

Epidemiology of human brucellosis in Isfahan, Iran*

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SUMMARY

Studies on brucellosis were carried out to elucidate the epidemiology of the disease in Isfahan province, where *Brucella melitensis* is highly prevalent in animals and man. A positive milk ring test of 32% and 23% was found in unvaccinated goats and sheep respectively. Card and tube agglutination tests showed an infection rate of about 12% in sheep and goats and 42% in cattle. *B. melitensis* was isolated from 8% of 677 samples of fresh cheese examined.

Of 1526 clinically suspected human cases, 476 showed laboratory evidence of brucellosis. Of these patients, 291 cases were from urban and 185 cases from rural areas. Cases from urban and rural areas were seen principally in the younger age groups. The median age of infection was 19·7 in urban and 15·7 in rural patients respectively. The infection was encountered mainly from April to August. This correlates with animal parturition and the greatest amount of sheep and goat milk production, which is introduced to the local market as fresh cheese. Raw dairy product consumption is the most probable way of *Brucella* transmission in urban patients. In rural areas, both dairy product consumption and contact with animals are sources of infection.

INTRODUCTION

Sheep and goats are the principal farm animals in Iran, and more than 50 million of these animals are raised in the country (FAO-WHO-OIE, 1972). A few organized dairy farms and breeding units are found around the large cities where pasteurized dairy products are consumed. Brucellosis among farm animals is widespread in most parts of the country and, in consequence, human brucellosis is common, especially in areas of high sheep and goat concentration.

Isfahan, located in the central part of Iran, is an agricultural province. Because of its climate and relatively rich pastures, approximately one-third of the country's total number of sheep and goats graze in this area. Animal and human brucellosis is widely prevalent in certain areas of Isfahan and of 4738 cases of human brucellosis in Iran reported to the World Health in 1964, 3811 (80%) were from this region (Ministry of Health, 1965).

An epidemiological study of human brucellosis was carried out among the rural

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and urban population in Isfahan province. This report covers an analysis of case histories in the areas studied together with the prevalence of animal brucellosis and contamination of dairy products with *Brucella* organisms for the period 1967–1972.

MATERIAL AND METHODS

Clinically suspected human cases from outpatient clinics in the city of Isfahan and from seven villages on its outskirts were brought to the attention of the investigation team by attending physicians. Laboratory diagnosis of brucellosis was based on card, tube agglutination tests and blood culture. Complement fixation tests were performed on those with negative or non-significant agglutinin titre by the above tests. A titre of 1/80 (160 i.u.) or greater by tube agglutination and 1/20 by complement-fixation test was considered positive.

Patients were interviewed for clinical symptoms, and epidemiological history forms were completed.

Unvaccinated sheep, goats and cows with a history of abortion and some animals belonging to the patients were tested for brucellosis. Milk ring, card and tube agglutination tests, as well as blood culture, were employed for the diagnosis of animal brucellosis.

Fresh cheese, cream, ice-cream and butter were collected from retail shops and distribution centres and cultured for *Brucella* isolation. Three recommended antibiotics (Alton & Jones, 1967) were added to trypticase soy agar, and this medium was used for the testing of dairy products. Trypticase soy agar plates were inoculated in duplicate with 10 mg. each of the sample and incubated at 37° C. One plate from each sample was incubated in 10% CO₂ atmosphere. The plates were discarded as negative after 10 days. Identification of *Brucella* strains isolated from human patients, animals and dairy products was made by sensitivity, physiological and serological methods.

RESULTS

Human brucellosis

Of 1526 suspected cases in the areas studied, 476 (31.2%) showed laboratory evidence of *Brucella* infection. Of these patients, 291 cases were from the city of Isfahan and 185 from the villages studied. All of those positive on card test were also positive by tube agglutination test at 160 i.u. of *Brucella* agglutinin or greater.

Seasonal distribution

The seasonal distribution of all cases is shown in Fig. 1. It can be seen that cases occurred throughout the year except during January. About 84% of the cases occurred during the period April–August. There was no difference between urban and rural cases in this respect. The attack rate during the 12-month period corresponded to sheep and goat parturition and milk production.

Age and sex

The age and sex distribution of clinical cases is shown in Table 1. This table indicates that brucellosis in urban and rural areas is principally a disease of the

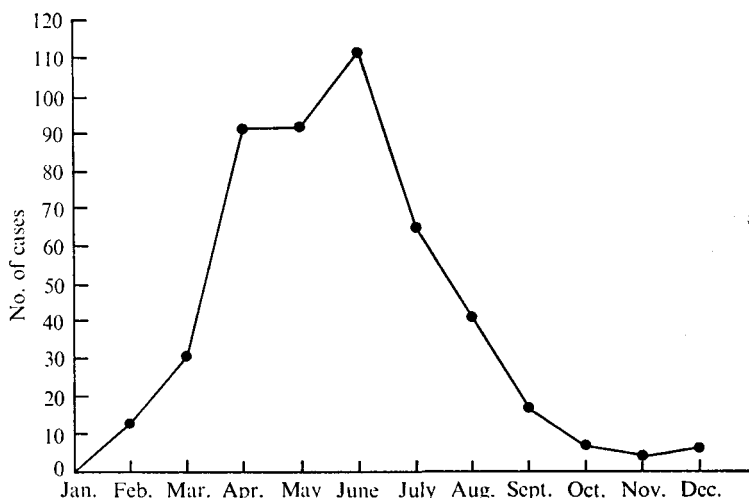


Fig. 1. Seasonal distribution of 476 brucellosis cases in Isfahan, Iran.

Table 1. Age and sex distribution of 291 urban and 185 rural cases of brucellosis

Age	Urban cases				Rural cases			
	Sex		Total	% of Total	Sex		Total	% of Total
	M	F			M	F		
0-4	4	1	5	1.7	7	4	11	5.9
5-9	16	10	26	8.9	14	18	32	17.3
10-14	35	25	60	20.6	26	19	45	24.3
15-19	47	11	58	19.9	13	19	32	17.3
20-24	12	9	21	7.2	7	5	12	6.5
25-29	11	11	22	7.6	3	6	9	4.9
30-34	13	4	17	5.8	5	4	9	4.9
35-39	12	5	17	5.8	5	6	11	5.9
40-44	12	11	23	7.9	2	4	6	3.2
45-49	5	8	13	4.5	5	1	6	3.2
50-54	4	10	14	4.8	4	2	6	3.2
55-59	1	1	2	0.7	3	—	3	1.6
60+	7	6	13	4.5	2	1	3	1.6
Total	179	112	291		96	89	185	

younger age groups. Of 291 urban cases, 149 (51.2%), and of 185 rural cases, 120 (64.8%), were found in the age group up to 20. This table also shows that the median age of infection for males and females in the city of Isfahan is 18.6 and 25 years respectively, while the median age of infection for both sexes in rural areas is about 15 years. In general, the median age of infection in urban areas is 19.7 and in rural areas 15.7.

Dairy product consumption

The consumption of five dairy products is shown in Tables 2 and 3. These data have been compared with 250 suspected cases with no *Brucella* agglutinin in

Table 2. *Reported consumption of five dairy products among outpatients from urban areas*

	No. interviewed	Fresh cheese (%)	Butter (%)	Raw milk (%)	Ice-cream (%)	Cream (%)
Serologically positive	291	91	59.8	18.5	46.7	26.1
Serologically negative	250	83.2	70.4	16.8	46.8	33.6
Total	541	87.4	64.7	17.7	46.8	29.6

Table 3. *Reported consumption of five dairy products among outpatients from rural areas*

	No. interviewed	Fresh cheese (%)	Butter (%)	Raw milk (%)	Ice-cream (%)	Cream (%)
Serologically positive	185	94	66.5	51.9	45.9	50.8
Serologically negative	250	98.8	66	13.2	12.4	30
Total	435	96.8	66.9	29.6	26.7	38.8

Table 4. *Correlation of occupation and reported animal contacts of cases of human brucellosis in urban and rural areas*

Occupation	Urban cases		Rural cases	
	No. of patients	No. reporting animal contact	No. of patients	No. reporting animal contact
Housewives	66	14	40	21
Farm workers	22	9	23	19
Butchers	1	1	2	2
Shepherds	—	—	10	10
Students	62	6	36	18
Tradesmen	15	3	34	7
Labourers	18	4	13	6
Pre-school children	20	—	27	22
Total	204	37	185	105

each of the groups studied. In rural areas, the main difference between the two groups is seen in those who consumed raw milk, ice-cream and cream (Table 3). Table 2 shows that dairy products are consumed equally by the two groups in the city of Isfahan. In general, the percentage of dairy product consumption, especially of fresh cheese, by all interviewed persons in both areas was very high.

Occupations and reported animal contacts

The occupations and reported animal contacts of urban and rural brucellosis cases is shown in Table 4. This table indicates that housewives and students are more affected in both urban and rural groups. About 56% of the patients in the villages reported animal contact while in the city only 18% had animal contact.

Table 5. Chief symptoms of 476 cases of human brucellosis in Isfahan

Symptom	No. of cases	% of total
Fever	366	76.9
Sweating	375	78.8
Joint pain & arthralgia	454	95.4
Headache	288	60.5
Fatigue	177	37.2
Cachexia	235	49.4
Chills	202	42.4
Constipation	119	25.0
Diarrhoea	7	1.5
Anaemia	268	56.3
Orchitis	36	7.6
Muscle pain	92	19.3
Total	476	

Table 6. Results of milk ring and tube agglutination tests

Type of test	Kind of animal	Number tested	Percent positive
M.R.T.	Sheep	1850	23
	Goats	1340	32
Tube agglutination	Sheep	1241	12.7
	Goats	736	12.2
	Cattle	326	42.3
	Camels	1	1/1

Brucella isolations

Positive blood cultures were obtained from 40 out of 123 acute brucellosis cases. Two patients still had a positive blood culture after their treatment was completed and the clinical symptoms had improved. All 40 strains isolated were *Brucella melitensis* biotype I, the species commonly found in sheep and goats and detected continuously in dairy products. *B. abortus* was not isolated from humans on any occasion during this study.

Clinical symptoms

The predominant symptoms in all brucellosis cases were pain in the joints, arthralgia, sweating, fever and headache (Table 5). Anaemia was seen but, because of parasitic infestation, is considered common in this area. More than one half of the cases reported symptoms lasting two months or more and 38 patients were ill for over a year.

Animal brucellosis

Sheep, goats, cattle and camels were tested for *Brucella* infection. Nearly all animals tested were from areas whose products are sold mainly in the city of Isfahan. Table 6 shows the results of milk ring and tube agglutination tests. Card and tube agglutination showed close agreement and a titre of 1/40 (80 i.u.) or greater was considered positive. In Table 7 the results of milk and blood culture are shown. One of the two strains from cow's milk and all of those from sheep and goat milk were *B. melitensis* biotype I. The other strain from cow's milk was *B. abortus* which was not typed.

Table 7. *Results of milk and animal blood culture*

Material examined	Kind of animal	Number of cultures	Number positive
Milk	Sheep and goats	115	3
	Cows	83	2
Blood	Cattle	72	0

Table 8. *Results of four dairy product cultures*

Dairy product	Number cultured	Number positive
Cheese	677	56 (8.3)
Cream	160	1
Ice-cream	295	0
Butter	146	0

Dairy product culture

In the examination of dairy products *B. melitensis* was isolated from a high percentage of cheeses examined (Table 8). Out of 677 fresh cheese specimens collected from retail shops and distribution centres, 56 (8.3%) were infected with *B. melitensis* biotype I. Only one isolation was made from 160 cream samples and all 295 ice-cream and 146 butter specimens were negative. The number of *Brucella* organisms per gram of cheese was calculated and ranged from 1000 to 131,000 with a mean of 17,750.

DISCUSSION

B. melitensis type I was isolated from human, animal and dairy products. Bovine brucellosis due to *B. abortus* is prevalent in the areas studied, but this organism could not be isolated from human patients. On the other hand, *B. melitensis* type I was isolated from a dairy cow. This indicates that, in an area where sheep and goat brucellosis is endemic, cattle could be one of the animal reservoirs for *B. melitensis*.

The consumption of fresh white cheese, which is produced from unpasteurized sheep and goat milk, is very popular in Iran. This cheese is often manufactured rapidly by adding dried rennin from young goat and sheep abomasum to milk, and is then consumed within a few days. In a previous study (Sabbaghian, 1973) *Brucella* organisms were isolated from cheese four weeks after collection from retail shops.

With the high prevalence of brucellosis in sheep and goats and the contamination of fresh cheese with *B. melitensis*, the high consumption of this dairy product (87.4%) seems to be the most important source of human infection. Raw milk, although consumed by only 17.7% of the interviewed group, is another possible source of infection.

Nicoletti & Amini (1971) and Sabbaghian, Ghiasseddin & Abolhassani (1973) have shown that in large cities in Iran, brucellosis is transmitted mainly through the consumption of dairy products. In another study, Feiz, Sohrabi & Sabbaghian (1972) have shown that animal contact is the main source of infection in a village

near Isfahan. The results of this study support the previous findings and indicate that in the city of Isfahan, brucellosis is transmitted mainly through the consumption of dairy products. Although 18% of the urban patients reported animal contact, most of these were from the suburbs of the city and usually maintained a few farm animals. In these cases, infection through animal contact cannot be ruled out.

In contrast to the urban situation, in rural areas about 56% of the patients reported animal contact. On the other hand, a considerable number of the people interviewed in villages reported consumption of unpasteurized dairy products. These findings suggest that, among the rural population in Isfahan province, both contact and non-contact brucellosis occurs.

Bothwell (1960) pointed out that, when the source of infection is dairy products, younger age groups are more frequently affected because of the high rate of dairy product consumption among these groups. In this study, we found that 51.2% of the confirmed urban brucellosis cases were in the age group of up to 20 years. Among the confirmed rural brucellosis cases, 64.8% were in the age group up to 20 years; this may be a result of possible infection by dairy products as well as by animal contact. In the urban and rural population, younger age groups are at a high risk of infection due to unpasteurized dairy products. In addition, the rural young groups are exposed to animal contact sources. In villages, most families keep some kind of farm animals in their own houses and children start working when they are about 7 years of age. Looking after the animals as shepherds is one of the main jobs for children in their spare time. Table 4 shows 50% of reported animal contacts among rural students.

A significant difference between male and female cases of brucellosis in the city of Isfahan could mislead us to suggest that the problem may be occupational. In fact this is not so; only 18% of the urban cases reported animal contacts, and consumption of dairy products is alike for both sexes in this country. A possible reason for the higher rate of brucellosis in males among the urban population may be that more males seek medical care and thus their number appears greater in reports compared with females, as is common in developing countries.

In this study group, housewives without animal contact formed about 25% of the total urban cases, and adult male infection was not higher than female; this suggests that the disease is not occupational in the city of Isfahan.

It has been reported (Boycott, 1964; Report, 1968) that occupational brucellosis is most commonly seen in males. This may not be applicable in all parts of the world, especially where jobs are not well oriented. In developing countries, women work on farms and the chance of coming in contact with farm animals is equal in both sexes. In our study, 21.6% of all rural cases were housewives and about 50% of them reported animal contact. This high percentage report of animal contact among housewives is indicative that, in the rural situation in Isfahan province, occupational transmission of brucellosis is not always confined to men.

In conclusion, in the city of Isfahan most brucellosis cases are transmitted directly through consumption of unpasteurized dairy products, but in the suburban villages both direct and indirect transmission occur.

This study shows that animal brucellosis is prevalent in Isfahan province and, since brucellosis is a zoonosis, control of the disease in animals would result in the reduction of human cases. The production of pasteurized dairy products and public education to elucidate the danger of consuming unpasteurized milk or cheese is recommended for control of human brucellosis.

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