NATURAL HISTORY OF BRUCELLOSIS IN AN ENDEMIC REGION IN DIFFERENT TIME PERIODS

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SUMMARY – The aim of the study was to determine the evolution and outcome of human brucellosis in an endemic region in relation to time interval. Retrospective analysis was employed to compare demographic, epidemiological, clinical, laboratory features and the outcome of patients with brucellosis, treated at University Department of Infectious Diseases in Skopje during two different periods of time. A series of 159 patients were studied in the first (1990-1991) and 138 in the second (2003-2005) study period. Patients treated in the second period were older (34.6±20.9 *vs.* 30.0±17.7 years; P=0.041) and acquired brucellosis less frequently on ingestion of incriminated food (34.8% *vs.* 47.2%; P=0.031). Focal forms were more evident in the second period (66.7% *vs.* 50.3%; P=0.004), mainly due to osteoarticular localization. Post-treatment follow up was more efficient in the second group (76.1% *vs.* 61%; P=0.005). There was no difference according to disease outcome in spite of different therapeutic trials during the two study periods. In conclusion, the established differences showed an improvement in the understanding of the disease by the general population as well as upgrading of some aspects considering medical activities. Nevertheless, this endemic region still lacks the most important measure, i.e. development and implementation of an appropriate national program for efficient control of the disease.

Key words: *Brucellosis – diagnosis; Brucellosis – education; Brucellosis – epidemiology; Brucellosis – prevention and control; Zoonoses – epidemiology; Macedonia*

Introduction

Brucellosis is one of the most widespread zoonoses in the world generating devastating economic losses and significant public health problems[1,2]. It is estimated that the real number of *Brucella* infected individuals is 26 times higher[3] than the 500 000 reported annually[4]. The reason for this is that the disease often remains underreported due to the lack or feeble diagnosis or failure in the reporting systems[5,6]. Among the most prevalent endemic regions in the world are the Near and the Middle East, Mediterranean Basin, Indian Subcontinent, Mongolia and several Asian states of the former Soviet Union[7].

The Republic of Macedonia situated on the Balkan Peninsula is a small country of 25 000 km^2 with 2 000

000 inhabitants. During the 1945-1979 period, only 14 cases of human brucellosis were reported in the country[8]. After the introduction of infected rams from Israel in the 1980s, there was a rapid increase in the incidence of human brucellosis in the country (Fig. 1), being pres-
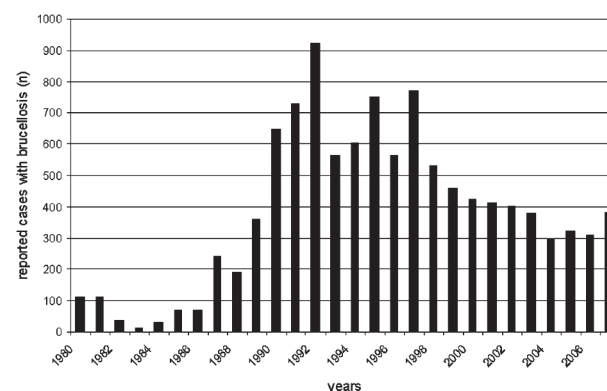

Fig. 1. Brucellosis in the Republic of Macedonia (data obtained from the National Health Care Institute).

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ently one of the highest in the world[7]. We have previously described some clinical features of human brucellosis in this endemic region[2,9-11].

The available literature offers no data analyzing the impact of time on the brucellosis characteristics in an endemic region (i.e. the time as a factor influencing brucellosis presentation and outcome). However, there are just few reports on studies in non-endemic areas, where epidemiological trends of human brucellosis were observed in relation to time period[12-15]. We present the impact that two different circumstances separated by a lapse of 12-15 years had upon the presentation (demographic, epidemiological, clinical and laboratory features) and outcome of brucellosis in a restricted endemic region with a high prevalence of the disease.

Material and Methods

This retrospective study included 297 patients with brucellosis diagnosed and treated at University Department of Infectious Diseases and Febrile Conditions in Skopje. Group 1 of 159 patients was diagnosed from January 1990 till December 1991, whereas the group 2 of 138 patients was diagnosed from January 2003 till December 2005. The diagnosis was based on clinical findings compatible with brucellosis, supported by detection of specific antibodies at significant titers and/or demonstration of an at least fourfold rise in antibody titer in serum samples obtained 3 to 4 weeks apart. Antibody titers were determined by standard tube agglutination (STA), Brucella Coombs[16,17] and Brucellacapt assays[18,19]. The corresponding titers for considering positive reactions were ≥ 1/160, ≥ 1/320 and ≥ 1/320, respectively. During these periods, bacteriologic studies for isolation of *Brucella* sp. were not performed in the Republic of Macedonia.

Patients were treated with various antimicrobial combinations, as follows: 1) oral tetracycline 2000 mg/day in adults, 30-50 mg/kg/day in children older than 8 years; 2) oral doxycycline 100-200 mg/day in patients older than 8 years; 3) oral rifampin 600-900 mg/day in adults, 15-20 mg/kg/day in children; 4) oral trimethoprim/sulfamethoxazole 160/800-320/1600 mg/day in adults, 10-12/50-60 mg/kg/day in children; 5) oral ciprofloxacin 1000 mg/day in adults; 6) intramuscular streptomycin 1000 mg/day in adults, 20-25 mg/kg/day in children; 7) intramuscular gentamicin 240 mg/day in adults, 5 mg/kg/day in children; and 8) intravenous ceftriaxone 4 g/day in adults, 80 mg/kg/day in children. When used, tetracy-

cline, doxycycline, trimethoprim/sulfamethoxazole, rifampin, and ciprofloxacin were administered for at least 45 days. Streptomycin was used for the first 14-30 days, and gentamicin for the first 10 days. Ceftriaxone was part of antimicrobial combination in some patients with neurobrucellosis and was administered for 14-30 days. The choice of antibiotics depended on the study period, tradition, clinical presentation, age, pregnancy, drug side effects, tolerability and/or availability. Antimicrobial therapy longer than 45 days was only administered in several patients from group 1. Alternatively, in all group 2 patients that manifested spondylitis, neurobrucellosis or endocarditis antibiotic therapy was administered for at least 3 months.

Demographic and epidemiological data, clinical manifestations, laboratory characteristics and patient outcome were recorded. Various types of focal forms were defined in our previous studies[2,9-11]. Therapeutic failure was defined as persistence of symptoms and signs attributable to the disease at the end of the second month of antibiotic therapy initiation. Concomitantly, relapses were defined as reappearance of symptoms and signs upon completion of the specific antibiotic treatment. Outcome was categorized as favorable in case of recovery achieved with the first therapeutic course or unfavorable in case of relapse or therapeutic failure. The outcome was only evaluated in patients with a follow-up period of at least 6 months post-therapy.

The χ^2-test was used for qualitative variables. For quantitative variables, Student's t-test and Mann-Whitney U test were used for normal and not normal distributions, respectively. P values <0.05 were considered significant.

Results

The clinical and epidemiological characteristics of brucellosis in populations corresponding to two different time periods are shown in Table 1. As presented, the majority of study patients were males and belonged to families with a history of brucellosis in at least one of their members. Direct contact with infected domestic animals seemed to be a significant factor for the acquisition of the infection, and was more frequent during the 2003-2005 period. Patients diagnosed and treated during the second time lapse were considerably older than those from the first time period. In the second study period, 21 (15.2%) patients were older than 60, as compared with only 10 (6.3%) patients in the first period

Table 1. Demographic, epidemiologic and clinical characteristics of brucellosis during two study periods

Parameter	Patients with brucellosis 1990-1991 (n=159)	Patients with brucellosis 2003-2005 (n=138)	P
Brucellosis family antecedents	106 (66.7)	79 (57.2)	0.095
Contact with animals	84 (52.8)	90 (65.2)	0.031
Male sex	116 (73)	106 (76.8)	0.446
Age (yrs) (mean±SD)	30.0 ± 17.7	34.6 ± 20.9	0.041
Days of illness prior to antibiotic treatment (median, range)	30 (2-300)	30 (4-360)	0.781
Fever	117 (73.6)	94 (68.1)	0.300
Arthralgia	121 (76.1)	103 (74.6)	0.770
Weight loss	37 (23.3)	33 (23.9)	0.896
Malaise	120 (75.5)	102 (73.9)	0.758
Sweating	107 (67.3)	97 (70.3)	0.579
Hepatomegaly	96 (60.4)	72 (52.2)	0.155
Splenomegaly	61 (38.4)	41 (29.7)	0.117
Focal form[*]	80 (50.3)	92 (66.7)	0.004

Data are in "n" (%) unless otherwise stated; [*]some patients had two or more concomitant focal forms

(P=0.012). The predominant clinical manifestations were arthralgia, malaise and fever. The only clinical manifestation that significantly distinguished the two time periods was the higher rate of focal forms, mainly osteoarticular and cutaneous, in group 2 (Table 2). Seasonal distribution of the disease onset showed predominance from March to June (Fig. 2).

The most common initial laboratory findings were elevated erythrocyte sedimentation rate, above 20 mm/h (52.8% *vs.* 59.4%; P=0.254) and normal leukocyte counts (91.8% *vs.* 84.1%; P= 0.116). In group 1, serolog-ic testing demonstrated STA and Brucella Coombs test median titers of 320 (range 80-1280) and 640 (range 80-1280), respectively. In group 2, the STA and Brucella Coombs test median titers recorded in 28 patients corresponded to 640 (range 80-1280) and 1280 (range 160-1280), respectively. In the rest of 110 patients, the diagnosis was established using Brucellacapt test (median 5120, range 160-5120).

During the 1990-1991 period, 149 (93.7%) patients were treated with a combination of streptomycin, tetracycline and trimethoprim/sulfamethoxazole. However,

Table 2. Focal forms of brucellosis in two study periods

Parameter	Patients with brucellosis 1990-1991 (n=159)	Patients with brucellosis 2003-2005 (n=138)
Spondylitis	8 (5)	16 (11.6)
Sacroiliitis	5 (3.1)	11 (8)
Peripheral arthritis	36 (22)	57 (41.3)
Other osteoarticular	2 (1.3)	2 (1.4)
Hepatic	4 (2.5)	4 (2.9)
Hematologic	14 (8.8)	11 (8)
Respiratory	8 (5)	6 (4.3)
Cutaneous	3 (1.9)	5 (3.6)
Cardiovascular	4 (2.5)	3 (2.2)
Urogenital	7 (4.4)	6 (4.3)
Neurologic	9 (5.7)	6 (4.3)

Data are in "n" (%)
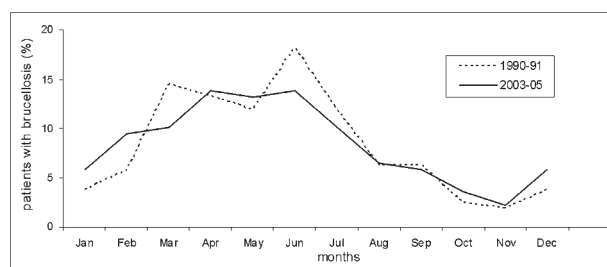
Monthly distribution of the onset of brucellosis in study patients during the 1990-1991 period (dotted line) and 2003-2005 period (full line).

Fig. 2. Monthly distribution of the onset of brucellosis during two study periods.

during the 2003-2005 period, this drug combination was abandoned and substituted by a combination of rifampin, doxycycline and trimethoprim/sulfamethoxazole in 100 (72.5%), rifampin and doxycycline in 11 (8%), and rifampin, doxycycline and gentamicin in 9 (6.5%) patients. In the rest of patients various antimicrobial combinations were used. The rate of efficient follow up of at least 6 months was higher in the second than in the first study period. The rate of favorable outcomes was comparable in the two groups (Table 3).

differences observed.

First of all, the attitude of the population towards the disease: information, health education, compliance, cooperation with medical, veterinary and other competent state authorities[3,20]. The declining trends in the disease acquisition by consumption of incriminated food, the higher prevalence of older patients (who actually carry out the most risky activities in animal husbandry), and longer patient follow up in the second study period could be to a great extent attributed to the improved understanding of the disease and its sequels in the general population. Our presumption on the shorter disease duration prior to therapy in the second study period was not justified. The main reason was personal decision of some patients to avoid visiting their doctors due to unsatisfactory compensation for their herds that were to be slaughtered afterwards[9], but also due to the mentality of the people themselves[5,21]. Even though no significant differences were observed during the two study periods, it is obvious that the disease continues to be predominant in males and in family members, mainly because of the traditional husbandry practices in the region. The seasonal distribution of brucellosis was also similar in the two study periods[22].

Table 3. Outcome and follow up of brucellosis patients in two study periods

Outcome	Patients with brucellosis 1990-1991 (n=159)	Patients with brucellosis 2003-2005 (n=138)	P
Follow up (>6 months)	97 (61)	105 (76.1)	0.005
Favorable	83 (85.6)	88 (83.8)	
Relapse	6 (6.2)	12 (11.4)	0.283
Therapeutic failure	8 (8.2)	5 (4.8)	

Data are in "n" (%)

Discussion

The purpose of the present study was to observe the evolution of human brucellosis in an endemic area through comparison of demographic, epidemiological and clinical characteristics as well as the outcome of the disease over a time lapse of 12 to 15 years and to give an appropriate explanation for the differences recorded. In this way, the follow up of the disease evolution enabled introspection of the relation between the disease and three vital (dynamic) segments of daily life. At the same time, these segments yield positive feedback to the disease and represent a mainstay for rationalization of the

Second issue concerns the relation of medical services and brucellosis: diagnosis and reporting, definition of symptoms and signs, therapeutic decisions and follow up of patients. The evident increase in the osteoarticular and cutaneous forms of the disease observed in the second time period could presumably be ascribed to enhanced carefulness of the physicians in patient management and examination, and to better, more sophisticated imaging techniques available. The use of different serologic tests (Brucellacapt instead of STA and Brucella Coombs test) in the 2003-2005 period was based on the decision of health management factors, as the result of easier provision of Brucellacapt but also

due to its easier performance, having in mind that there were no differences found when Brucella Coombs and Brucellacapt tests were compared[18,19,23]. The absence of bacteriologic diagnosis of brucellosis during the study periods deprived us of vital information on the possible species modifications and changes in its sensitivity to the antimicrobials used in the region.

Albeit there were no significant differences in the disease outcome, it is obvious that the rate of disease relapses was higher in the second period. It might be partly a result of better and longer follow up of patients, as well as of therapeutic choice (rifampin instead of streptomycin), which does not represent a curiosity in the literature[24]. Also, the lower rate of therapeutic failures in the second period could be explained by prolonged treatment duration in all patients with spondylitis, endocarditis and central nervous system involvement.

The third segment is the state policy-disease relationship, i.e. implementation and harmonization of national brucellosis surveillance and control programs. Having in mind that the control of brucellosis is mainly a veterinary responsibility[25], these programs should provide for recruitment of qualified veterinarians, collaboration between the veterinary and public health sectors, funds, international techniques and scientific collaboration, implementation of regulation/legislation activities to control marketing and movement of animals[3,26,27], as well as for tight control of the manufacture and marketing of food of animal origin. Unfortunately, in our country the strategy of brucellosis control has to date been based exclusively on partial test-and-slaughter of infected animals.

In conclusion, in spite of evident improvement in the control of brucellosis, there is still no reason to be satisfied with the achieved. During the 15-year period, some positive results have been reached; however, the continuingly high incidence of the disease, the disease transmission without animal contact in one third of patients, the long disease duration prior to therapy, the high incidence of focal forms, and the high rate of relapses and therapeutic failures point to the need of hard, relentless and organized work in many segments of daily life to be able to overcome the problem of human brucellosis. Also, clinicians should pay due attention to better follow up of the disease and longer treatment for some focal forms of the disease.

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Sažetak

NARAVNA POVIJEST BRUCELOZE U ENDEMSKOM PODRUČJU KROZ RAZLIČITA VREMENSKA RAZDOBLJA

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Cilj studije bio je utvrditi razvoj i ishod bruceloze kod ljudi u jednom endemskom području u odnosu na vremenski pomak. Demografski, epidemiološki, klinički i laboratorijski podaci te ishodi u bolesnika s brucelozom liječenih na Klinici za infektivne bolesti u Skopju kroz dva vremenska razdoblja uspoređeni su retrospektivnom analizom. U prvom razdoblju (1990.-1991.) ispitano je 159 bolesnika, a u drugom razdoblju (2003.-2005.) 138 bolesnika. Bolesnici iz drugog razdoblja bili su stariji (34,6±20,9 prema 30,0±17,7 godina; P=0,041) i rjeđe su obolijevali od bruceloze zbog unosa određene hrane (34,8% prema 47,2%; P=0,031). Žarišni oblici bili su očitiji u drugom razdoblju (66,7% prema 50,3%; P=0,004), uglavnom zbog koštano-zglobne lokalizacije. Praćenje nakon liječenja bilo je bolje u drugoj skupini (76,1% prema 61,0%; P=0,005). Usprkos različitim terapijskim pristupima tijekom ispitivanih razdoblja nije zabilježena nikakva razlika u ishodu bolesti. Dakle, utvrđene razlike pokazale su napredak u vidu boljeg razumijevanja bolesti u općoj populaciji, kao i poboljšanja u nekim vidovima medicinskih postupaka. Ipak, u ovom endemskom području još uvijek nedostaje najvažnija mjera, a to je stvaranje i provedba boljeg nacionalnog programa za kontrolu ove bolesti.

Ključne riječi: *Bruceloza – dijagnostika; Bruceloza – izobrazba; Bruceloza – epidemiologija; Bruceloza – prevencija i kontrola; Zoonoze – epidemiologija; Makedonija*