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Brucellosis in 418 patients from the Balkan Peninsula: exposure-related differences in clinical manifestations, laboratory test results, and therapy outcome

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Occupational exposure;
Relapse;
Risk factor

Summary
Objective: The aim of this study was to describe some demographic, clinical and laboratory characteristics, and to evaluate the outcome, in patients with brucellosis in an endemic area in the Balkan Peninsula, and to reveal the differences between patients with and without occupational exposure.
Methods: The study was carried out at the Clinic for Infectious Diseases in Skopje over a period of seven years. Four hundred and eighteen patients with brucellosis were enrolled and classified into two groups: patients with (251) and without (167) occupational exposure.
Results: Two hundred and twenty-eight (54.5%) of the patients had a positive family history. The most common clinical manifestations were arthralgia (81.8%), sweating (71.5%), localized disease (67.7%) and subjective fever (68.4%), whereas elevated values of C-reactive protein (78.9%) and circulating immune complexes (75.8%) were the most frequent laboratory abnormalities. Relapses and therapeutic failure were registered in 16.2% and 10.4%, respectively. Male gender, positive family history and arthralgia were more prevalent in those with occupational exposure, while pediatric age, fever and anemia were inversely correlated with occupational exposure.
Conclusions: Human brucellosis is a serious problem in the Republic of Macedonia presenting with a high percentage of localized forms, relapses and therapeutic failures. The risk factor for acquiring the disease had no influence on the outcome.

Introduction

Brucellosis is one of more than 166 recognized zoonoses,[1] considered by the Food and Agricultural Organization (FAO),

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the World Health Organization (WHO) and the Office International des Epizooties (OIE) as the most widespread zoonosis globally.[2] In endemic regions brucellosis is recognized to have an important impact on human and animal health, economic development, agricultural trade and even tourism.[3]

Humans acquire the infection through the consumption of products of infected animals such as unpasteurized milk, cheese and insufficiently cooked or raw meat.[3] Infection may also result from the entry of the bacteria from diseased animals or their secretions through skin lesions, conjunctiva or from inhalation of contaminated dust or aerosols.[3,4] This is especially common in occupationally exposed persons.[5,6]

In humans, brucellosis behaves as a systemic infection with a very heterogeneous clinical spectrum, which led Simpson in the 1940s to remark: ''no disease, not excepting syphilis and tuberculosis, is more protean in its manifestations''.[7] In some situations, focal forms, relapses, a protracted clinical course, and therapeutic failures are found even when the disease has been recognized in a timely manner and has been adequately treated.

The aim of this study was to describe some demographic, clinical and laboratory characteristics, and to evaluate the outcome, in patients with brucellosis in an endemic area in the Balkan Peninsula in relation to mode of exposure (occupational and non-occupational).

Patients and methods

Patients

The study included 418 consecutive patients with brucellosis who were diagnosed and treated at the Clinic for Infectious Diseases in Skopje in the period January 1998—December 2004. The Clinic for Infectious Diseases is the only academic tertiary care hospital in the Republic of Macedonia, serving a population of 2 million people.

Diagnosis of brucellosis

Brucellosis was diagnosed, as previously described,[8,9] on the basis of clinical symptoms and signs compatible with brucellosis, supported by detection of specific antibodies (standard tube agglutination test and anti-brucella Coombs' test) at significant titers and/or demonstration of an at least fourfold rise in antibody titer in serum specimens obtained 3 to 4 weeks apart.

Patient data

Demographic, clinical, and laboratory data, and information on history of occupational exposure to brucellosis were collected for all patients according to the study protocol. Erythrocyte sedimentation rate, complete blood count, blood chemistry profile, circulating immune complexes, and C-reactive protein were examined in all patients.

A radiographic study of the spine, sacroiliac joints, and other osteoarticular locations was performed for each patient with relevant symptoms and signs, and a radionuclide bone scan with technetium^{99} methylene diphosphonate and/or computed tomography (CT) or magnetic resonance imaging (MRI) were performed in cases with a clinical suspicion of deep osteoarticular location. All patients underwent chest X-ray, electrocardiography, and abdominal ultrasound investigation. Cardiovascular or urogenital involvement was excluded/confirmed by ultrasound investigation of the respective regions. Computed tomography of the brain, lumbar puncture, and electromyography were performed for the clinical suspicion of neurobrucellosis.

Treatment

The patients were treated with various antimicrobial combinations consisting of: oral doxycycline 100—200 mg/day in patients $\geq$8 years old; oral rifampin 600—900 mg/day in adults, 15—20 mg/kg/day in children; oral trimethoprim/sulfamethoxazole 160/800—320/1600 mg/day in adults, 10—12/50—60 mg/kg/day in children; oral ciprofloxacin 1000 mg/day in adults; intramuscular gentamicin 240 mg/day in adults, 5 mg/kg/day in children; intravenous ceftriaxone 4 g/day in adults, 80 mg/kg/day in children.

Doxycycline, rifampin, trimethoprim/sulfamethoxazole and ciprofloxacin when used, were administered for at least 45 days, gentamicin for the first 7—10 days, and ceftriaxone only in patients with brucellar meningitis during the first 21 days. The choice depended on several conditions: clinical presentation, age, pregnancy, drug side effects, tolerability and/or availability. In patients with spondylitis, neurobrucellosis, endocarditis and those with therapeutic failure, the duration of treatment lasted from 3 months to up to one year.

Definitions

Occupational exposure to brucellosis was defined as direct exposure to infected sheep, goats or cows, their blood, secretions, excretions, or tissues for any patient, up to 6 months before the onset of illness.

Various types of localized forms of brucellosis were defined according to criteria from previous studies: neurobrucellosis,[10,11] osteoarticular,[8,12] hematologic,[13] respiratory,[14] hepatic,[15] endocarditis,[11] orchitis and epididymitis.[11]

Therapeutic failure was defined as the persistence of symptoms and signs attributable to the disease at the end of a 45-day therapy, and relapse as the reappearance of disease symptoms and signs after the antibrucellar treatment was completed. Outcome was categorized as favorable (recovery during the first therapeutic course) or unfavorable (relapse, therapeutic failure). The outcome was evaluated only in patients who had a follow-up period of at least 6 months post-therapy.

Follow-up

The patients were hospitalized until clinical improvement was achieved. Laboratory and serological controls were conducted on the 15th and 40th day of treatment. Over the following three months, these check-ups were done once a month, and then every 3—6 months. If necessary, controls were made over shorter time periods if signs or symptoms of relapse appeared or if there was worsening of the existing signs and symptoms. In cases of relapse, the same diagnostic and therapeutic procedures were performed as during the initial episode.

Table 1 Demographic and clinical characteristics in 418 patients with brucellosis, according to occupational exposure

Parameter	Occupational exposure ($N = 251$)	Non-occupational exposure ($N = 167$)	p
Male gender	188 (74.9)	100 (59.9)	0.001
Positive family history	154 (61.4)	74 (44.3)	0.001
Age, years (mean $\pm$ SD)	36.3 $\pm$ 18.7	35.4 $\pm$ 20.4	0.643
Illness duration prior to therapy, days (median; range)	30; 4–360	30; 3–360	0.488
Fever (symptom)	161 (64.1)	125 (74.8)	0.021
Headache	130 (51.8)	91 (54.5)	0.588
Arthralgia	216 (86.1)	126 (75.4)	0.006
Weight loss	68 (27.1)	34 (20.4)	0.116
Malaise	168 (66.9)	115 (68.9)	0.679
Sweating	183 (72.9)	116 (69.5)	0.444
Temperature at admission >37.5 °C (sign)	148 (59.0)	98 (58.7)	0.954
Hepatomegaly	130 (51.8)	77 (46.1)	0.255
Splenomegaly	78 (31.1)	38 (22.8)	0.063
Lymphadenopathy	75 (29.9)	43 (25.7)	0.358
Focal form	163 (64.9)	120 (71.8)	0.139

Data are n (%) unless otherwise stated.

The evaluation of examined data was stratified according to the mode of disease acquisition. Patients were classified into two groups: (a) occupationally exposed and (b) non-occupationally exposed.

Statistical analysis

Quantitative parameters that had normal distribution were presented using mean and standard deviation, and for those without normal distribution median and range values were performed. The Chi-squared test was used for qualitative variables. For quantitative variables the comparison was performed using Student's t-test (normal distribution) and Mann–Whitney U test (not normal distribution). A p value <0.05 was considered significant. Statistical analysis was preformed using Statistical Package for Social Sciences (SPSS) version 8.0.

Results

Occupational exposure to brucellosis was recorded in 251 (60.0%) of 418 examined patients with brucellosis. In 54 (21.5%) subjects in this group, fresh cheese and raw milk consumption were also recorded. The non-occupational group consisted of 167 (40.0%) patients, and in 119 (71.3%) of them ingestion of fresh cheese was recorded as the most probable means of disease acquisition. Drinking of raw milk and ingestion of undercooked goat and lamb meat was a scarce event. However 48 (28.7%) patients in this group denied ingestion of any suspect food.

The mean age of the patients was 36 $\pm$ 19 (range 1–82) years. In 113 (27.0%), the diagnosis was established within two weeks after the onset of symptoms, and an additional 216 (51.7%) patients were diagnosed between two weeks and two months following onset. The most common clinical

Table 2 Laboratory and serological data in 418 patients with brucellosis, according to occupational exposure

Parameter	Occupational exposure ($N = 251$)	Non-occupational exposure ($N = 167$)	p
Erythrocyte sedimentation rate >20 mm/h	151 (60.2)	110 (65.9)	0.355
Anemia	94 (37.4)	80 (47.9)	0.034
Leukopenia	29 (11.6)	15 (9.0)	0.401
Leukocytosis	12 (4.8)	9 (5.4)	0.780
Lymphocytes >45%	85 (33.9)	69 (41.3)	0.122
Thrombocytopenia	10 (4.0)	6 (3.6)	0.838
Alanine aminotransferase >40 U/L	79 (31.5)	59 (35.3)	0.412
Circulating immune complexes >0.05 g/L	191 (76.1)	126 (75.4)	0.880
C reactive protein >5 mg/L	205 (81.7)	125 (74.8)	0.094
Standard tube agglutination test (median; range)	640; 80–1280	640; 80–1280	0.443
Anti-brucella Coombs test (median; range)	1280; 80–5120	1280; 160–5120	0.377

Data are n (%) unless otherwise stated.

Table 3 Localized disease in 418 patients with brucellosis, according to occupational exposure

Parameter	Occupational exposure (N = 251) [a]	Non-occupational exposure (N = 167) [b]
Osteoarticular	142 (56.6)	93 (55.7)
Hematologic	14 (5.6)	15 (9.0)
Urogenital	20 (8.0)	9 (5.4)
Respiratory system	9 (3.6)	16 (9.6)
Nervous system	7 (2.8)	8 (4.8)
Hepatic	4 (1.6)	8 (4.8)
Cardiovascular system	6 (2.4)	3 (1.8)
Cutaneous	4 (1.6)	3 (1.8)

Data are n (%).
[a] Thirty-seven patients with two or more concomitant localized forms.
[b] Thirty-three patients with two or more concomitant localized forms.

manifestations were arthralgia (81.8%), sweating (71.5%) and subjective fever (68.4%).

Male gender and the presence of two or more infected family members were significantly more prevalent in the group that acquired the illness by means of occupational exposure. Arthralgia and no fever (symptom) too, were more frequent in this group (Table 1). Although there was no difference in age distribution overall, a significant number of patients without contact with animals were younger than 14 years when compared to the group with animal contact; 38 (22.8%) and 32 (12.8%), respectively (p = 0.007).

The most frequent laboratory abnormalities in the examined patients were elevated values of: C reactive protein (in 78.9%), circulating immune complexes (in 75.8%), and erythrocyte sedimentation rate (in 62.4%). According to laboratory parameters, the only statistically significant difference between the groups was for anemia (Table 2).

Osteoarticular brucellosis was by far the most common localized form (Table 3). Some of these cases and some of the patients with respiratory manifestations are discussed elsewhere. The most common hematological manifestation was anemia, although thrombocytopenic purpura and pancytopenia were also present. Urogenital localization was mainly presented with orchitis and epididymitis, but pyelonephritis, cystitis, and acute renal failure were present too. Radiculitis, meningitis, myelitis, and peripheral and cranial neuritis were the neurological manifestations recorded. Cardiovascular forms consisted of myocarditis, endocarditis and vasculitis, and various types of rashes represented skin localization (Table 3).

Table 5 Outcome and follow-up in 418 patients with brucellosis, according to occupational exposure

Outcome	Occupational exposure (N = 251)	Non-occupational exposure (N = 167)	p
Follow-up (>6 months)	196 (78.1)	149 (89.2)	0.003
Favorable	143 (73)	110 (73.8)	0.490
Relapse	35 (17.9)	21 (14.1)	
Therapeutic failure	18 (9.2)	18 (12.1)	

Data are n (%).

Most of the patients (78.7%) were treated with a combination of doxycycline, rifampin and trimethoprim/sulfamethoxazole. In the rest of the patients other therapeutic regimens were used (Table 4). No significant difference was evident between groups according to the therapeutic regimen used (p = 0.064).

A follow-up period longer than 6 months was recorded in 345 (82.5%) cases, with a higher percentage in the non-occupationally exposed patients (p = 0.003) (Table 5). Relapses occurred in 16.2% and therapeutic failure in 10.4% of the patients. A favorable outcome during the first therapeutic course was found in 73.4%. The outcome was almost the same in both groups.

One patient died as a direct consequence of brucellosis. Three others died during the follow-up period, but the cause was not brucellosis (myocardial infarction in two and hepatic malignancy in one).

Discussion

The Republic of Macedonia is a small, developing country in the central part of the Balkan Peninsula, with semi-nomadic sheep and goat farming a predominant occupation, and a diet that often consists of cheese prepared from raw milk. This country represents an endemic area where brucellosis prevails as a dominant zoonosis, and is a cause for high morbidity and huge economic loss.[9] The annual incidence of human brucellosis in this country has been higher than 20 cases per 100 000 population for almost 20 years.[8,9] The absence of an animal vaccination program, erroneous implementation of testing and slaughtering of infected animals, and inadequate collaboration between the doctors and veterinarians are the

Table 4 Antimicrobial combinations used in 418 patients with brucellosis, according to occupational exposure

Therapeutic protocol	Occupational exposure (N = 251)	Non-occupational exposure (N = 167)	p
Doxycycline + rifampin + trimethoprim /sulfamethoxazole	190 (75.7)	139 (83.2)	0.064
Doxycycline + rifampin	24 (9.6)	6 (3.6)	
Doxycycline + rifampin + gentamicin	12 (4.8)	4 (2.4)	
Other	25 (10.0)	18 (10.8)	

Data are n (%).

main reasons for the high incidence of the disease. At the same time, the absence of adequate regulation/legislation activities and the apathy of the farmers for collaboration due to insufficient and irregular compensation for sacrificed animals, have also hindered attempts to control brucellosis. Both *Brucella melitensis* biotypes 2 and 3 have been identified from some Macedonian patients in laboratories abroad.

The present study is the largest one in the Balkan Peninsula,[16–18] and unique in comparing patients according to the mode of disease acquisition. To our knowledge, this is the only study showing method of exposure as a factor influencing presentation and outcome.

Often the mode of disease acquisition remains an enigma.[19] Direct contact with infected animals is proven in 11–90%,[20–22] and ingestion of contaminated food in 22–94%[11,15] of patients. In 53–62% of those with direct contact, additional risk factors are also ingestion of inadequately thermally processed products or airway transmission.[12,23] However, in 12–57% patients, the origin of infection remains unknown.[11,21,24] This study has shown that the main risk factor for brucellosis in the Republic of Macedonia is not the consumption of animal products like in other endemic countries,[4,24,25] but working with animals, mainly sheep and goats.

Experimental studies in some species of monkeys have indicated that the minimum oral, inhalation and subcutaneous infective doses of *B. melitensis* are about 5000, 1300 and 325 organisms, respectively.[26] It is estimated that in humans the minimum infective doses are comparable.[6] The inoculum size due to livestock exposure is different from that in ingestion. At the same time, occupational exposure offers frequent, repetitive contact with the source of infection. Wallach et al. stated that the frequency of contact with probable sources of infection, rather than the type of work, appeared to be the main contributory factor for contagion.[5] Studies in abattoir workers, on the other hand, have shown that repeated exposure to *Brucella* organisms might promote immunity that would protect them from the severe symptoms of brucellosis.[27] Finally, recurrent contacts with infected animals may lead to hypersensitivity.[28]

Taking into account the above-mentioned findings, one would expect to observe variations in certain clinical and laboratory findings, as well as in the outcome, depending on the occupational or non-occupational form of disease acquisition. Our expectations were also encouraged by the observation of Young who stated that: "susceptibility to infection depends upon various factors, including... the size and route of inoculum...".[29] Hasanjani Roushan et al. too, have indicated that the size of inoculum and route of acquisition are important factors in determining the clinical presentation and evolution of brucella infection.[11]

As expected, this study showed a significantly higher association of male gender and age above 14 years with occupational exposure, due to the increased involvement of men and adults in livestock breeding. It also showed a more frequent familial involvement in occupationally exposed patients, which reflects the fact that livestock breeding in our country is usually a family trade. The decreased incidence of anemia in occupationally exposed brucellosis patients was unexpected since this population has lower economic standards and an insufficient dietary intake of nutrients. It is possible that this is attributable to the higher altitudes in which these patients with direct contact with animals live.

Regarding the significantly high percentage of localized forms of the disease, up to this date only one report has shown them to occur more frequently than we have.[24] In both studies, this was as a result of a higher incidence of osteoarticular localizations. The occurrence of cutaneous and respiratory forms in patients with occupational exposure was not high, which is contrary to expectations,[20] but agrees with the observation that the mode of entry is not associated with the types of clinical manifestations.[27] Considering that the therapeutic protocol comprised two or three drugs, with good patient compliance and with a duration of no less than 45 days, the percentage of relapses was on the upper limit described in various reports over the past decades.[17,20,29] It is possible, and has to be taken into account, that some of the relapses were due to re-infection.[17,20,27,30]

In conclusion, in the Republic of Macedonia, where brucellosis remains an infection with serious sequelae and public health implications, we observed some differences in demographic, clinical and laboratory parameters, but no differences in the outcome, among patients with and without occupational exposure. All types of specialists must be familiar with this disease, so that early recognition might result in lower morbidity. Special efforts need to be made in the areas of disease control and prevention in animals and in food control, and public-wide education is necessary.

Conflict of interest: No conflict of interest to declare.

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