A Survey on Human Brucellosis in Iran

G. Samar[1], P. Vahdani[2], N. Aminian[1*] and E. Zowghi[3*]

1- Faculty of Medicine, University of Tehran, Tehran, Iran; 2- Faculty of Medicine University of Shahid Beheshti, Tehran, Iran; 3- Brucellosis Department, Razi Vaccine and Serum Research Institute, PO Box 11365-1558, Tehran, Iran.

Summary

A study on human brucellosis was conducted at Imam Khomaini Hospital in Tehran IRAN in a period of 12 months, extending from 23 September 1993 to 22 September 1994. Of 65 cases, clinically suspected of being brucellosis, 60 showed laboratory evidence of brucellosis. The disease was more frequently diagnosed among adults than children. The common clinical features were fever, sweating, anorexia, muscle pain, arthralgia, splenomegaly and hepatomegaly. Serological evidence of Brucella *antibodies in RBPT, SAT and 2-ME test proved to be reliable in confirming suspected cases of brucellosis.* Brucella melitensis *biovar 1 was isolated, by culture, from 13 (21.7%) blood and 17 (28.3%) bone marrow samples. The infection was encountered mainly in spring (33.3%) and summer (36.7%). In these seasons, due to lambing and kidding, sheep and goats milk become available. Consumption of unpasteurised dairy products and contact with animals are, most likely, responsible for the transmission of* Brucella *to humans. The patients were treated successfully with combinations of co-trimoxazole/ rifampicin, doxycycline/ rifampicin, tetracycline/streptomycin or doxycycline/co-trimoxazole/rifampicin.*

Introduction

Human brucellosis is a zoonosis caused by three important *Brucella* species, namely, *B. abortus*, *B.* suis and *B. melitensis*. The respective primary host for these species are cattle, swine and sheep or goats (Salata and Ravdin, 1985). Many countries of the world have seen a significant reduction in the

* *Correspondent author*

incidence of brucellosis. Usually, this has been achieved by a strict sanitary control of animal husbandry and animal products. However, the disease is still a major health problem in countries where the control of infection in domestic animals has been unsuccessful (Hall, 1982; Benenson, 1990). In animals, in the course of infection the micro-organism can be found in the blood, liver, kidneys and spleen. It also localises in the pregnant uterus, causing abortion, and in the mammary glands where organisms are shed into the milk (Nielson and Duncan, 1990). The disease is, usually, transmitted to human beings by direct contact with infected animals or by ingestion of infected dairy products, but, it may also be contracted via the respiratory tract, abraded skin and conjunctiva (Gilbert *et al.*, 1980; Filstein *et al.*, 1980). In countries where milk and dairy products are always pasteurised before consumption, *Brucella* infection has remained as a professional disease and it affects persons who are in close contact with animals (Hall, 1982). It is also considered a serious threat to the laboratory workers who are exposed to the risk (Kaufmann *et al.*, 1980; Jaime and Jaume, 1987).

Brucellosis is an endemic disease in Iran. The disease constitutes a very important sanitary problem in both rural and urban areas of the country. It is estimated that about 50000 new cases of human brucellosis occur every year, mainly due to *B. melitensis* (Anonym, 1994). Sheep and goats, the main hosts of *B. melitensis*, with a population of more than 70 million, are the principal farm animals in Iran. Epidemiological studies on human brucellosis, in areas where the disease is prevalent, have revealed that in rural areas both direct and indirect transmissions are the potential means of infection, whereas, in urban areas the consumption of unpasteurized dairy products remain a common source of infection (Sabaghian and Nadim, 1974). Diagnosis of brucellosis is difficult and challenging due to its protean manifestations, involvement of different organs and variable clinical courses. The clinical manifestations of human brucellosis vary from acute/sub-acute systemic or localised infections to chronic infections (Al-Dubooni *et al.*, 1986; Shehabi *et al.*, 1990; Abramson and Rosenvasser, 1991; Trujillo *et al.*, 1994). As a contribution to overcome the diagnosis and treatment difficulties, the experience gained on diagnosis and treatment of 60 cases of human brucellosis are described in this paper.

Materials and methods

Patients: The study started on 23rd September 1993 and ended on 22nd September 1994 at Imam Khomaini Hospital in Tehran, Iran. Any patient suspected, clinically, of having *Brucella* infection was serologically and bacteriologically tested. A questionnaire on age, sex, place of residence, current occupation, kind of complaints, date of onset and duration of illness,

history of coming into contact with animals and consumption of unpasteurized milk or dairy products was filled for each patient. The diagnosis of brucellosis was based on clinical features plus isolation, by culture, of *Brucella* organisms from either blood or bone marrow and/or positive reaction to serological tests.

Serology: The antigens for Rose Bengal plate test (RBPT) and serum agglutination test (SAT) were prepared and standardised at Razi Vaccine and Serum Research Institute according to the method recommended by Alton *et al* (1988) using *B. abortus*, either Strain 19 or Strain 99. Each patient's serum was tested by RBPT, SAT and 2-mercaptoethanol test (2-MET) according to procedures recommended by WHO (Alton *et al.*, 1975). Optimal dilution of samples was determined to avoid the prozone phenomenon in SAT. The RBPTs were deemed positive, with any degrees of agglutination, and negative when agglutination did not occur. The titre of ≥1:80 in SAT or ≥ 1:40 in 2-MET were considered positive.

Bacteriology: Samples of blood or bone marrow were inoculated into *Brucella* broth medium in bottles of 50-70 ml capacity. After inoculation, the bottles were incubated for 4 to 6 weeks. During this time, subcultures were made on *Brucella* agar medium every 3rd day. All cultures were incubated at 37°C, one series in ordinary incubator and another series in an incubator with an atmosphere of 5-10% carbon dioxide. Slopes were examined 3 to 4 days later for *Brucella* colonies. From any colonies resembling those of *Brucella* subcultures were made for further investigations. The *Brucella* isolates were biotyped as previously been described (Zowghi and Ebadi, 1982), using the techniques recommended by Alton *et al.* (1988) and Corbel *et al.* (1978).

Antimicrobial drugs: Four regimens of drug combination, namely, co-trimoxazole/rifampicin, doxycycline/rifampicin, tetracycline/streptomycin or doxycycline/co-trimoxazole/rifampicin were used. The following dosages were administered. Rifampicin for children: 20 mg/kg/day orally for 6 weeks. Rifampicin for adults: 600-900 mg/day orally for 6 weeks. Doxycycline for adults: 200 mg/day orally for 6 weeks. Tetracycline for adults: 2 g/day orally for 6 weeks. Streptomycin for adults: 1 g/day I.M. for 3 weeks. Co-trimoxazole for children: 8 mg trimethoprim plus 40 mg sulphamethoxazole/Kg/day orally for 6 weeks. Co-trimoxazole for adults: 160 mg trimethoprim plus 800 mg sulphamethoxazole/ 3 times a day for 6 weeks.

Results

Of 65 patients, that had been tested for brucellosis by serology and bacteriology, 5 were negative. Sixty patients showed positive reaction to RBPT. Of these, 2 patients had negative reactions to SAT and 2 other showed a weak initial titre of 1: 40. Also, 4 patients had negative response to 2-ME test and 3 showed weak titres of 1:20. Table 1 shows the results of SAT and 2-ME reactions in 60 patients. *B. melitensis* biovar 1 was isolated from the blood of 13/60 (21.7%) and the bone marrow of 17/60 (28.3%) patients. All but 4 patients had a *Brucella* antibody titre ≥ 1:80 in SAT.

The seasonal distribution of cases is shown in Table 2. It can be seen that cases were unequally distributed through out the year. About 70% of the cases occurred during the spring and summer. During these seasons parturition of sheep and goats takes place and the milk from these animals becomes available and abundant. Of the 60 patients, 28(47.7%) were females and 32(53.3%) were males. The patients' age distribution is shown in Table 3. Of 60 patients, 51(85%) cases were in age groups of 11 to 50 years. This indicates that brucellosis, in Iran, is principally a disease of the young and the middle age persons. The consumption of unpasteurized milk and dairy products such as fresh cheese, cream, and ice-cream, or contact with animals were responsible for the disease in 43(71.7%) patients. On the other hand, 17(28.3%) patients did not have a history of ingesting unpasteurized dairy products or contact with animals. The occupation of patients is shown in Table 4. According to this table housewives, students, labourers and farm workers have been those more exposed to the causative agent. According to our definitions for the types of illness, 42 cases (70%) were classified as acute (less than 3 months of illness) and only 1 case (1.7%) as chronic (more than 12 months of illness). The clinical features of the patients are shown in Table 5. The predominant symptoms in all cases were fever, chills, sweating, arthralgia, weakness, anorexia, muscle pain, headache, splenomegaly and hepatomegaly. Table 6 shows the clinical response to various regimens of antimicrobial treatments.

Discussion

Human brucellosis in Iran is predominantly due to infection with *B. melitensis* (Zowghi and Ebadi, 1985). In spite of the prevalence of bovine brucellosis due to *B. abortus* this species could not be isolated from human patients. On the other hand, *B. melitensis* biovars, especially biovar 1, had previously been isolated from dairy cows (Zowghi and Ebadi, 1985). This indicates that, in areas where sheep and goats brucellosis is prevalent, cattle may be one of the animal reservoirs for *B. melitensis*. Therefore, it was

presumed that most, if not all, of the patients in this study were suffering from *B. melitensis* infection. The mode of acquiring the infection was difficult to establish in these cases. Nevertheless, more than 40 patients had probably contracted brucellosis by ingestion of unpasteurized milk and dairy

Table 1. *The results of serological tests of 60 patients with positive reaction to Rose Bengal plate test.*

Titre	SAT No. positive (%)	2-MET No. positive (%)
Negative	2 (03.30)	4 (06.70)
1:20	0 (00.00)	3 (05.00)
1:40	2 (03.30)	4 (06.70)
1:80	3 (05.00)	15 (25.00)
1:160	10 (16.70)	25 (41.50)
1:320	8 (13.30)	4 (06.70)
1:640	16 (26.70)	4 (06.70)
1:1280	17 (28.30)	1 (01.70)
1:2560	1 (01.70)	0 (00.00)
1::5120	1 (01.70)	0 (00.00)
Total	60 (100)	60 (100)

Table 2. *Seasonal distribution of brucellosis in 60 patients.*

Season	Number of cases	% of the total
Spring	20	33.3
Summer	22	36.7
Autumn	9	15.0
Winter	9	15.0
Total	60	100.0

Table 3. *Age distribution of 60 brucellosis patients*

Age (years)	Number of patients	% of the total
0-10	1	31.7
11-20	16	20.0
21-30	11	18.3
31-40	14	15.0
41-50	10	10.0
51-60	3	3.3
61-70	5	1.7
Total	60	100.00

Table 4. *The occupation of 60 brucellosis patients.*

Occupation	Number of patients	% of the total
Housewife	19	31.7
Student	12	20.0
Labourer	11	18.3
Farmer	9	15.0
Employer	6	10.0
Soldier	2	3.3
Businessman	1	8.3
Total	60	100.00

Table 5. *Clinical signs of 60 brucellosis patients.*

Sign	Number of cases	5 of the total
Fever	55	91.7
Sweating	43	71.7
Chills	42	70.0
Weakness	37	61.7
Anorexia	33	55.0
Weight loss	31	51.7
Headache	27	45.0
Arthralgia	45	75.0
Vomiting	7	11.7
Nausea	15	25.0
Splenomegaly	26	43.3
Hepatomegaly	11	18.3
Muscle pain	33	55.0
Bone pain	34	56.7
Nervous pain	5	8.3
Abdomen pain	6	10.0
History of abortion	1	1.7

products or through contact with animals. Cases were observed in all seasons of the year with a peak in spring and summer, a period coinciding with the time of parturition and maximum milk yield by sheep and goats.

Human brucellosis is well known for its variety of clinical manifestations and complications. Effective antimicrobial treatment requires prompt clinical and laboratory diagnoses (Filstein and Potter, 1980; Taylor and Perdue, 1989; Shebahi and Shakir, 1990; Abramson *et al.*, 1991). In our study, most of the patients showed acute or sub-acute forms of the disease with the commonly characterised clinical features such as fever, sweating, chills, weakness, anorexia, arthralgia, muscle pain and headache with, to a lesser extent, involvement of spleen and liver. It should be noted that brain abscess, hepatic cirrhosis and liver abscess are very uncommon complication of brucellosis (Williams and Grossly, 1982; Bashir *et al.*, 1985). Our findings that brucellosis occur more often in adults than in children (Table 3) concur with the results of studies from other countries in the area (Al-Dubooni *et al.*, 1986; Khan, 1986; Lulu *et al.*, 1988; Shehabi *et al.*, 1990). From the results that housewives and students were the top most affected groups, one may infer that the disease in the city of Tehran is not an occupational one.

Table 6. *Antimicrobial regimens for treatment of 60 brucellosis patients*

Groups	Therapy	No. of patients	% of the total
1	Cotri. + Rifa	23	38.3
2	Doxy. + Rifa	25	41.7
3	Tetra. + Strep.	8	13.3
4	Doxy/Cotri + Rifa	4	6.7
Total		60	100.00

Cotri. = Co-trimoxazole Rifa.=rifampicin Doxy.=doxycline Strep.= streptomycin

It is emphasised that RBPT was more sensitive than SAT and 2-ME and had a greater value as an initial screening test. The initial *Brucella* antibody titre of ≥ 1:80 in SAT proved to be the most useful procedure in reaching an early and accurate diagnosis of brucellosis in the most of patients (93.4%). Also, 2-ME test, that measures IgG, has been found useful to determine the activity of disease and the response to treatment.

The difficulty to isolate *Brucella* organisms, from blood or bone marrow at any stage of the illness, might have partly been due to the previous antibiotic treatments. For recovering *B. melitensis,* culture of bone marrow was shown to be more successful (28.3%) than that of blood (21.7%). Culture of blood and bone marrow are particularly important when brucellosis is suspected but no specific clinical features are associated or serum antibody titres are low (Farid *et al.*, 1980; Young, 1986). All the patients treated with one of the four drug-combinations appeared to be clinically cured, judged by the disappearance of all major signs and symptoms of the infection. None of the patients treated with the listed drugs relapsed. In conclusion, our results indicated that rifampicin plus doxycycline or co-trimoxazole is a useful alternative to streptomycin and tetracycline in the treatment of human brucellosis

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