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TYPING OF BRUCELLA STRAINS ISOLATED IN IRAN

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SUMMARY

The results of typing of various strains of **Brucella** isolated in a decade, starting 1971, are reported.

Isolates of **Brucella abortus** were of biotypes 1, 2, 3, 4, 5, 6, 8, 9,. Bictype 3 being considered as the endemic one and biotypes 5 and 9 the most prevalent, next to biotype 3. **Brucella abortus** biotype 7 has never been isolated.

Brucella melitensis biotypes 1 and 2 were mostly isolated from sheep, goats, and even from cattle, of the infected areas. Biotype 3 comprised a small portion of the isolates.

The isolates from pigs were shown to be mainly biotype 1 and a few biotype 2 of **Brucella suis.**

Brucella canis and **Brucella ovis,** which had not been reported in Iran, were not isolated.

INTRODUCTION

Brucellosis is a zoonotic, chronic and infectious disease, which is caused by various species of **Brucella,** that morphologically appear as cocobacilli about 0.6-1.5 micron in length and 0. 5-0. 7 micron in diameter. These microorganisms are gram negative, non- motile, non-sporing and all aerobic. They need to be cultivated in specific nutritious media. The differences in biological and biochemical charactristics of the causative organisms of brucellosis isolated from human and animals have led to the identification of various species. The main recognised types are **Brucella abortus** in bovine, **Brucella melitensis** in sheep and goats, **Brucella suis** in pigs. The recent isolates of **Brucella neotoma** in desert wood rats, **Brucella canis** in doge and **Brucella ovis** in sheep must be added to the list.

The **Br. melitensis, Br. abortus, Br. suis** and **Br. canis** cause Malta fever in human.

At present the identification of species of **Brucella** is carried out according to the following two methods:

a) Oxidative Metabolic Test (Joint FAO / WHO Expert Commitee on

Brucellosis 1971) that is accomplished by cultivating the organisms in special apparatuses, such as Warburg, in the presence of 8 aminoacids and 5 carbohydrates. The charactristics of strains and their biotypes can be determined on the basis of the degree of oxidation and oxygen consumption.

b) Phage typing, using Tibilisi (Tb) and Weybridge phages.

According to Stableforth and Jones (1963), Alton and Jones (1967), Aldrick (1968) , Alton et al (1975), the various species of **Brucella are classified as follows:**

Brucella melitensis	**3 biotypes**
Brucella abortus	**9 Biotypes**
Brucella suis	**4 biotypes**
Brucella neotoma	**1 biotype**
Brucella canis	**1 Biotype**
Brucella ovis	**1 biotype**

In Iran the causative agent of brucellosis were isolated from the human blood culture in 1932, bovinés foetus in 1944, sheep and goats milk in 1950 and subsequently from pigs aborted foetus in 1971 (Delpy and Kaveh 1945, Kaveh 1952, Ardalan and Ebadi 1977). Here in this communication the results of biotyping of the strains isolated during a decade, starting 1971, are reported.

MATERIALS AND METHODS

Reference Strains -- The freeze dried reference strains of **Br. melitensis** 16 M and **Br. abortus** 544 had been suplied by Weybridge Institute (Surrey, England). The reference **Tibilisi phage** (Tb) was propagated for daily tests and used in Routine Test Dilution (R. T. D.) and R. T. D. X 10^4. The tests performed in the different parts of the world have confirmed that this phage, originally isolated in the U. S. S. R., can lyse only **Br. abortus** strains in R. T. D., whearas it can lyse **Br. abortus** as well as **Br. suis** in R. T. D. X 10^4. (Alton and Jones 1967, Alton et al 1975). The Weybridge phage is recently used and since in R. T. D. it is able to lyse **Br. abortus** together with **Br. suis**, thus Tb phage was substituted by it in R. T. D. X 10^4. None of these phages were effective on **Br. melitensis**. (Morris and Corbel 1973, Corbel and Thomas 1976).

Monospecific Sera -- The monospecific sera, Anti-A and Anti-M were prepared and tested according to Alton and Jones (1967).

Isolates – The various species of **Brucella** were mostly being isolated from suspected specimens of foetus, placenta, vaginal swab, lymph nodes, milk and human blood in a period lasting from 1971 to 1980. A total of 1972 strains of **Br. abortus,** 1107 strains of **Br. melitensis** and 62 strains of **Br. suis** have been subjected to the identification procedures.

Cultures – All cultures were performed according to the method

recommended by WHO (Alton and Jones 1967, Alton et al 1975-Laboratory Techniques in Brucellosis). To initiate the growth, 10 percent Co2 was supplied.

The production of H2S was evaluated by using lead acetate paper in tubes of nutrient brucella agar medium. The smooth and rough colonies of strains were distinguished by the usage of acriflavin. When sufficient cultures were obtained they were examined in parallel with control cultures, for the following charactristics:

Sensivity to dyes and reagents incorporated in brucella agar, at the concentrations shown below:

Thionin 1: 25000 , 1: 50000 , 1: 100000

Basic Fuchin 1: 50000 , 1: 100000 (Alton and Jones 1967, Alton et al 1975, Morris et al 1973).

Ability of phages to lyse cultures – Originally the phage Tibilisi (Tb) was used at R. T. D. and at R. T. D. X 10^4, the former concentration lysing smooth **Br. abortus** cultures and the latter lysing smooth **Br. abortus** and Br. suis cultures. (Alton and Jones 1967, Alton et al 1975, Ekers 1978).

Agglutination with mono-specific sera – This was carried out with monospecific sera of Anti-A and Anti-M.

RESULTS

Brucella abortus

Table 1 demonstrates the results of typing of **Br. abortus** isolates, in respect to the year and the number. One can see in this table biotype 7 has never been, so far, isolated in Iran. Biotypes 6 and 8 were isolated on one occasion each, in 1972 and 1974, respectively. Biotype 4 has been isolated only on 5 occasions. Biotype 3 which appears to be the most prevalent one is followed by biotypes 5 and 9 in regard to their incidences.

Brucella melitensis

Typing procedures were performed on 1107 specimens and the results are shown in table 2. According to the results presented in table 2, the biotypes 1 and 2 were widely spread and are, therefore, of considerable importance, but biotype 3 was encountered on rare occasions. It is noteworthy that, in 28 cases biotype 1 and in 37 cases biotype 2 of **Br. melitensis** were isolated from cattle being kept close to the sheep farms, where samples had been collected.

Brucella suis

To investigate the rate of **Brucella** infection in pigs, a series of lymph node cultures were carried out. In 62 cases the **Brucella** was isolated. The isolates after being typed at the Razi Institute and confirmed by the Weybridge Institute, were found to be mostly of **Br. suis** biotype 1, with a few of biotype 2. Three cases were identified as **Br. abortus** and 3 cases were also suspectedly determined as **Br. melitensis.**

Table 1-Typing of Brucella abortus strains isolated in the period 1971-1980

Br. abortus	Biotypes									Total
Year	1	2	3	4	5	6	7	8	9	
1971	6	7	21	–	2	–	–	–	–	36
1972	14	–	10	–	62	1	–	–	–	87
1973	–	12	97	5	5	–	–	–	–	119
1974	11	1	307	–	117	–	–	1	6	503
1975	1	–	36	–	55	–	–	–	–	92
1976	2	–	35	–	–	–	–	–	–	37
1977	1	6	273	–	19	–	–	–	35	334
1978	–	1	372	–	2	–	–	–	24	399
1979	8	–	22	–	–	–	–	–	1	31
1980	1	6	273	–	19	–	–	–	35	334
Total	44	33	1446	5	341	1	–	1	100	1972

Table 2-Typing of Brucella melitensis strains isolated in the period 1971-1980

Br. melitensis	Biotypes			Total
Year	1	2	3	
1971	32	8	2	42
1972	100	11	–	111
1973	34	14	2	50
1974	161	20	–	181
1975	84	58	–	143
1976	83	44	–	127
1977	53	16	–	69
1978	173	17	–	190
1979	88	38	–	126
1980	53	16	–	69
Total	851	242	4	1107

DISCUSSION

The **Brucella** strain isolated from a bovine foetus in 1944 (Delpy and Kaveh 1945), by taking its specific charactristics into consideration, had been desiganted **Br. abortus** Iran strain, prior to being biotyped. Later on this was identified as **Br. abortus** biotype 3. For somtimes this biotype was the sole kind of isolate from brucellosis cases and, in epizootilogical point of view, it was considered the main and the most important biotype. Other biotypes such as biotype 5 which used to be widely spread in countries like Britain, were not isolated until much later.

It appears the most probable that they are newly introduced biotypes, through unrestricted and careless importation of cattle from different parts of the world. However the multiplicity of biotypes is still a phenomenon, more or less, limitted to dairy farms around Tehran where industrial dairy farming is mainly located. In regions such as Isfahan, Khorasan and West-Azarbaijan, biotype 3 still remains the dominant one.

In Iran, **Br, melitensis** was first isolated from a sheeps foetus in Isfahan in 1950 (Kaveh 1952) and subsequently its biotype 1 was sporadically isolated in different regions of the country from sheep and goat foetuses as well as from milk and cheese. **Br. melitensis** biotype 2 is of considerable importance and has been frequently isolated. **Br. melitensis** biotype 3 seems to have a very low incidence, since only 4 cases have been identified in the period covering the study.

Br. melitensis biotype 1 is responsible for the disease in the regions of Isfahan, Khorasan, Guilan, Khoozistan, Yazd, Kermanshahan and East-Azarbaijan, whereas in Tehran, West-Azarbaijan and Mazandaran, biotypes 1 and 2 are considered the cause of the disease.

As the pig-breeding during the period of this work was very limited, now being given up completely, the sampling was restricted to a pig slaughter-house in Tehran.

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