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ORIGINAL ARTICLE

Isolation and identification of Brucella organisms in Iran

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ABSTRACT

Background: Brucellosis is a zoonotic, chronic and infectious disease, which is caused by bacteria of genus Brucella. The present study investigated exactly what species and biovars of Brucella are responsible for brucellosis in Iran.
Materials and methods: The various species of Brucella were mostly being isolated from suspected specimens of animal fetus, placenta, vaginal swab, lymph nodes, milk, and human blood and bone marrow cultures. A total of 618 strains of B. abortus and 2413 strains of B. melitensis have been subjected to the identification procedures.
Results: Of 3031 isolates, 618 and 2413 were B. abortus and B. melitensis, respectively. Strains of B. abortus were isolated from cattle (612 cases) and sheep (6 cases). These isolates were biovars 1 (70 cases), 2 (1 case), 3 (511 cases), 4 (1 case), 5 (30 cases) and 9 (5 cases). Biovar 3 is considered as the endemic one and biovars 1 and 5 are the most prevalent. B. melitensis strains were isolated from sheep and goats (1717 cases), cattle (109 cases), camel (5 cases), dogs (4 cases) and human beings (497 cases). These isolates were biovars 1 (2102 cases), 2 (205 cases) and 3 (106 cases). B. suis, B. neotomae, B. ovis and B. canis were not isolated.
Conclusion: In many regions of Iran B. abortus biovar 3 still remains the dominant one, however, for B. melitensis biovar 1 is the most prevalent one.

Keywords: *Brucella, Epidemiology, Biovar, Iran.*
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INTRODUCTION

Brucellosis is a zoonotic, chronic and infectious disease, which is caused by bacteria of genus Brucella. Currently six species of Brucella are recognized: B. abortus, B. melitensis, B. suis, B. neotoma, B. ovis and B. canis (1-4). In Iran, B .abortus and B. melitensis are more prevalent.

Brucella vary in the frequency with which they infect particular host species. Thus B. abortus infects cattle, but is sometimes transmitted to many other hosts including sheep, goats and human beings. B. melitensis primarily infects sheep and goats, but can be transmitted to other hosts and is the most important cause of brucellosis in men (1,2,4,5).

Recently, as a result of DNA—DNA hybridization studies, it has been proposed that genus comprises a single species "Brucella melitensis" (4,6-8). Nevertheless, the six currently recognized species of Brucella are differentiated according to their pattern of utilization of amino acid and carbohydrate substrates in oxidative metabolism tests and their susceptibility to lysis by Brucella phages. Within these species, a number of biovars have been defined on the basis of requirement for supplementary CO_2, H_2S production, growth in the presence of thionin and

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basic fuchsin and agglutin-nation with monospecific A, M and R antisera.

Therefore, the various species of Brucella are classified as: B. abortus with 7 biovars, B. melitensis with 3 biovars, B. suis with 5 biovars, and B. ovis, B. neotoma and B. canis (2,3,9,10).

In Iran, the causative organism of brucellosis was isolated from human blood culture in 1932, bovine fetus in 1944, and sheep and goats milk in 1950 (11,12). Previously, we reported biovars of Brucella isolated up to 1980 (13), now, we extended our experiment till 2000.

PATIENTS and METHODS

Reference strains of B. abortus 544, B. melitensis 16M and B. suis 1330 were originally provided by the Central Veterinary Laboratory, Weybridge, and are held as freeze-dried cultures.

Reference phages, Tbilisi (Tb), Weybridge (Wb), Firenze (Fi), Berkely (Bk2) and Izatnagar (Izl) are routinely propagated for use in Brucella typing (2,10,14).

Monospecific sera, anti-A and anti-M were prepared in rabbits according to Alton et al (1,2)

The various species of Brucella were mostly being isolated from suspected specimens of animal fetus, placenta, vaginal swab, lymph nodes, milk, and human blood and bone marrow cultures. A total of 618 strains of B. abortus and 2413 strains of B. melitensis have been subjected to the identification procedures.

All cultures were performed according to the method recommended by WHO (1,10). To initiate the growth, 10 percent CO_2 was supplied, the production of H_2S was evaluated using lead acetate paper in tubes of brucella agar medium. The smooth and rough colonies of strains were distinguished by the usage of acriflavin and crystal violet tests. When sufficient cultures were obtained, they were examined in parallel with control cultures, for the following characteristics: sensitivity to dyes and reagents incorporated in brucella agar medium, at the Thionin: 1/25000, 1/50000 , 1/100000; Basic fuchsin: 1/50000, 1/100000. Ability of lysis by Tb and Wb phages evaluated in Routine Test Dilution (RTD) and RTD*10. Also, Fi, BK2 and Izl phages were used in RTD. Agglutination with antisera was carried out with monospecific anti-A and anti-M sera (1,2,9,10).

RESULTS

B. abortus and B. melitensis are the only Brucella species that have been isolated in Iran. B. abortus biovars 1, 2, 3, 4, 5, and 9 (=7) have been identified. Biovar 6 has not been isolated. B. abortus biovars were mostly (612 cases) isolated from cattle, but also a few cases (6 cases) were isolated from sheep. A total of 618 isolates of B. abortus have been examined, and the following biovars were reported: biovar 1 (70 cases), 2 (1 case), 3 (511 cases), 4 (1 case), 5 (30 cases), and 9 (5 cases). Biovar 3 is considered endemic in our population.

Strains of B. melitensis were isolated from sheep and goats (1717 cases), cattle (190 cases), camel (5 cases), sheep-dogs (4 cases) and human beings (497 cases). A total of 2413 isolates of B. melitensis have been identified. These isolates were biovars 1 (2102 cases), 2 (205 cases) and 3 (106 cases). According to the results, biovar 1 is endemic and widely spread, however, biovars 2 and 3 were also found in some cases.

Surprisingly, B. suis, B. neotomae, B. ovis and B. canis were not isolated.

DISCUSSION

In Iran, the Brucella strain was first isolated from a bovine fetus in 1944 (11). Having biotyped, it was identified as B. abortus biovar 3. For many years, this biovar was the only isolated one and in epidemiological point of view, it was considered as the main and the most important one. Other biovars

were not isolated until recently. Probably they are newly introduced biovars through unrestricted and careless importation of cattle from different parts of the world. However, the multiplicity of biovars is still a phenomenon, more or less, limited to dairy farms around Tehran (capital city), where industrial dairy farming is mainly located. In regions such as Isfahan, Khorasan and Azarbaijan (central, east-north and west-north of Iran), biovar 3 still remains the dominant one (15,16).

In Iran, B. melitensis was first isolated from a sheep in Isfahan in 1950 (12) and subsequently its biovar 1 was sporadically isolated in different regions of the country from sheep and goats as well as cattle, camel, sheep-dogs, and human being. Meanwhile, B. melitensis biovars 2 and 3 are of considerable importance and have been frequently isolated from sheep, goats and human beings. B. melitensis biovar 1 is responsible for the disease in regions of Isfahan, Khorasan, Guilan (north), Khoozestan (south), Yazd (central) and Kermanshah (west), whereas in Tehran and Azarbaijan, biovars 1, 2 and 3 are the responsible ones. Surprisingly, B. suis, B. neotoma, B. ovis and B. canis were not isolated in Iran (17-22).

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