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Epidemiological Characteristics of Brucellosis in Serbia, 1980-2008

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Aim To analyze the epidemiological characteristics of human brucellosis in Serbia from 1980 to 2008 and the most important factors affecting its emergence and spread.

Methods Public sources of data on brucellosis were used, including official reports of infectious diseases and epidemics, as well as monthly and annual reports of the Serbia and Vojvodina Institutes of Public Health.

Results From 1980 through 2008, there were 1521 human brucellosis cases in Serbia. The annual number ranged from 2 in 2000 to 324 in 1991. Infections occurred more often in men (67% of cases) than in women (odds ratio, 2.17; 95% confidence interval, 1.57-3.00; $\chi^2 = 24.52$, $P < 0.001$). The largest number of patients over the entire study period (1184) was recorded in Kosovo and Metohija, which accounted for 78% of the total number of patients. The maximum incidence rate in Kosovo and Metohija was 12 per 100 000 in 1991. In Vojvodina, the first autochthonous human cases of brucellosis were recorded in 1999, and 101 affected persons were registered by the end of 2008. During the period 1994-2008, the largest number of patients in Serbia was recorded from June to September (310 of 623 cases, 50%). The disease was most prevalent among people aged 30-49 years, accounting for 81 of 177 (46%) of the cases in Serbia from 1999 to 2008.

Conclusion Brucellosis has been a significant public health concern in Serbia. This problem may be solved by joint efforts of all relevant factors, first of all human and veterinary medical services.

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Brucellosis is still a significant infectious disease. It primarily affects domestic animals, but humans are often infected due to direct contact with animals or ingestion of contaminated dairy products. The disease is spread all over the world with about 500 000 new human cases occurring annually (1). Human brucellosis is more prevalent in western parts of Asia, India, the Middle East, southern Europe, and Latin American countries (1). It mostly occurs in rural and nomadic communities where people live close to animals. Worldwide, reported incidence of human brucellosis in endemic disease areas varies widely, from <0.01 to >200 cases per 100 000. In Europe, brucellosis affects mainly the Mediterranean countries, but the epidemiology of this infection has changed over the past decade due to various sanitary, socioeconomic, and political factors, and international travel (1). The low incidence rate reported in known brucellosis-endemic areas may reflect absent or deficient surveillance and reporting systems (2).

Many countries in the world, especially those with significantly developed small ruminant and cattle breeding sectors, are concerned about the spread of the disease. Large losses in livestock, long-term treatment of infected people, and the costs of brucellosis control and eradication are some of the reasons why the disease is a drain on the economy.

In the former Yugoslavia, brucellosis was first recorded in Istria and near the Slovenian coast in 1947 but a few years later it was eliminated (3). In 1978, it appeared in Macedonia (4), where an epidemic broke out in 1980. Since no adequate action for elimination and eradication was taken, a rapid increase in the number of human cases emerged in the former Yugoslavia. Since then, brucellosis has become a significant concern in the country. By the end of the 1990s, brucellosis was reported throughout Macedonia, Kosovo and Metohija, and the southern part of central Serbia.

The aim of this study was to determine the frequency and distribution of human brucellosis cases in Serbia from 1980 to 2008 and the most important factors affecting its emergence and spread.

METHODS

Studied regions

Serbia is divided into 150 municipalities and 24 cities. Of the 150 municipalities, 83 are located in central Serbia, 39 in Vojvodina, and 28 in Kosovo. Of the 24 cities, 17 are in central Serbia, 6 are in Vojvodina, and 1 in Kosovo.

Serbia has two autonomous provinces: Vojvodina in the north, which includes 39 municipalities and 6 cities; and Kosovo and Metohija in the south, with 28 municipalities and 1 city. The Autonomous Province of Kosovo and Metohija has been under UN administration (UNMIK) since June 1999. The area between Vojvodina and Kosovo is central Serbia, which is not an administrative division, and it has no regional government of its own.

Data sources

Public sources of data on brucellosis for 1980-2008 were used, including the official reports on infectious diseases and epidemics, monthly and annual reports of the Institute of Public Health of Serbia and the Institute of Public Health of Vojvodina, as well as reports of local health departments in Serbia. The incidences in this study are reported as cases per 100 000 inhabitants. The source of population data was the Statistical Office of the Republic of Serbia (http://webrzs.stat.gov.rs/axd/en/index.php). Record keeping on brucellosis cases exists since 1984, when the Law on Infectious Diseases was passed. Data on diseases of brucellosis in Kosovo and Metohija were available up to the beginning of 1999. Data on age and sex distribution were available for 177 cases, regional distribution for 1275 cases, and seasonal distribution for 623 cases.

Patients were considered to be suffering from brucellosis if, according to the World Health Organization case definition, they showed intermittent or irregular fever of variable duration, profuse sweating, and fatigue, as well as positive reaction to a serological test used for brucellosis diagnosis (standard agglutination test, enzyme-linked immunosorbent assay, complement fixation test, Coombs test, or fluorescent antibody test) (5). Bacterial isolation and characterization were not routinely performed for the people in our data set; instead, they were confirmed to have brucellosis based on serological tests. Two or more connected cases of human brucellosis were considered as an outbreak.

Statistical analysis

Data were analyzed by the χ^2 goodness-of-fit test, the χ^2 test with Yates correction, and the Fisher exact test using EpiInfo version 6 (6).

RESULTS

In 1980-2008, 1521 human brucellosis cases were recorded in Serbia. The number of cases reported annually is shown in Figure 1.

The distribution of recorded cases of human brucellosis was 1184 (78%) in Kosovo and Metohija, 236 (16%) in central Serbia, and 101 (6.6%) in Vojvodina.

In 1980, 120 cases of human brucellosis were recorded in the former Yugoslavia: 112 cases in Macedonia and 8 cases in Kosovo and Metohija. In fact, 1980 was the first time that brucellosis was recorded in Kosovo and Metohija. From 1981 to 1984, no cases of human brucellosis were recorded in Serbia. Since 1985, the number of cases started to increase in Kosovo and Metohija. The first cases in central Serbia were recorded in 1988.

The number of cases of human brucellosis ranged from 2 in 2000 to 324 in 1991. In 1991, the maximum incidence was reported for whole Serbia (3.3 per 100 000), Kosovo and Metohija (12 per 100 000), and central Serbia (1.15 per 100 000). That year, an outbreak of brucellosis was reported for the first time.

The first human cases in Vojvodina were reported in 1999, after 30 years without a single recorded autochthonous case of brucellosis in this area. Human brucellosis was diagnosed in two workers on a sheep farm in the municipality of Kovin. Sero-epizootic surveys indicated the presence of brucellosis among animals. By the end of 2008, the total number of affected persons was reported to be 101, with a maximum incidence of 1.72 per 100 000 in 2004. Mean annual incidence in Vojvodina for 1999-2008 was 0.46 per 100 000 inhabitants, three times higher than that in central Serbia (0.15 per 100 000 inhabitants).

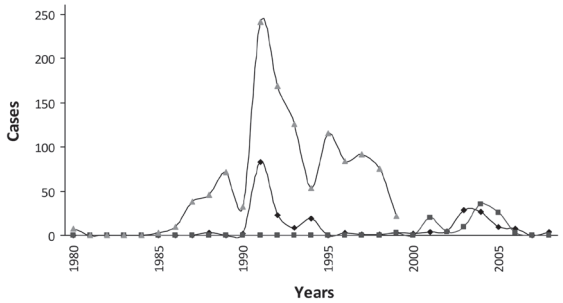
During the study period, only one case of death caused by brucellosis was reported in Kosovo and Metohija, and it occurred in 1993.

Figure 2 presents brucellosis incidence in Serbia. The incidence increased from 1985 and peaked in 1991. After this, the incidence tended to decrease. The epicenter of the first wave was in Kosovo and Metohija, and of the second wave in Vojvodina. The incidence started to increase again in 2002 and reached a peak in 2004. Over the period of 1991-2000 in Serbia, a substantial decrease in the reported cases was observed, from 324 to 2 (3.29 to 0.02 per 100 000), whereas after the year 2000 the number of reported cases increased and brucellosis incidence reached another peak in 2004 (from 0.02 in 2000 to 0.82 per 100 000 in 2004).

During the study period, most of the cases in Serbia (total of 1521) were recorded in districts of Kosovo and Metohija (Prizrenski 367 [24%], Kosovski 159 [10%], and Kosovsko-pomoravski 137[9.0%]); in central Serbia (Pčinjski 58 [3.8%]); and Vojvodina (Južno-banatski 57 [3.7%]) (Figure 3). In the district of Belgrade, 38 (2.5%) cases were reported, with the first 19 reported in 2003.

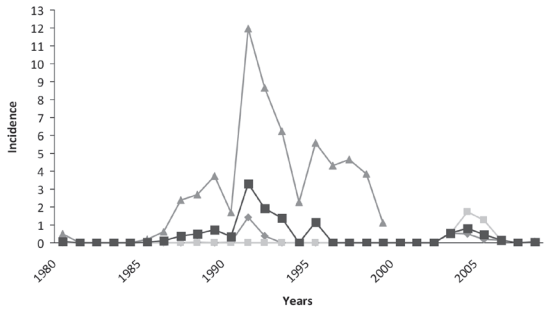
Figure 4 presents the age and sex distribution of patients. Male patients amounted to 67% (119 of 177) of the cases. The odds ratio of male to female patients was 2.17 (2.17; 95% confidence interval, 1.57-3.00; $\chi^2 = 24.52$, $P < 0.001$), in-

Figure 1.



Reported brucellosis cases in Serbia, 1980-2008. Rhomb – Central Serbia; square – Vojvodina; triangle – Kosovo and Metohija. Data for Kosovo and Metohija are available only until 1999 because the Institute of Public Health of Serbia stopped receiving reports when Kosovo and Metohija came under the administration of UNMIK.

Figure 2.



Brucellosis incidence in Serbia, 1980-2008. Rhomb – Central Serbia; square – Vojvodina; triangle – Kosovo and Metohija; cross – entire Serbia. Data for Kosovo and Metohija are available only until 1999 because the Institute of Public Health of Serbia stopped receiving reports when Kosovo and Metohija came under the administration of UNMIK.

dicating that men acquired *Brucella* spp. infection more often than women.

Brucellosis cases were reported in all age categories. For 177 patients, the mean age was 41.7 ± 15.4 years. Only 12 (6.7%) patients were younger than 19 years, 40 (23%) were younger than 29 years, and 56 (32%) were over 50 years. The age group of 30-49 (working population) accounted for 81 (46%) of these 177 cases.

The monthly distribution of cases in the 15-year period (1994-2008) is presented in Figure 5. There is a significant seasonal distribution of cases (n = 623, $\chi^2_{11} = 114.316$, $P < 0.001$). The largest number of patients from 1994 to 2008 (310 of 623, 50%) was reported from June to September, with a smaller peak (58 of 623, 9.3%) in March.

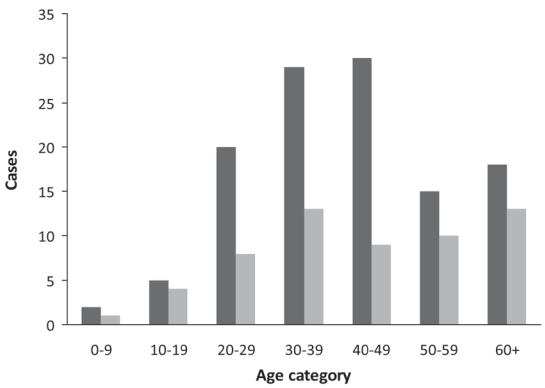
From 1991 to 2008, 47 outbreaks of human brucellosis resulting in 304 cases were reported in Serbia (Table 1). In 14 (30%) outbreaks, the major mode of transmission was contact with the infected animals, while 33 (70%) outbreaks were food-borne. In food-borne outbreaks, 221 (73%) cases were recorded. In contact outbreaks, 83 (27%) cases were recorded.

We compared the relative frequency of food-borne and contact outbreaks during two time periods in Serbia. For the first period, from 1991-1999, we mostly have the data from Kosovo and Metohija. For the second period, from 2000-2008, we only have the data from central Serbia and Vojvodina, since data for Kosovo and Metohija are available only until 1999. These two time periods were chosen because the first human cases in Vojvodina were reported in 1999, and because brucellosis in central Serbia was a consequence of brucellosis in Kosovo (before 2000) and Vojvodina (after 2000). There were significantly more food-borne than contact outbreaks in the 1991-1999 period than in the 2000-2008 period (25:5 vs 8:9, Yates-corrected $\chi^2 = 5.20$, $P = 0.023$). From 2000 to 2008, a larger number of contact than food-borne outbreaks occurred in Vojvodina

Figure 3.

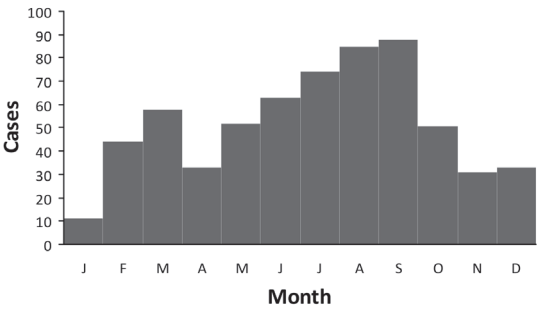
Regional distribution of brucellosis cases (n = 1275) in Serbia, 1991-2008.

Figure 4.



Age and sex distribution of brucellosis cases (n = 177) in Serbia, 1999-2008. Dark gray bars – men; light gray bars –women.

Figure 5.



Seasonal distribution of brucellosis cases (n = 623) in Serbia, 1994-2008.

TABLE 1. **Sources and modes of brucellosis transmission in Vojvodina, 1999- 2006.**

Sources and modes of transmission	No. of human brucellosis cases (%)
Contact with infected sheep	35 (50)
Contact with infected goats	3 (4.3)
Contact with infected animals	11 (15.7)
Consuming sheep cheese produced from uncooked milk	14 (20)
Consuming uncooked goat milk	3 (4.3)
Accident during collecting specimens from infected animals	1 (1.4)
Laboratory work with specimens from infected animals	3 (4.3)
Total	70 (100)

than in central Serbia (8:1 vs 1:4, Fisher exact two-tailed test, $P=0.023$).

Approximately two-thirds of the outbreaks in Serbia during the study period were reported from 1991-2000, including 25 of 33 (76%) food-borne outbreaks (accounting for 184 of 221 [83%] cases) and 5 of 14 (36%) contact outbreaks (accounting for 16 of 83 [19%] cases). This is the period when surveillance and reporting was regularly conducted in Kosovo and Metohija. From 2001 through 2008, 8 of 33 (24%) food-borne outbreaks (37 of 221 [17%] cases) and 9 of 14 (64%) contact outbreaks (67 of 83 [81%] cases) were reported.

We analyzed sources and modes of brucellosis transmission during outbreak investigations in Vojvodina from 1999 to 2006 (Table 1). Thirty-five (50%) of the 70 infected individuals had direct or indirect contact with infected sheep. Fourteen (20%) of 70 patients consumed sheep cheese produced from uncooked milk. In addition, the total group of 70 people included 3 (4.3%) laboratory workers who were in contact with specimens from infected animals, and one person (1.4%) who was exposed accidentally during specimen collection.

DISCUSSION

The total number of human brucellosis cases in Serbia from 1980 to 2008, based on official reports, was 1521. Despite being a serious infectious disease, brucellosis cases often remain unrecognized and are frequently labeled as a "fever of unknown origin," even by hospital physicians. As a result, the actual number of cases is unknown and may be far larger than officially reported.

In 1980, brucellosis was imported to Kosovo from Macedonia due to several favorable factors: overlapping common pastures on the Šar mountain (in the municipalities of Prizren, Dragaš, and Vitina in Kosovo) and in the municipality of Negotino (Republic of Macedonia), a semi-nomadic lifestyle of raising small ruminants and cattle, and making cheese from uncooked milk. The reason for the increase in incidence in 1991 was the military conflict in the former Yugoslavia, which led to population and animal migration and uncontrolled trade of food. The first cases in Belgrade occurred as a consequence of an epizootic in domestic animals. This epizootic was the result of importing small ruminants and cattle from territories where brucellosis was enzootic.

In Vojvodina, brucellosis was imported with small ruminants and cattle from endemic areas, ie, Bosnia and Herzegovina and Kosovo. Due to war and economic crisis in Serbia, veterinary monitoring of imported small ruminants and cattle was not adequately performed. Since 1970, in the territory of Vojvodina, regular serological check-ups of cattle were performed, but sheep and goats were not included. Such practices led to reappearance of the disease. Inadequate implementation of measures against epizootics contributed to faster spreading of this zoonosis to the rest of Vojvodina. The first human cases in Vojvodina were reported in 1999 and by the end of 2008 the total number of affected persons was reported to be 101, with a maximum incidence of 1.72 per 100 000 in 2004. In the period after 1999, brucellosis appeared in the areas of central Serbia bordering Vojvodina.

Serbia is a country with moderate incidence of brucellosis. Together with Bulgaria, it has the lowest brucellosis incidence in the Balkans. In 2006, the incidence in Serbia (0.15 per 100 000) was lower than in the European Union countries (0.20 per 100 000) (7). On the other hand, the number of human brucellosis cases in Serbia was greater than in Bulgaria, where 22 cases were reported during 1992-2004 and 105 during 2005-2007 (8).

Brucellosis is a major concern for most of the countries in the Balkans. We found a maximum incidence in Serbia to be 3.3 per 100 000; within Serbia, maximum incidences were 12 per 100 000 in Kosovo and Metohija and 1.15 per 100 000 in central Serbia. These incidences were lower than in most of the Balkans and Mediterranean countries. In Macedonia, an incidence of 18.6 and 24 per 100 000 was reported in 2007 and 2008, respectively (9). From 1980 to 2009, the annual average

incidence was 20 per 100 000 inhabitants (10). In Albania, an incidence of 25 per 100 000 inhabitants was reported in 2008 (11). In Bosnia and Herzegovina, the incidence increased from 3.8 to 33.43 per 100 000 inhabitants 2004-2008 (12). In comparison, from 1993 to 2000 in Greece, there was an increase in the incidence from 1.1 to 5.0 per 100 000 (from 112 to 548 cases). From 1997 to 1999 in a rural area of northwestern Peloponnese in Greece, the overall incidence was even higher – 1110 per 100 000 (13). After 2000, a remarkable decrease in the number of reported cases in Greece was observed, resulting in an incidence of 2.2 per 100 000 in 2003 (14). Nevertheless, there is evidence that foci of brucellosis still exist in Greece. A local surveillance system implemented in northwestern Greece recorded 152 newly diagnosed cases during a 2-year study period (from April 1, 2002 to March 31, 2004). The age- and sex-adjusted mean annual incidence rate for the population of the study area was 17.3 cases per 100 000 inhabitants (15). In Serbia, after a decrease in human brucellosis incidence from 1991 to 1999, the incidence increased from 2000 to 2004. This increase in human brucellosis after 2000 was due to emergence of a new brucellosis focus in Vojvodina.

Men in Serbia were two times more likely to be infected than women. These findings are consistent with the results of epidemiological studies on human brucellosis conducted in other parts of the world, where brucellosis is considered an occupational disease (16,17). The higher frequency of *Brucella* spp. infection in men can be attributed to their professions, since in rural areas, men usually deal with cattle (3). Sheep and goats are milked by hand and through this activity people come in direct contact with infected milk, especially during the lambing season, when the concentration of infectious agent in milk is high. In Greece, brucellosis is also an occupational disease, and it was diagnosed three times more often in men (12). In contrast, in Germany, brucellosis has evolved into a food-borne disease, and unpasteurized goat cheese is most frequently reported vehicle of exposure (18). Thus, in Germany from 1995 to 2005, both sexes were equally represented among patients: 54% men and 46% women (18).

Most patients in Serbia are in the age category from 30 to 49 years, which reflects the fact that brucellosis is associated with occupational exposure. Similar to our findings, the largest number of cases in central Greece occurred among men aged 30-50 years (16). In contrast, no such age clustering has been observed in Germany, where brucellosis is not an occupational disease (18).

In Serbia, human brucellosis is more frequent in the summer months. The number of human brucellosis cases increased from January through March when it reached the first, smaller peak. Then it continued to increase from May through September, reaching its second peak. In Serbia, brucellosis infections changed with the seasons, increasing when contacts between people and animals intensified (March and September). It is a time of births and abortions in sheep, goats, and other domestic animals. In central Greece, another region where brucellosis is an occupational disease, two peaks were recorded: one in March and another in May (16). In Germany, where human brucellosis is imported disease, seasonality of this disease is connected with summer vacations. The largest number of cases in that country was recorded in August and September (18).

Before 2001, brucellosis was mainly a food-borne disease, but after that year, it became predominantly a contact and occupational disease. 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.

The largest number of contact outbreaks in the present study was reported in Vojvodina. Compared with the rest of Serbia, Vojvodina has a more developed livestock breeding sector and more funding for livestock activities. Thus, it is understandable that contact outbreaks are predominant in Vojvodina.

During investigations of outbreaks, serological testing of animals connected to human brucellosis cases was performed by competent veterinary institutes in the regions under study. In our study, we focused on the epidemiological situation and we did not analyze the epizootiological situation.

The main sources of human brucellosis infection in Serbia were occupational exposure and ingestion of contaminated food products. The groups in which the occupational risk of infection is greatest include those whose work brings them in direct contact with infected animals or their products. These include farmers, shepherds, and their family members who help with the animals. An additional important category includes laboratory workers who may be exposed to contaminated specimens and to *Brucella* cultures during the course of diagnostic procedures. Food-borne transmission is usually the main source of brucellosis in urban populations. Ingestion of fresh milk

or dairy products prepared from unheated milk is the main source of infection for most populations. The cheese-making process may actually concentrate the *Brucella* organisms, which can survive for up to several months in this type of product (4).

Uncontrolled trade and migration of animals is the main mode of spreading brucellosis. Based on data from the annual reports of the Public Health Institutes of Republic Serbia and Vojvodina, brucellosis was imported to Kosovo and Vojvodina by importing infected animals. This suggests that controlling the animal trade is the main mode of control and prevention of brucellosis spread. Animals should be individually identified by brand, tattoo, or ear tag. Unauthorized sale or movement of animals from an infected area to other areas should be forbidden. Similarly, importations into clean areas must be restricted to animals that originate from brucellosis-free areas, that have no herd history of the disease, and that have obtained negative results in recently performed diagnostic tests (19).

The spread of brucellosis out of Kosovo and Metohija and into other regions of Serbia is a classic example of spreading old and creating new zooanthroponotic foci as a result of migrations of people and animals. The results implicate that brucellosis has been a significant public health concern in Serbia. This problem may be solved by joint efforts of all relevant factors, mainly human and veterinary medical services.

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