Original Article

Epidemiological and Clinical Aspects of Human Brucellosis in Central Greece

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SUMMARY: The confirmed cases of human brucellosis in the area of Larissa in Central Greece from 2003 to 2005 were analyzed to assess the features of the affected population and to determine the factors influencing the acquisition of infection. Data of patients infected by *Brucella* spp. concerning age, gender, occupation, date of diagnosis and the observed symptoms were collected from regional hospitals, health centers and private practitioners. The incidence of human brucellosis in the area was 32.49 cases/100,000 inhabitants. Males, due to their professions, were affected more often by brucellosis than females. The majority of the cases were attributed to direct contact with animals or their products. Only in 8.49% of the cases was the infection attributed to the consumption of dairy products. The urban population is not at potential risk for acquiring brucellosis because all commercialized dairy products in Greece are produced from pasteurized milk. The occurrence of human brucellosis shows seasonality, with the majority of the cases diagnosed from December to May. Direct contact with animals, the season of the year and gender were the risk factors influencing the acquisition of infection. Fever, arthralgia, profuse sweating and anorexia were the symptoms most often observed.

INTRODUCTION

Bacteria of the genus *Brucella* are major zoonotic pathogens responsible for considerable human morbidity in areas where they are endemic in livestock. Humans are infected either by direct contact with infected animals and their products or by the consumption of contaminated milk or dairy products (1). A person is in direct contact with animals or their raw products when he or she is working on a farm where there are animal species susceptible to brucella infection, handles or milks them by hand, is generally in close contact with animals potentially infected by brucellosis, or handles raw animal products like milk, meat or skins. For these persons, the usual route of infection is through skin abrasions, although in some cases infection can be acquired by the inhalation of infected aerosols. In some countries, the expansions of animal movement and commerce as well as the intensification of animal management have contributed to an increase in the incidence of human brucellosis (2).

In parts of the world where *Brucella melitensis* infection is endemic in sheep and goats, like Mediterranean littoral, Middle East, Southwest Asia and parts of Latin America, human brucellosis is often observed. In areas where *Brucella* spp. infection is often present in animals but the pasteurization of raw milk is practiced and dairy products are made from pasteurized milk, the incidence of human brucellosis is not that high, and direct contact with infected animals or their products is considered the most common way of infection for humans. In these areas, brucellosis is regarded as an occupational disease for persons (farmers, butchers, slaughterhouse workers, milk factory workers, veterinarians, etc.) who, due

to their professions, are in contact with animals and handle their raw products (3). In contrast, in areas where measures for consumer protection are not routinely practiced, the market for dairy products is neither well organized nor inspected and the consumption of unpasteurized raw milk or dairy products made from unpasteurized milk is a common nutritional habit, brucellosis is a community health problem with a high incidence, and the main route of infection is through the digestive system (4).

In Greece, a significant increase in the prevalence of infection due to *B. melitensis* in sheep and goats was observed from 1995 to 2000, followed by a marked increase of the incidence in humans, reaching its highest value in 2000 (5,6).

The aim of the present study was to conduct an epidemiological study of confirmed cases of human brucellosis in the area of Larissa in Central Greece from 2003 to 2005. The cases of human brucellosis in this part of Greece were studied and analyzed in relation to the age, gender and occupation of the patients as well as the period of year in which they were diagnosed in order to assess the features of the affected population and determine the risk factors influencing the acquisition of infection. Information about the most common clinical symptoms observed by the patients was also collected in order to assess the clinical aspects of the disease.

MATERIALS AND METHODS

Collection of data: The data used in the study were collected in the National Reference Laboratory for Brucellosis (NRL) in Larissa from regional hospitals, health centers and private practitioners in the area. Although brucellosis is a notifiable disease in Greece, for this study, more data than officially reported were needed, and these were collected directly from their sources in the area.

The collected data came from persons who were suspected to be suffering from brucellosis and were under investigation for its confirmation. For every person, a data capture sheet

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was filled out in which the demographic data, as well as the occupation, age, gender and date of diagnosis were reported. In addition, the day of the onset of symptoms, the day each patient visited the physician and the day that brucellosis was confirmed or ruled out were reported. The symptoms observed by the patient were checked off on a list in the data capture sheet.

The data from persons to whom brucellosis was not confirmed by laboratory testing were used as negative controls in binary regression analysis, so in conjunction with the data from persons suffering from brucellosis, the risk factors influencing *Brucella* spp. infection were determined.

In the present study a patient was considered to be suffering from brucellosis if, according to the World Health Organization (WHO) case definition, that person "showed intermittent or irregular fever of variable duration, profuse sweating, fatigue and other symptoms as well as positive reaction to a serological test used for brucellosis diagnosis (standard agglutination test, enzyme-linked immunosorbent assay [ELISA] [IgA, IgG, IgM], complement fixation test, Coombs' test or fluorescent antibody test)" (7).

All persons included in the present study had never suffered from brucellosis before, so the confirmed cases studied were new infections and not relapses. The collected data capture sheets were coded to ensure the confidentiality of the patients' data.

Data analysis: The collected data were analyzed and the diagrams were created by Medcalc (version 8.0 for Windows; Medcalc Software, Mariakerke, Belgium) (8). The proportions were compared by chi-square test and the age medians by the non-parametric Wilcoxon test, since age data did not follow the normal distribution (9). The risk factors influencing the acquisition of infection by humans during the study period in this area in Central Greece were determined by binary logistic regression analysis using the forward stepwise likelihood ratio method using SPSS software (version 12.0; SPSS, Inc., Chicago, Ill., USA).

RESULTS

During the study period in the area of Larissa in Central Greece, 821 persons were suspected to be infected by *Brucella* spp., and the infection was confirmed and reported in 271 of them. Taking into account the number of confirmed cases of human brucellosis and the population in the prefecture, which was 278,000 inhabitants (10), the annual incidence of human brucellosis in this area of Central Greece for the 3-year period was calculated at 32.49 cases/100,000 inhabitants.

Male patients represented 74.9% (203 out of 271) of the cases, which differs significantly ($P < 0.05$) from that of females, whereas the ratio of male to female patients was calculated as 2.99/1 (203/68), indicating that men acquired *Brucella* spp. infection more often than women.

The monthly distribution of confirmed cases for the 3-year period is presented in Figure 1. The reported cases of human brucellosis increased from November to May and decreased from June to October. Of the cases reported in the study, 75.27% (204 out of 271) were diagnosed during winter and spring (December to May), whereas the remaining 24.73% were diagnosed during summer and autumn (June to November).

Figure 2 presents the age distributions of the patients according to gender. Taking into account that the arithmetic mean is influenced from extreme values and is not repre-
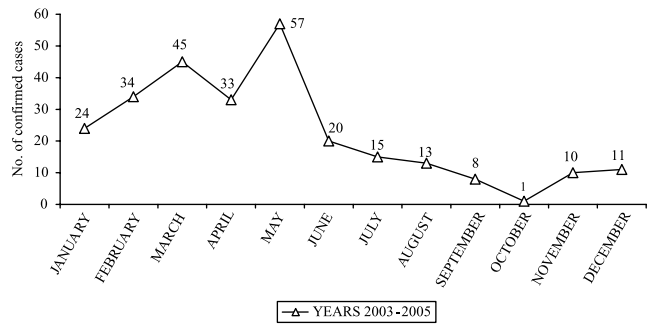

Fig. 1. Distribution of confirmed cases of human brucellosis according to the month of the year for the years 2003 - 2005.
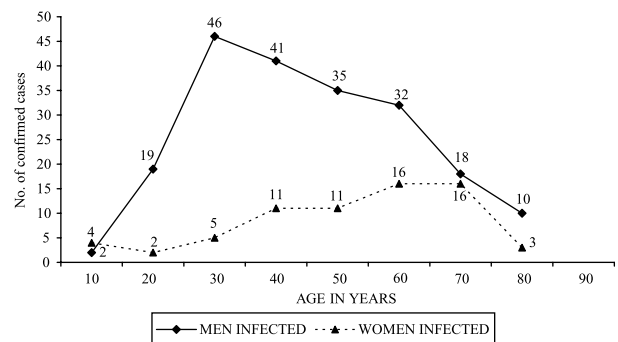

Fig. 2. Distribution of human cases of brucellosis according to age and gender of patients for the years 2003 - 2005.

sentative, the medians of ages and their 95% confidence intervals (95% CI) were calculated for men and women separately, which were 40 (37 - 42) and 51 (44 - 55) years, respectively. As the Wilcoxon test reveals, the median of men's ages differs significantly ($P < 0.05$) from that of women's ages.

The distribution of patients' professions is presented in Table 1. The professions are classified by those in which the person was in direct contact with animals or animal products, and those in which there was no contact. The data reveal that 91.51% of the patients in this area were practiced professionals who were in direct contact with animals or animal products.

For persons who were not in direct contact with animals or

Table 1. Distribution of cases of brucellosis according to the profession of patients

Profession without direct contact with animals or animal products		Professions with direct contact with animals or animal products	
Profession	No.	Profession	No.
Private employee	5	Infants/Farmer family	4
Stretcher-bearer	1	Animal dealer	2
Construction worker	5	Cooker	1
Civil engineer	1	Veterinarian	5
Teacher	1	Slaughterhouse worker	6
Driver	2	Cheese factory worker	9
Pilot	1	Veterinary laboratory assistant	1
Gymnast	1	Students/Farmer family	15
Policeman	1	Housekeeper/Farmer family	19
Electrician	1	Sheep/Goat Farmers	182
Shopkeeper	1	Butcher	5
Housekeeper	2		
Total	22	Total	249

Table 2. Frequency of observed clinical symptoms

Symptom	No. (% of total no.)
Anorexia	118 (43.5)
Arthralgia	186 (68.6)
Fatigue	219 (80.8)
Fever < 38°C	169 (62.4)
Fever > 38°C	102 (37.6)
Headache	97 (35.8)
Myalgia	121 (44.6)
Profuse sweating	159 (58.7)
Weight loss	45 (16.6)
Depression-Irritation	37 (13.6)

animal products, the conducted case studies revealed that they acquired the infectious agent from consuming homemade feta cheese. During the study period, infection by *Brucella* spp. was confirmed in four infants aged <7 years and in 15 students aged <18 years in families that were involved in sheep and goat farming.

Using as controls the data from persons for whom brucellosis was not confirmed (550 out of 821) and the data from patients (271 out of 821), the factors influencing the acquisition of infection in humans were determined by binary logistic regression analysis using the forward likelihood ratio method. The factors contributing significantly ($P < 0.05$) to the acquisition of infection during the study period in this area in Central Greece and included in the equation were: gender, contact with animals or animal products and period of the year.

The factors were identified for gender, 0 = female, 1 = male; for contact, 0 = no contact, 1 = direct contact with animals or animal products; and for period of the year, 0 = summer-autumn (June to November), 1 = winter + spring (December to May).

The regression equation yielding a significant P value ($P < 0.05$) is:

$$logit(p) = -2.898 + 0.794 \times gender + 1.605 \times contact + 0.486 \times period \ of \ the \ year$$

The predictive accuracy of the model is high because it correctly predicted 67% of the confirmed cases of human brucellosis in the area. The odds ratio and their 95% CI of the independent variables used in the model are for gender (2.21 [1.59-3.08]), contact with animals and animal products (4.98 [3.09-8.03]) and period of the year (1.63 [1.16-2.28]), respectively. These express the relative amount that the odds of a positive outcome are higher when each independent variable has the characteristic of interest.

From the collected data, the mean and standard deviation (SD) of the time elapsed from the day the first symptoms of brucellosis were observed by the patient until the first visit to the physician, and from the visit to physician until definite diagnosis, was calculated at 25.5 ± 28.0 days and 2 ± 1.93, whereas the values of these intervals ranged from 3 to 133 and 1 to 14 days, respectively.

The frequencies of clinical symptoms observed by the patients are presented in Table 2. Although the symptoms reported from patients were always in combinations of more than two, Table 2 presents the number of cases in which a particular symptom was reported. The vast majority of patients reported fatigue and arthralgia, whereas in most cases the reported fever was mild and <38°C.

DISCUSSION

Although control and eradication programs for brucellosis in small ruminants have been implemented during the last few decades in most Mediterranean countries, brucellosis remains endemic in most of them (4). In the area of Larissa as well as in the entire mainland of Greece, a control program based on the vaccination of young and adult sheep and goats with Rev-1 vaccine has been in place since 1999. Before the beginning of the vaccination campaign, the prevalence of *B. melitensis* infection among sheep and goat flocks in Greece was 27.82% and in the area of Larissa 40.94% (11). Although the prevalence of *B. melitensis* infection decreased after the implementation of the vaccination campaign, in many areas of the country it remains high. Due to this, human brucellosis is often diagnosed in the rural population (12).

Human brucellosis in the area of Central Greece is mainly an occupational disease and not a health problem for the whole population. The disease mainly affects persons who, due to their profession, are in direct contact with animals or animal products like farmers, slaughterhouse workers, milk factory workers, animal dealers and veterinarians. It must be pointed out that the consumers in Greece are not at high risk for acquiring brucellosis because, according to European Union legislation, milk for human consumption must be pasteurized, all dairy products must be made from pasteurized milk, and homemade dairy products from unpasteurized milk are not allowed in the market (13).

In the area of Larissa, infants and school-age children of farmers' families acquired infection due to direct contact with animals. Such cases of *Brucella* spp. infection have been reported by other researchers and are attributed to the fact that farmers' children often accompany their parents on the farms and handle newborn lambs or kids born from infected dams, and thus acquire the infectious agent from the contaminated fur of the animals. In addition, school-age children often help their families with flock management, acquiring the infectious agent by direct contact with animals (14,15).

The annual incidence of human brucellosis in the area of Larissa in Central Greece during the study period was almost 15 times higher than that reported for the whole country (16). This can be attributed to the high prevalence of *B. melitensis* infection in sheep and goats in this area, since it is accepted that the incidence of human brucellosis is positively correlated with the prevalence of brucellosis in animals (17,12). Additionally, the large number of sheep and goats reared in this area of Central Greece, about 900,000 animals (Ministry of Agriculture, unpublished data), also contributes to the high incidence of human brucellosis.

Human brucellosis in this area of Greece was diagnosed three times more often in males than in females. This finding is in agreement with the results of epidemiological studies for human brucellosis conducted in other parts of the world where brucellosis is considered an occupational disease. The higher frequency of *Brucella* spp. infection in men can be attributed to their professions, since in rural areas, men deal mainly with animal husbandry (18-20). Additionally, in Greece, the vast majority of sheep and goats are milked by hand, which is done mainly by men who come in direct contact with potentially infected milk, especially during the lambing season when excretion of the infectious agent in milk is high.

In areas where brucellosis is a common health problem in the urban population and the infection is acquired through

the consumption of infected milk or dairy products, there is no difference in the incidence among men and women, since the probability of acquiring the infectious agent is almost the same among the sexes. In these areas, the occurrence of childhood brucellosis often happens because children's nutrition is based on milk and milk products (14,15).

The incidence of human brucellosis increased from November to July and decreased from August to October, whereas the majority of cases were diagnosed from December to June and peaked in May. The seasonality of human brucellosis can be attributed to the seasonality of parturitions in small ruminants. In Greece, sheep and goats give births in two periods. The first period is from October to the middle of December, and the second is from the middle of January to March. Taking this into account, it can be concluded that the maximum number of cases of human brucellosis were observed in the interval of one to two months after the end of the parturition period of sheep and goats. This delay in the appearance of the peak represents the disease's incubation period and the interval from the onset of symptoms until definite diagnosis. The seasonality of human brucellosis for European countries has been reported by other researchers, who found that >70% of the cases of human brucellosis occurred from March to June, with the peak observed from May to June (21,22). The seasonality of human brucellosis is more noticeable when brucellosis is mainly an occupational disease and infection occurs by direct contact with animals. This occurs because during the period of parturitions, the number of animals excreting the infectious agent increases, so the environmental contamination builds up progressively. Under such circumstances, the probability of infection increases dramatically for personnel working in heavily contaminated environments, who can acquire infection by other routes, such as through the respiratory system instead of through skin abrasions or mucus membranes.

Concerning the patients' age distribution in the area of Larissa, the age median of the females is significantly higher than that of the males. This finding is almost identical with the findings reported in epidemiological studies conducted in other countries (17,21). The difference observed in the medians of age between men and women can be attributed to the fact that in rural areas, especially in families rearing animals, the older women usually help men with flock management, and are mainly responsible for the care of goats kept in houses as well as with the preparation of cheese for the family. These activities bring them in direct contact with potentially infected animals or milk.

The risk factors influencing the acquisition of *Brucella* spp. infection by humans during the study period in the area of Larissa were gender, period of the year and direct contact with animals. The latter appears to be the most important among the other factors.

The time interval from the onset of symptoms until the disease's definite diagnosis was almost a month. Data analysis revealed that this interval was due to patients choosing to delay visiting a physician, and not to the inability of physicians to suspect and diagnose brucellosis. The latter finding is supported from the collected data, which revealed that the majority of patients were suspected by physicians to be suffering from brucellosis and underwent laboratory investigation to establish the diagnosis. The delay by patients in visiting a physician can be attributed to the mentality of people living in rural areas, especially of shepherds and farmers, who avoid visiting physicians and choose to wait instead for symptoms to go away.

The symptoms reported by the patients were the typical symptoms of brucellosis such as fever, arthralgia, profuse sweating and anorexia. In most of the cases, the infection started with a fever <38°C, revealing that the disease started with relatively mild symptoms. It must be pointed out that brucellosis is often observed with mild symptoms in persons who are in close contact with animals and are at high risk of exposure to the infectious agent (like farmers), compared to other patients who are not exposed to the infectious agent (23).

The findings of the present study reveal that human brucellosis in Central Greece is an occupational disease with high impact on persons who, due to their professions, are in direct contact with animals and animal products. This indicates that the urban population is not at potential risk for acquiring *Brucella* spp. infection, since all commercialized dairy products are produced from pasteurized milk. In order for the incidence of human brucellosis to decrease, a program of health education for people at risk must be implemented. Additionally, appropriate measures for the control of brucellosis prevalence in small ruminants must be implemented with the highest degree of efficacy by the relevant veterinary authorities.

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