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Human Brucellosis in Macedonia – 10 Years of Clinical Experience in Endemic Region

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Aim To present our 10-year clinical experience with brucellosis patients at the University Clinic for Infectious Diseases and Febrile Conditions in Skopje, Republic of Macedonia.

Methods A total of 550 patients with brucellosis treated between 1998 and 2007 were retrospectively assessed for their demographic, epidemiological, and clinical characteristics and outcomes.

Results Of the 550 patients, 395 (72%) were male. The median age was 34.5 years (range, 1-82). Direct contact with infected animals was recorded in 333 (61%) patients and positive family history in 310 (56%). The most frequently seen symptoms were arthralgia (438, 80%), fever (419, 76%), and sweating (394, 72%). The most common signs were fever and hepatomegaly, which were verified in 357 (65%) and 273 (50%) patients, respectively. Focal brucellosis was found in 362 patients (66%) and osteoarticular in 299 (54%). Therapeutic failures were registered in 37 (6.7%) patients. Of the 453 (82%) patients who completed a follow-up period of at least 6 months, relapses occurred in 60 (13%).

Conclusion Due to non-specific clinical manifestation and laboratory parameters, brucellosis should be considered one of the differential diagnoses of any patient suffering from obscure involvement of various organs in a brucellosis-endemic region. High percentage of relapses and therapeutic failures in spite of the use of currently recommended therapeutic regimens indicates the seriousness of this zoonosis and the need to control it.

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Brucellosis is a zoonosis caused by intracellular bacteria of the genus *Brucella* (1). The disease is widespread in many countries of the Mediterranean basin, and together with hydatidosis, trichinellosis, and leishmaniasis, it is considered to be a typical Mediterranean zoonosis (2). Human brucellosis is a multisystem disease whose patients present with nonspecific symptoms (3) and a high risk of complications, a protracted clinical course, and relapses (4).

The main clinical characteristics of human brucellosis have been well known for a long time. Marston had been the first to give an accurate description of brucellosis as a disease entity even before the etiological agent was detected (5). The monographs published by Hughes in 1897 (6) and Spink in 1956 (7) contain perhaps the most detailed and still accurate data on this topic. Today, there is a lot of information about the characteristics of human brucellosis available from various parts of the world, and the description of its characteristics varies widely.

For almost 30 years, brucellosis has been a dominant zoonosis in the Republic of Macedonia, causing a high morbidity and huge economic losses. However, the main reasons for persistence of the disease are not only husbandry practices and traditional food and living habits (2), but also an inadequate strategy of brucellosis control (8). The aim of our study was to present more detailed insights into the predominant demographic, epidemiological, clinical, and laboratory features of brucellosis patients, and their outcomes, during a 10-year period in the endemic region of the Republic of Macedonia.

METHODS

This retrospective study included 550 patients who were diagnosed with brucellosis and treated at the University Clinic for Infectious Diseases and Febrile Conditions in Skopje between January 1998 and December 2007. The diagnosis was based on clinical findings compatible with brucellosis (arthralgia, fever, sweating, malaise, hepatomegaly, splenomegaly, signs of focal disease), supported by detection of specific antibodies at significant titers and/or demonstration of at least a 4-fold rise in antibody titer in serum samples obtained 3-4 weeks apart. Antibody titers were determined by standard tube agglutination (STA), *Brucella* Coombs test (9,10), or the Brucellacapt assay (11). The corresponding titers considered positive were ≥1/160, ≥1/320, and >1/320, respectively. During the study period, bacteriological isolation was not a routine practice in the Republic of Macedonia.

Demographic and epidemiological data, clinical symptoms and signs, laboratory characteristics and outcome of the patients were analyzed. If a focal form of the disease was suspected after clinical examination, further investigations were performed, such as x-rays, electrocardiography, ultrasound investigations, lumbar puncture, radionuclide bone scan, computerized tomography, magnetic resonance imaging, and electromyography.

The patients were treated with various combinations of two or three of the following drugs: (a) oral doxycycline at 100-200 mg/d in patients ≥8 years of age; (b) oral rifampin at 600-900 mg/d in adults or 15-20 mg/kg/d in children; (c) oral trimethoprim (TMP)-sulfamethoxazole (SMZ) combination therapy at TMP doses of 160-320 mg/d and SMZ doses of 800-1600 mg/d in adults, or TMP doses of 10-12 mg/kg/d and SMZ doses of 50-60 mg/kg/d in children; (d) oral ciprofloxacin at 1000 mg/d in adults; (e) intramuscular gentamicin at 240 mg/d in adults or 5 mg/kg/d in children, and (f) intravenous ceftriaxone at 4 g/d in adults or 80 mg/kg/d in children. Doxycycline, TMP-SMZ, rifampin, and ciprofloxacin were administered for 45-60 days; gentamicin, for the first 7-14 days. Ceftriaxone was part of the antimicrobial therapy in patients suffering from neurobrucellosis and was administered for 14-30 days. In patients with spondylitis, neurobrucellosis, endocarditis and those with therapeutic failures, antimicrobial treatment was prolonged for at least 3 months.

Osteoarticular involvement was considered to be present if there were any inflammatory signs in peripheral osteoarticular locations (swelling, pain, functional disability, heat, and redness of the joints), or inflammatory pain in deep osteoarticular locations concurrently with pathologic findings on x-rays, radionuclide bone scans, computerized tomography, or magnetic resonance imaging (12). Orchitis and epididymitis were diagnosed by the presence of swelling and tenderness of the testis and epididymis, respectively. Hepatic involvement was defined as more than a 2-fold increase in alanine aminotransferase levels above the reference values (13). Neurobrucellosis was defined as presence of neurological dysfunction, not otherwise explainable, in combination with pathologic laboratory findings in the cerebrospinal fluid, ie, >10 cells/mL or protein concentrations >0.45 g/L and detection of anti-*Brucella* antibodies (14,15). Endocarditis was confirmed by auscultation of a cardiac murmur and detection of valvular vegetations using trans-thoracic echocardiography (15). Respiratory complications were defined by the protocol described by Pappas et al (16).

Hematological parameters were described according to the criteria published by Troy et al (17). However, hematological involvement was considered to be present only in patients with manifestations due to hematological discrasia.

Therapeutic failure was defined as persistence of symptoms and signs attributable to the disease after two months of antibiotic treatment, and relapse as the reappearance of symptoms and signs after completion of antibrucellar treatment. Relapses were evaluated only in patients who had a follow-up period of at least 6 months post-therapy, whereas therapeutic failures were estimated in all treated patients irrespective of the follow-up period.

Patients were hospitalized until clinical improvement was achieved. Laboratory and serological controls were conducted on the 15th and 40th day of the treatment. In the follow-up period, these controls were done once a month during the first three months, and then every two to six months until the patient stopped coming for check-ups. Controls were conducted earlier if signs and symptoms aggravated or re-appeared after clinical cure. In the case of a relapse, the same diagnostic and therapeutic procedures were performed as in the initial episode of the disease.

Age, illness duration prior to diagnosis, treatment duration and follow-up period are presented using median and range values. All other parameters are presented as frequencies and percentages. Statistical analysis was performed using the SPSS, version 8.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

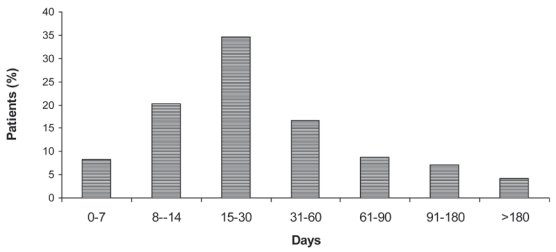
The patients were 1 to 82 years old (median, 34.5). Most of them belonged to the age group 15-40 years (n=252 patients, 46%), followed by the age group 41-60 years (n=132, 24%). The number of patients younger than 14 (n=86, 16%) was nearly the same as the number of those older than 60 years (n=80, 15%). The male/female ratio was 395/155 (72/28%). Four female patients were pregnant at the time when the diagnosis was established.

An occupational exposure was reported by 333 (61%) patients. The ingestion of potentially contaminated food was mentioned by 155 (28%) patients. In 62 (11%) patients, the mode of transmission remained obscure. Three hundred and ten patients (56%) belonging to 103 families, had a confirmed family history of brucellosis in at least one of their members. Patients presented at the hospital most frequently during the spring (n=216, 39%) and summer (n=183, 33%). The nadir of brucellosis was in winter (n=95, 17%) and fall (n=56, 10%).

The median illness duration prior to diagnosis was 30 days (range, 3 to 360). More than 60% of patients were diagnosed within 30 days, and 80% within 60 days after the onset of symptomatic disease (Figure 1). The clinical characteristics, laboratory findings, and initial serological titers at the time of admission are shown in Table 1 to Table 3, respectively. Osteoarticular involvement was the most frequent focal complication, found in 299 (54%) patients. Patients presented with peripheral arthritis (n=187, 34%), sacroiliitis (n=68, 12%), spondylitis (n=78, 14%), and miscellaneous osteoarticular forms (n=11, 2%). The genitourinary, respiratory, hematological, nervous, hepatobiliary, cardiovascular, and other organ systems were affected, respectively, in 36 (6.5%), 34 (6.2%), 32 (5.8%), 19 (3.4%), 15 (2.7%), 10 (1.8%), and 6 (1.1%) patients.

Eighty-six patients (16%) were treated with a combination of two drugs; in the remaining 464 (84%) patients three drugs were used. Three hundred and nineteen (58%) patients were treated with a combination of doxycycline, rifampin, and trimethoprim/sulfamethoxazole; 62 (11%) with a combination of doxycycline, rifampin, and gentamicin; 53 (10%) with a combination of doxycycline and rifampin; and 31 (6%) with a combination of rifampin and TMP/SMZ. In the remaining 85 (15%) patients, other therapeutic regimens were used. Treatment duration was median 45 days (range 45-360). In 102 (18%) patients, treatment duration was prolonged for at least 90 days. Corticosteroids were used in 15, 12, 11, and 4 patients presenting with meningo/myelo/radiculo/neuritis, thrombocytopenia/ pancyto-

Figure 1.



Illness duration before diagnosis in 550 patients with brucellosis diagnosed and treated during 1998-2007 at the University Clinic for Infectious Diseases and Febrile Conditions in Skopje.

penia, orchitis/orchoepididymitis, and vasculitis as a consequence of brucellosis, respectively.

The outcome of the pregnancy in the 4 expecting patients was spontaneous deliveries in 3 (1 premature, 2 in-term) and 1 abortion. One patient with brucellar endocarditis underwent a cardiac surgery and valve replacement. Two patients died within the treatment period: the first fatal outcome was a result of a myocardial infarction, and the second of renal and respiratory complications due to brucellosis. Therapeutic failures occurred in 37 (6.7%) patients. The median follow-up of our patients was 10 months (range, 0-84) post-therapy. A follow-up period of ≥6 months was recorded in 453 (82%) cases, 60 (13%) of which relapsed.

DISCUSSION

Most of the patients with brucellosis in our study were men and belonged to the age group 15-40 years. Age distribution of the study population is important because it may influence the clinical expression of brucellosis, including the frequency and type of signs, symptoms, complications (18,28-30).

The age distribution of the patients in our study did not differ significantly from that of patients in other studies (19,28). The frequency of brucellosis among children (≤14 years) in our study was similar to that reported in other studies (20,28); in fact, in several other studies, nearly one of every

TABLE 1. Frequency of clinical characteristics in patients with brucellosis, as reported in our and other studies

	Percent of patients reporting condition						
Literature reference	fever (symptom)	malaise	night per-spiration	arthralgia	fever	hepato-megaly	spleno-megaly
Aygen et al (14)	80	90	84	82	39	21	14
Yinnon (18)	96	33	ND*	35	ND	55	69
Memish et al (19)	91	ND	19	66	84	6	7
Andriopoulos et al (20)	100	97	96	87	ND	25	51
Shehabi et al (21)	100	ND	88	60	ND	54	61
Demiroglu et al (22)	80	86	78	87	ND	ND	ND
Tasbakan et al (23)	94	96	92	82	97	38	60
Pourbagher et al (24)	55	76	53	85	ND	6	8
Trujillo et al (25)	100	77	96	81	ND	10	12
Buchanan et al (26)	ND	95	93	17	95	ND	ND
Kokoglu et al (27)	78	ND	72	78	41	27	36
Present study, No. (%)	419 (76)	377 (68)	394 (72)	438 (80)	357 (65)	273 (50)	159 (29)

*Abbreviation: ND – not determined.

TABLE 2. Frequency of hematological and biochemical features in patients with brucellosis, as reported in our and other studies*

	Percent of patients with the given feature								
Literature reference	erythrocyte sedimentation rate >20 mm/h	C-reactive protein >5 mg/L	alanine aminotransferase >40 U/L	leucopenia	leucocytosis	lymphocytes >45%	thrombocytopenia	anemia	pancytopenia
1	ND	ND	24	2	ND	40	5	ND	2
13	ND	ND	31	27	ND	ND	15	31	2
14	59	ND	ND	8	6	68	ND	55	ND
18	ND	ND	40	31	3	55	3	51	ND
24	49	23	10	ND	ND	ND	ND	30	ND
28	ND	74	39	14	8	32	ND	70	ND
29	42	48	18	6	4	ND	ND	ND	ND
30	77	ND	40	19	9	41	8	7	3.5
Present study, No. (%)	337 (63)†	389 (79)‡	180 (33)	50 (9.1)	45 (8.2)	132 (24)	58 (12)§	148 (27)	3 (0.6)§

*Abbreviations: ND – not determined.
†Measured in 535 patients.
‡Measured in 490 patients.
§Measured in 500 patients.

TABLE 3. Number (and percent) of patients showing the indicated anti-*Brucella* antibody titers as measured using the serum tube agglutination (STA) test, Coombs test, or Brucellacapt at the time of admission to hospital*

Titer	STA†	*Brucella* Coombs†	Brucellacapt‡§
≤1/80	24 (7)	0	0
1/160	34 (10)	28 (8)	4 (2)
1/320	51 (15)	43 (12)	8 (4)
1/640	82 (24)	67 (20)	20 (10)
1/1280	153 (44)	206 (60)	20 (10)
1/2560	ND	ND	29 (14)
1/5120	ND	ND	125 (61)

*Abbreviation: ND – not determined.
†Performed in 344 patients.
‡Performed in 206 patients.
§The numbers do not add up to 100% because of rounding.

four patients fell into this age group (3,18,19,21,31). In an Iranian study, 56% of the examined patients were younger than 14 years (32). The frequency of patients older than 60 years varied from 2% (33-35) to 32% (36). A high frequency in older age groups was also found in a study conducted in northwestern Greece (37).

Consistent with our results, the majority of studies indicate that men are infected more often, ie, in 55% (15,19,38-42) or even 96% of the cases (34). Nevertheless, sex distribution of patients with brucellosis varies widely: several studies have indicated either equal distribution (21,28,43,44) or, in some cases, predominance of female patients (14,22,29,45).

Gotuzzo et al detected a more severe course of the disease in women, especially those with brucellar arthritis (46). However, the complication rate seems to be higher in men (15). Headaches and lethargy are more frequently observed in women and splenomegaly in males (3).

Age and sex distribution found in this study is a result of regional habits, mostly due to husbandry practices that make working-age men most vulnerable to brucellosis.

Nevertheless, brucellosis can be found in women, as well as marginal age groups due to consumption of food products of animal origin (eg, young cheese from sheep or goats) that are not adequately thermally processed.

The epidemiology of brucellosis often remains obscure and is not well defined. In patients with professional exposure, besides the skin and conjunctival contact, the infection can be acquired both by airborne transmission and by ingestion of contaminated animal products (45). Hence, risk assessment is often subjective. Some authors think that occupational exposure risk is the most important (28,41), others favor the transmission by animal food (described in 63%-92% of the patients) (20,23,24,33,38,45), whereas in several studies the mode of transmission remains unknown for up to 57% of the patients (15,47). It is of particular importance to understand the mode of brucellosis transmission since this knowledge helps to apply appropriate countermeasures.

Our study confirmed that screening the family members of a brucellosis patient is an important issue. Family history of brucellosis was reported in 9% to 51% of patients (15,24,46,48). Family screening leads to early diagnosis of the disease, which may prevent complications (49,50).

Brucellosis duration prior to diagnosis is an important parameter in assessing the clinical course and outcome. It has been recognized that brucellosis duration without adequate treatment is directly correlated with the complication rate and unfavorable outcome (41). A long period of symptomatic disease before therapeutic intervention was significantly more frequent in patients with brucellar spondylitis than in patients without spondylitis (12,48). The percentage of relapses among patients suffering from the disease for more than a month before therapy was higher than that among patients suffering from the disease for less than a month (29). Contradictory to these findings, other studies demonstrated that treatment beginning less than 10 days after disease onset led to higher relapse rates (51,52). In endemic countries, brucellosis may be diagnosed earlier because physicians are familiar with the clinical signs and symptoms and take it more often into consideration as a differential diagnosis. However, this is not always the case. In some series, 16% of patients were diagnosed with a delay of more than three months after the onset of symptoms (41,47). The diagnostic delay could be a result of either misdiagnosis or postponed visit to a medical professional (8,53).

Clinical and laboratory features are often not pathognomonic for human brucellosis and differ widely (Table 1 and Table 2). In addition, the number of patients suffering from focal involvement ranges from 6% to 92% (20,21,42,54) and is usually about 30% (14,15,38,41,55). In our study, two-thirds of the enrolled patients had focal manifestations and osteoarticular forms predominated, similar to other reports (1,15,20,28,44). The wide variation in the frequencies of clinical manifestations may reflect the characteristics of the examined population, the nature of the

causative agent, geographic variations of the disease, the stage of disease, the diversity of case definition criteria, the diagnostic procedures, and the type of study (retrospective or prospective) (12).

The serum agglutination test is an important diagnostic tool, bearing in mind that bacterial isolation methods are time-consuming, show lack sensitivity, and pose risk to laboratory personnel (56). The combination of the serum agglutination test and Coombs tests in diagnosing the disease may help to overcome the problem of false-negative results (1,57). Recently, the Brucellacapt test has started to replace other serological tests (58,59). Serological evaluation of our results at the time of admission to hospital generally showed high anti-*Brucella* antibody titers, especially in the Coombs test and the Brucellacapt. In the case of inconclusive serological results but a high clinical suspicion of brucellosis, the patients were retested after 2-4 weeks to assess possible seroconversion (19).

The primary goal of brucellosis therapy is to control the illness and prevent complications, relapses, and sequels (60). The currently recommended treatment regimens are based upon the recommendations of the World Health Organization in 1986 (61), updated by experts in the field at the Conference for Treatment of Human Brucellosis held in Ioannina, Greece, in 2006 (62). Nevertheless, a great diversity of therapeutic protocols exists. The selection of antimicrobial agents depends on the clinical presentation, age of the patient, pregnancy, drug side effects, and co-morbidity; in addition, in regions with scarce resources, therapeutic decisions are also determined by treatment costs and drug availability. The triple antimicrobial combination is rarely implemented and its use is traditionally restricted to patients with neurobrucellosis, endocarditis, or abscesses (3,23,63,64). Nevertheless, the highest cure rate can be achieved with a triple therapy (3) and adding a third drug to the standard regimen seems to be beneficial (63). In two reports, children treated with three drugs had a better outcome than those treated with two drugs (65,66). Almost 84% of the patients in the present study were treated with a combination of three antimicrobial agents, based on our previous experience that three-drug therapy was better than two-drug therapy (unpublished data).

In the first epidemiological study on human brucellosis in Malta during the period 1901-1907, when understanding of the disease was very limited and treatment was inadequate, Eyre reported that the mortality rate among the civilian population was approximately 10%, and 2.3% among soldiers and marines (7,67). In the recent decades with the use of appropriate antimicrobial treatment, the mortality rate has been lower than 1% (4,14,29,41,68). However, there are exceptions, in non-endemic countries as a result of the decreasing knowledge about the disease and in endemic regions of the developing world due to lack of medical care. For instance, in Germany, mortality rates increased from 0.4% (1978-1981) to a maximum of 6.5% (1998-2001) (69). In Nigeria, a mortality rate of 5.4% was reported (70). Of course, mortality rates might be biased by the selection of patients with specific clinical manifestations; for example, the rate was 2.1% in a series of patients with vertebral osteomyelitis in Spain (71). *Brucella* endocarditis is the main cause of death attributable to this disease (72-74), but many other causes have been described, eg, endotoxic shock with disseminated intravascular coagulation (75), rupture of a mycotic aneurysm (76), aortitis with mesenteric thrombosis (41), myocarditis (77), dissemination with multifocal liver and lung nodules (78), thrombocytopenia (79), and neurobrucellosis (14,29,41). We had only one mortality case as a direct consequence of brucellosis. The main reason for fatal outcome was development of renal and respiratory failure, which can be added to the above list of rare causes of fatal outcome in patients with brucellosis.

Therapeutic failures and relapses are inevitable characteristics of the disease. Unfavorable outcomes usually have to be ascribed to the inability to eradicate the bacteria from their intracellular niche. In adequately treated patients, relapses occur in 0 to 16% of the cases (14,31,33,38,41,45,80-82). The frequency of relapses found in our patients is similar to those mentioned in other reports (27,35,83-85). In our study, only patients with a follow-up period of at least 6 months post-therapy were included when assessing the relapse rate. If we included the patients with a follow-up period shorter than 6 months, eg, those who had never showed up for a check-up or who had come to only one, we would have underestimated the relapse rate, given that relapses most frequently appear in brucellosis during the first 6 months after the end of treatment (52,86). Possible reasons for relapse are inadequate choice of antibiotics, short treatment duration, and a lack of compliance. However, the relapse rate may be biased by the inability to distinguish re-infections from relapses and short follow-up periods (12). Therapeutic failures, which occurred in 6.7% of patients in our study, are mostly associated with brucellar spondylitis (12,41,48,87). According to literature data, the frequency of therapeutic failures varies from 0 to 15% (17,22,23,33,38,41,42,55,80).

Our study has two main limitations. First, we did not use *Brucellae* isolation, which is important for disease diagnosis, determination of *Brucella* species, detection of relapses, and follow-up of antimicrobial sensitivity. Second, this study was retrospective and data were sometimes missing about biochemical tests or other diagnostic findings such as magnetic resonance imaging, which has since then become the preferred procedure for patients with spondylitis, but which was performed in our study in only 29 of 78 (37%) patients with this condition. In addition, since the study was retrospective, the study group included many different therapy combinations and longer follow-up for some of the patients was not possible.

In summary, brucellosis is still a serious public health problem in the Republic of Macedonia. For early diagnosis, considering family history of the disease is important and special emphasis should be placed on asking about occupational exposure and family members suffering from brucellosis. Keeping in mind that clinical and laboratory characteristics of the disease are heterogeneous and nonspecific, brucellosis has to be considered in the differential diagnoses of patients with complex organ involvement referred to hospitals in endemic regions and in travelers returning from endemic regions. The high relapse and therapeutic failure rates, despite the use of an adequate therapy, show the seriousness of this zoonosis and the absolute need to control it in the animal reservoir.

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