS-CLÉS : Bovins - Brucellose - Caprins - Enquêtes épidémiologiques - Iran - Ovins - Techniques sérologiques.

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INVESTIGACIONES SEROLÓGICAS SOBRE LA BRUCELOSIS EN BOVINOS, OVINOS Y CAPRINOS EN IRÁN. — E. Zowghi y A. Ebadi.

Resumen: Se ha estudiado la prevalencia de la brucelosis en bovinos, ovinos y caprinos en Irán en una encuesta serológica efectuada de 1970 a 1984. La cantidad de muestras de sangre examinadas ascendió a 175 676 en los bovinos y 110 817 en ovinos y caprinos. Los porcentajes de muestras seropositivas eran del 17,6% en los bovinos y del 14,7% en ovinos y caprinos. Los resultados probaron el interés de utilizar simultáneamente diversas pruebas serológicas para conseguir una interpretación precisa.

PALABRAS CLAVE: Bovinos - Brucelosis - Caprinos - Encuestas epidemiológicas - Irán - Ovinos - Técnicas serológicas.

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REFERENCES