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Epidemiology of human brucellosis in a defined area of Northwestern Greece

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SUMMARY

Despite a European co-financial programme for control and eradication of brucellosis in Southern Europe, there is evidence that foci of brucellosis still exists in Greece and other Southern European countries. Human brucellosis cases are probably underreported in these countries. A local surveillance system was implemented in a defined region of Northwestern Greece, in order to record and study all human brucellosis cases, using several sources of retrieval. A total of 152 newly diagnosed cases were recorded during a 2-year study period (from 1 April 2002 to 31 March 2004). The age- and sex-adjusted mean annual incidence rate for the population of the study area was 17·3 cases/10^5 inhabitants. Incomplete application of the control and eradication programme in livestock, and the possible illegal trafficking of animals and their products across the Greek–Albanian border could be responsible for the persistence of foci of brucellosis in the area.

INTRODUCTION

Brucellosis is a zoonotic infection associated with reproductive failure in animals and febrile disease in humans [1]. It represents one of the most widespread zoonoses and remains an important public health and an economic problem worldwide [2, 3].

Brucellosis is most prevalent in the Mediterranean basin, Arabian Peninsula, the Indian subcontinent, in parts of Mexico and Central and South America [4]. Some Northern and Central European countries along with the United States succeeded in the eradication of the disease [5, 6]. However, brucellosis still represents one of the major zoonoses among Southern and Eastern European countries [7, 8].

Since 1999 a European co-financial programme for the control and eradication of brucellosis in Southern European countries has been implemented by veterinary services. This programme is based on females and young replacement ovine and caprine vaccination with Rev-1 vaccine (conjunctival route) as part of *Brucella melitensis* control. Males are not vaccinated, but tested 'periodically' and slaughtered if they are positive. Some cattle that share common pastures with small ruminants are vaccinated with Rev-1 vaccine and they are kept for beef production. Dairy cattle are tested with two serological tests per annum or when they are in lactation, with three bulk milk ELISAs per annum [9]. However, in Greece and other Southern European countries, there is evidence that the real situation existing in caprine, ovine and cattle flocks, preserves brucellosis focus, in spite of the European programme for the control and eradication of the disease [7, 8]. This situation is related to uncontrolled animal movement and the transport of dairy products, especially in regions which border countries where the structures of veterinary control and epidemiological surveillance are

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of a low level, as well as to incomplete vaccination of animals [7, 9].

Brucellosis can be transmitted to humans by direct or indirect contact with animals and their products [11, 12]. Human-to-human transmission is extremely rare [13]. Human brucellosis is a reportable disease in all European countries, but underreporting of the disease is likely to lead to an important underestimation of the real dimension of the problem in many countries. In fact, a considerable number of brucellosis cases in humans are expected in areas where infection remains endemic among animals [8].

Public health surveillance of infectious diseases in Greece is based on a system of obligatory declaration of identified cases. The physician making the diagnosis of brucellosis has to report the case to the regional public health authorities within a week. It is estimated that a large number of brucellosis cases are not recorded through this surveillance systems. Only a small number of cases are reported annually in the study area. The aim of this study is to investigate the real occurrence and distribution of human brucellosis, its clinical and epidemiological characteristics, as well as the socioeconomic impact of the disease in a defined area of Northwestern Greece. The study is based on a regional surveillance system implemented in the area. The area studied is the region of Epirus, located close to the Greek–Albanian frontier.

METHODS

The region of Epirus lies in Northwestern Greece and includes four districts (Ioannina, Arta, Preveza and Thesprotia). The total population was 353 820 inhabitants according to the National Census of 2001. Rural residents represented 63·5% of the total population. Approximately one third of the active population works in farming and stock-farming. The livestock of the region consists of approximately 1 132 000 sheep and goats and 40 500 cattle, according to data supplied by the Ministry of Agriculture for the year 2003. Approximately 55% of sheep and goats and 35% of beef production cattle were vaccinated with Rev-1 vaccine up to 2003. Roughly 82% of dairy cattle flocks were serologically tested, and only 1·45% of them were found positive for *Brucella*, according to the Ministry of Agriculture.

Cases were recorded from three sources: (i) Both in-patients and outpatients referred to the hospitals of the study area. (ii) Patients referred to the health centres of the area. (iii) Patients referred to private microbiological and bio-pathological laboratories established in the area. A central database was created in the Department of Hygiene and Epidemiology, at the University of Ioannina. All hospitals, health centres, and private laboratories of the study area were contacted every month in order to record all potential new cases of brucellosis. A total of six hospitals, 15 health centres, and 38 private laboratories were established in the area during the study period.

Diagnosis was confirmed when a case met two of the following conditions: (*a*) positive serological test [standard tube agglutination test (SAT), in titres $>1:320$; Rose Bengal plate test (RBPT), or enzyme-linked immunosobent assay (ELISA)], or positive blood culture; (*b*) clinical syndrome consistent with brucellosis and (*c*) a clinician's decision for anti-brucellosis treatment.

All patients diagnosed during the period from 1 April 2002 to 31 March 2004 in the study area were included in the study. Incident rates were calculated considering only those patients who were first diagnosed during the study period and were residents in the study area (incident cases). Population data were based on databases of the National Statistical Service (National Census 2001). Age- and sex-adjusted rates were obtained by the direct method using the Greek population.

Information on patients was obtained by interview, using a standard questionnaire. A detailed explanation of the study was provided to all the patients, as well as to the physicians involved in the study. Each questionnaire was coded to ensure confidentiality of the participant's responses. Information collected included age, sex, residence, occupation, time of diagnosis, time of disease onset, symptoms, and possible routes of transmission, diagnostic tests, medication, and hospitalization. All patients were followed-up for 6 months, to record repetitive serological examinations and courses of anti-brucellosis treatment.

Information on the cost of diagnosis and treatment of brucellosis cases (diagnostic examinations, medication, and hospitalization) was obtained from the accounts department of the local hospitals, as well as from the insurance health booklets of the patients.

RESULTS

The main characteristics of brucellosis patients diagnosed during the period from 1 April 2002 to 31 March 2004 in the defined region of Northwestern

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Table 1. *Demographic and clinical characteristics of brucellosis cases diagnosed during the period 1 April 2002 to 31 March 2004, in the region of Epirus*

Total number of cases	152
Men/women	96/56
Age at diagnosis (years) (mean ± S.D.) [range]	50·0 (18·6) [9–88]
Men (mean age, years ± S.D.)	47·2 (19·7)
Women (mean age, years ± S.D.)	54·3 (16·1)
Nationality	
Greek	127 (83·6%)
Albanian	25 (16·4%)
Clinical symptoms	
Fever	102 (67·1%)
Shivering	96 (63·2%)
Arthralgia	110 (72·4%)
Gastrointestinal symptoms	20 (13·2%)
Respiratory symptoms	53 (34·9%)
Musculoskeletal symptoms	75 (49·3%)
Fatigue	113 (74·3%)
Night sweats	110 (72·4%)

Greece are presented in Table 1. A total of 152 cases were recorded. Among them, 104 were recorded at the hospitals of the study area, 46 in the rural health centres, and two were recorded from private laboratories. In six cases the disease was culture-confirmed following an isolation of *Brucella* spp. from blood culture.

Two cases were identified without any laboratory confirmation, based only on the clinical syndrome and a physician's decision for anti-brucellosis treatment. Men represented a 1·7-fold higher number of patients than women, and a lower mean age. During the period studied only 21 cases were reported to the Ministry of Health by the local health authorities.

A total of 119 cases (78·3%) were in residence in the study area, representing a mean annual crude incidence rate of 16·8 cases/10^5 inhabitants. Eight cases were residents of neighbouring Greek areas and 25 (16·4%) were immigrants, officially residents of South Albania. Figure 1 shows the incidence of the disease during the study period, by sex and age group. The mean annual incidence rates are higher in the ⩾65 years age group for men and in the 45–64 years age group for women. The age- and sex-adjusted mean annual incidence rate for the population was 17·3 (95% CI 13·8–20·8) cases/10^5 inhabitants.

The incidence of brucellosis varied widely among the four districts of the region of Epirus. The highest mean annual incidence rate was observed in the District of Thesprotia (52·1 cases/10^5 inhabitants), and

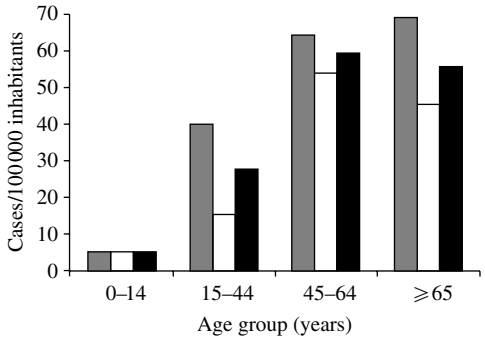

Fig. 1. Mean annual incidence rates (cases per 100 000 inhabitants) by sex and age group. ▩, Women; □, men; ■, total.

Fig. 2. Geographic distribution of brucellosis cases. •, 1–2 cases; ▪, 3–5 cases; ■, 6–10 cases; ●, regional capital.

the lowest in the District of Ioannina (11·8 cases/10^5 inhabitants). The geographical distribution of the cases of brucellosis is shown in Figure 2.

The monthly frequency of brucellosis cases is presented in Figure 3. There is a peak in the number of cases in spring, with the highest number occurring in April. A fall in the number of cases is observed in the autumn. The seasonal distribution of brucellosis cases presents differences between the two years of the study period.

A total of 48 patients were hospitalized because of brucellosis infection, and 13 of them were hospitalized for a second time. The mean duration of hospitalization for these 48 patients was 10·9 days (S.D. = 8·5, range 1–32 days). The total direct cost of diagnosis and treatment (diagnostic examinations, medication,

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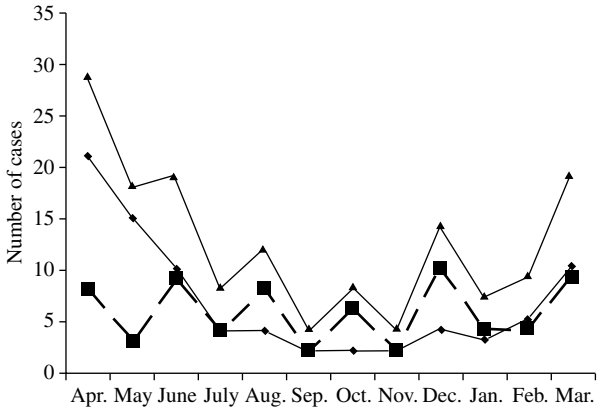

Fig. 3. Seasonal distribution of brucellosis cases in Northwestern Greece from April 2002 to March 2004. —●—, First year; – –■– –, second year; —▲—, total period.

and hospitalization) could be estimated for 134 of the 152 patients, and was approximately €56 700, given a mean cost of €423 per case. Around 90 % of this cost was covered by the public health insurance funds, which mostly cover the expenses of hospitalization and a large part of the expenditure for medication and diagnostic examinations in Greece. The remaining represents private expenditure.

Brucella spp. was transmitted by direct, indirect or mixed forms of contact with the animals and their products. A total of 114 patients reported direct contact with animals, 41 reported contact with the REV-1 vaccine and 56 patients reported contact with an aborted fetus. Consumption of home-produced milk and cheese was mentioned by 77 patients, and seven, at the same time, reported consumption of vegetables from local gardens fertilized with manure. Concurrent contact with animals and their home-produced products was mentioned by 62 patients. There were no data available for 25 cases.

Concerning the distribution of high-risk activities among the cases, 92 patients were shepherds (60·5 %), two were veterinarians or assistants (1·3 %), four were abattoir workers (2·6 %), and one was tannery worker (0·6 %) (Table 2).

Table 2. *Distribution of professional activities among 152 cases*

	n	(%)
Shepherds	92	(60·1)
Abattoir worker	4	(2·6)
Veterinarians and assistants	2	(1·3)
Skin workers	1	(0·7)
Other	43	(28·3)
Unknown data	10	(6·6)

DISCUSSION

The World Health Organization estimates the number of the new cases of brucellosis at more than 500 000 per year worldwide [14]. Recent studies suggest that brucellosis still remains endemic in large areas of Greece, as well as in other Southern European countries [8]. According to the results of the present study, 152 new brucellosis cases were diagnosed between April 2002 and March 2004 in the region of Epirus, indicating that brucellosis still remains a considerable and underestimated public health problem in the area. It is likely that foci of infection persist among animals because of the incomplete application of a control and eradication programme. An important number of flocks remain unvaccinated or incompletely vaccinated. In addition, within the vaccinated flocks the coverage of vaccinated animals (including young and adult females) varies greatly, which can lead to low flock immunity. A number of flocks were vaccinated 3 or 4 years ago and have never been re-visited, resulting in a high proportion of unvaccinated animals. The main reason lay in the lack of personnel in the veterinary services, and the geographical distribution of flocks. In addition, vaccines sometimes arrived late, after the end of vaccination period. As a consequence, only a limited number of animals were vaccinated [15, 16]. Thus, we assume that animal brucellosis is relatively frequent at the farms of the study area, although the percentage of infected animals is not known.

Another explanation could be related to the possible illegal trafficking of animals and their products across the Greek–Albanian border. The geographical distribution of the cases indicates that most occurred in the northwestern part of the region, bordering Albania. The illegal transport of flocks is common along this borderline. The nomadic way of flocks' breeding in the country allows the dissemination of *Brucella* infection in spite of the control and eradication programmes against brucellosis implemented in the area.

Clinical symptoms of brucellosis cases in our region are similar to those reported by previous studies. Fatigue, night sweats, fever and shivering, and musculoskeletal symptoms were the most common manifestations. Brucellosis has a wide range of clinical

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manifestations. The variety of clinical symptoms and the possible negative results of agglutination tests or the lack of laboratories in some health centres makes diagnoses difficult. In some cases diagnosis was made possible due to experienced physicians practising in endemic areas who were able to recognize the disease's early symptoms. As a consequence it is expected that a small number of brucellosis cases could remain undiagnosed [17, 18].

In this study we used several sources for case ascertainment, within the framework of a regional surveillance system implemented in the area, in order to reduce a potential underestimation of brucellosis cases and avoid bias. The establishment of a local network for case ascertainment could be considered as the most appropriate way to study the epidemiology of brucellosis, as a large number of brucellosis cases are not reported to the local and national health authorities. During the period studied only 21 cases were reported to the Ministry of Health, although we recorded 152 cases. However, it is possible that a small number of brucellosis patients could still escape the recording system. A number of patients, mainly in rural areas, where health services are less developed, could remain undiagnosed. This could be true mainly for milder cases of the disease. It is unlikely that even a small number of patients resident in the study area could be diagnosed and treated outside this area. On the other hand we consider that practically all cases diagnosed during the study period were recorded, as our recording system included all public and private laboratories established in the area.

Studies from other Mediterranean countries suggest a characteristic seasonal variation of brucellosis cases, with higher occurrence in the spring and the beginning of summer, and a decrease in autumn and winter [19, 20]. This could be related to seasonal activities leading to a close relationship between animals and human, as is the case during the lambing season [21]. We found a similar seasonal distribution of cases when considering the total observation period of 2 years. However, there were differences in the seasonal distribution between the two years of the study period. These differences could be related to the existence of several focuses of infection among animals, dispersed throughout the whole area, giving some clustered cases among humans in different times and under different circumstances [22]. Thus, the study of space–time clustering of cases, and its association with several transmission ways would be of interest.

The incidence rates were lower among women, and among children and young adults. This finding is consistent with the findings of other studies carried out in Mediterranean countries [23]. The age and sex distribution of cases could be related to the main mode of transmission, which seems to be direct contact with animals by shepherds, especially in the lambing season, when aborted fetus or infected placenta are handled. Children are probably infected when consuming uncontrolled milk and milk products [24]. It is also possible that children accompany their parents at the farms, so they could also be infected by direct animal contact [25]. In this study only seven children and adolescents aged 9–17 years were infected, and all of them came from shepherds' families with $\geqslant 6$ members. In two of these cases, another member of the family was also infected during the study period. Elderly and middle-aged men and women represent the large majority of cases, this group may have more frequent close contact with animals because of their activities [26, 27].

The findings of our study suggest that the socioeconomic impact of brucellosis in the area is important and underestimated. A considerable number of patients were hospitalized and the mean direct cost for the total number of cases was about €373 per patient. The costs for diagnosis and treatment are mostly covered by public health insurance funds. It is difficult to estimate the indirect cost of brucellosis as measured by lost days of work, and the socioeconomic and psychological impact for the families and the communities. It should be pointed out that the official cost of health services and medications is significantly lower in Greece than in other European countries. The same diagnostic examinations and treatment would represent a several-fold higher cost in many other European countries.

In conclusion, the present study suggests that human brucellosis remains a considerable and underestimated public health problem in Northwestern Greece, despite the control and eradication programme implemented in the area. The cases recorded within the framework of our study represent a sevenfold higher incidence than the cases reported to the Ministry of Health, during the same time period. The large majority of these cases occurred in shepherds. The relatively high occurrence of the disease in the study area could be related to low rates of vaccination among animals, and to illegal trafficking of flocks and animal products across the Greek–Albanian border.

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