

Outbreak investigation of brucellosis in Thassos, Greece, 2008

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In spring 2008, the Hellenic Center for Disease Control and Prevention was notified about human brucellosis cases in Thassos, a Greek island that had been up to that point under a brucellosis eradication programme. Following the verification of the outbreak a 1:1 case–control study was conducted in the island. The study revealed that consumption of locally produced raw cheese was a risk factor for *Brucella melitensis* infection (odds ratio (OR): 15.1, 95% confidence interval (CI): 6.56–34.7). *Brucella melitensis* biotype 3 was identified in two clinical samples. As a result of the outbreak, the island is no longer officially considered as an area with farms free of brucellosis and is currently under a brucellosis control programme. The investigation of this outbreak demonstrated that control and eradication of brucellosis is not only a question of designing a strategy, but rather of ensuring its continuous, strict implementation. Furthermore, it revealed the lack of appropriate education of the public regarding the risks associated with raw, non heat-treated cheese consumption.

Introduction

Brucellosis is a common disease worldwide, representing a serious public health problem in many countries, especially those around the Mediterranean Sea [1–6]. In Greece, since 1998, the strategy of confronting brucellosis in small ruminants consists of two main components: (i) the implementation of a control programme (mass vaccination policy) in the mainland of the country and (ii) the implementation of an eradication programme in the islands, based on a test and slaughter policy [7,8].

On 16 May 2008, the Hellenic Centre for Diseases Control and Prevention (HCDCP) was notified about seven human cases of brucellosis in Thassos, the northernmost Greek island, located in the region of East Macedonia and Thrace. Thassos is only 10 km away from the mainland and has 13,720 inhabitants (census 2011) and an estimated population of goats

and sheep of around 60,000. There are no cattle herds on the island. At the time of the notification, Thassos was under a brucellosis eradication programme for livestock, which had been in place since 1998 [9]. Routine serological testing of animals conducted by the competent veterinary services had not resulted in any reports of animal cases since 2005. According to information from the mandatory notification system, no laboratory-confirmed human cases among the residents of Thassos or human cases epidemiologically linked to the island had been reported since 2005.

During the following days, more cases were notified from the island and an outbreak was verified. An outbreak investigation was initiated in order to identify the source of infection, investigate possible ways of the pathogen introduction into the island, control the outbreak and prevent the occurrence of future outbreaks in the area.

Methods

Epidemiological investigation

A 1:1 case–control study was conducted. Ninety-eight cases and 98 controls were invited to participate. A case was defined as any resident of Thassos aged ≥ 18 years who, from January until the end of August 2008, presented at least two of the following symptoms: fever, night sweats, arthralgia, malaise, headache, and who also had a positive Standard Agglutination Test (SAT) in a Wright test titre of at least 1:160. Controls were selected via simple random sampling of the general adult population using the municipality registries and a random number table. Person-to-person interviews were taken locally via a structured questionnaire, by healthcare professionals of the regional primary healthcare centre. The questionnaire included demographic information, clinical symptoms and exposure to possible risk factors for up to two months before symptom onset for cases, and in weeks 14 to 23 of 2008 for controls, such as participation in common meals, possession of or contact with farm animals,

manure handling, visits to areas with farm animals/breeding environments, consumption of dairy products and site of purchase thereof. The main hypothesis based on the available descriptive epidemiological data was that the consumption of a traditional cheese variety that is produced locally around Orthodox Easter had been the source of the outbreak.

Epidata (v3.1, Denmark) was used for data entry and Stata 11.0 software (STATA, College Station, Texas, USA) for data analysis. Each binomial exposure was analysed individually (univariate analysis). Odds ratios (OR) and the corresponding 95% confidence intervals (CIs) were calculated in Stata v11. When zero counts were reported for the control group, Cornfield's approximation was used. Variables that were statistically significant at the $\alpha=0.2$ level in the univariate analysis and to which at least 20% of the cases had been exposed were included in the multivariable analysis. The latter was conducted through the use of multiple logistic regression using backwards elimination techniques.

Laboratory and environmental investigation

Blood samples were obtained from eight patients in order to confirm the aetiological agent of the outbreak and identify the *Brucella* species involved. Further laboratory investigation including agglutination with specific antisera was performed for identifying the biotype of the bacterium. The Prefectural Public Health Directory of Kavala carried out hygienic inspections in shops and local cheese producers of the area. The Prefectural Veterinary Directorate tested herds in the area for *Brucella* spp. during the second half of May 2008.

Results

Epidemiological investigation

Ninety-eight cases and 63 controls responded (response rates: 100% and 64% respectively). Age did not statistically significantly differ between the two groups. Fifty-five cases (56%) and 19 controls (30%) were male. The most common clinical manifestations reported by cases are presented in Table 1. Malaise and fever were the two predominant symptoms, followed by night sweats, arthralgia and headache. Back pain was less frequent, being present in approximately one quarter of the cases.

TABLE 1

Clinical manifestations among brucellosis cases in Thassos, Greece, February–August 2008 (n=98)

Symptom	N (%)
Fever	62 (63)
Night sweats	60 (61)
Arthralgia	52 (53)
Back pain	28 (29)
Malaise	74 (76)
Headache	50 (51)

The distribution of week of symptom onset for the cases is depicted (Figure). The geographical distribution of cases and controls was similar. Most cases were residents of villages A (60%) and B (24%), while the retrospective percentages among controls were 62% and 27%.

Exposures that were ultimately included in the multivariable model are presented in Table 2 (univariable analysis results). After adjusting for age and sex and controlling for covariation of independent variables in the multivariable analysis, only consumption of fresh cheese from a local producer was a risk factor (OR:15.09, 95% CI: 6.56–34.7).

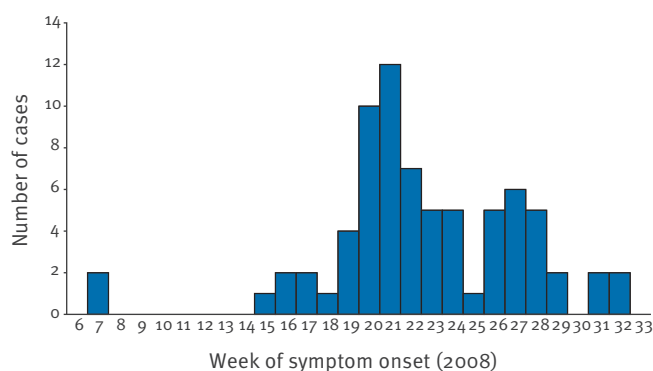
Laboratory and environmental investigation

Brucella melitensis was isolated in five of eight blood samples that were acquired from patients. In two of the positive samples, agglutination with specific antisera, revealed *B. melitensis* biotype 3, while no biotype testing was carried out in the other three positive samples. The environmental investigation in July 2008 did not pinpoint any specific establishment or market unit that could be linked to the outbreak. During the second half of May 2008, before the implementation of a mass vaccination control programme on the island, 30 herds were randomly tested for *Brucella* spp. including 4,585 sheep and goats. A total of 488 animals belonging to 18 (60%) of the 30 herds were found positive, resulting in a seroprevalence of brucellosis of 11% among sheep and goats. Further investigations for the identification of a specific biotype were not conducted.

In August 2008, epidemiological evidence showed that cheese consumption from a specific breeder of the island was linked to the occurrence of brucellosis; 79 of the 85 (93%) cases who had consumed fresh non heat-treated cheese, made with goat and sheep milk, could remember that the cheese had been purchased from that breeder. Cheese products were actually distributed by the specific breeder to consumers personally

FIGURE

Notified cases of brucellosis, by week of symptom onset, Thassos, Greece, 4 February–17 August 2008 (n=98)



The week of symptom onset is not known for 24 cases. These cases are not shown on the Figure.

and not through market stores. During the environmental investigation in July, raw unpasteurised cheese had been found in his premises but he claimed it was intended for personal consumption after maturation.

Measures implemented

In May 2008, the Prefectural Public Health Directorate of Kavala orally informed the residents about the basic preventive measures for the disease such as consuming only properly processed milk and dairy products and disseminated written information regarding the nature, the transmission route, and the main clinical manifestations of brucellosis. Written information on preventive measures was also given to local market stores and to taverns, to the Health Care Center of Prinos and to the General Hospital of Kavala. Local authorities also focused on informing local breeders and producers on correct practices and the risks associated with raw cheese consumption.

Following the results of the environmental investigation, the veterinary services of the Prefecture of Kavala moved on to a massive vaccination programme among young and adult female goats and sheep and to the slaughter of male animals which tested positive for *Brucella* spp..

In August, when the epidemiological link was made, all raw cheese in the premises of the implicated breeder had been consumed or discarded and his herds had already been included in the implemented massive vaccination programme of animals.

Discussion

In spring and summer 2008, there was an outbreak of brucellosis in the Greek island of Thassos that had been under a veterinary eradication programme since 1998. According to the available data from HCDCP, a similar outbreak had never been notified in the past. HCDCP worked closely with the Prefectural Public Health Directory of Kavala to identify the aetiological agent of the outbreak, timely control the outbreak and

take specific measures for the prevention of similar future incidents.

The results of the analytical epidemiological study showed that the consumption of raw non heat-treated cheese was the main risk factor for brucellosis infection, a finding that is consistent with previous reports in the literature [10-15]. Most cases, according to the epidemic curve, were exposed to the pathogen between week 15 and week 32 of 2008. Based on the incubation period of brucellosis (1–9 weeks), the time of exposure was, most probably, between week 14 and week 23 of 2008. The consumption of raw cheese is a local custom among the island inhabitants around the celebration of Orthodox Easter as part of a local tradition; in 2008, Easter was in the end of week 17. According to veterinary authorities, the most probable cause of re-emergence of brucellosis on the island was the illegal importation of animals from the mainland. However, we cannot be sure that this was actually the way that brucellosis was imported in Thassos.

A possible limitation of the study is that no breeders were included in the control group. This may have been a random result or due to the fact that breeders did not agree to participate. However, we believe that not having breeders in the control group could have actually led to an underestimation of the association between consumption of raw cheese from the specific breeder and the disease's occurrence. There are numerous breeders in the island that have small flocks and produce their own milk and cheese. One could thus assume that breeders mainly consume their own milk products and less so products of other producers. Thus, breeders would be less likely to consume raw cheese from the specific breeder involved in the outbreak compared to the general population. Even though some of the breeders in the cases' group had consumed raw cheese from the specific breeder during visits to friends or relatives, almost a third of them had become sick via other routes; this is why the consumption of cheese from the specific breeder does not

TABLE 2

Independent risk factors for acquisition of brucellosis in Thassos, Greece, February–August 2008

Risk factor	Cases (N=98) n (%)	Controls (N=63) n (%)	Odds ratio	95% Confidence interval
Men	55 (56)	19 (30)	3.03	1.48–6.31
Contact with farm animals (sheep, goats, cattle or pigs)	27 (28)	3 (5)	11.61	3.35–61.3
Visit to area with farm animals	29 (30)	17 (27)	1.50	0.72–3.22
Visit to breeding flocks	27 (28)	2 (3)	16.2	3.82–143.2
Manure handling	36 (37)	14 (22)	2.50	1.18–5.45
Goats' manure	27 (28)	10 (16)	2.23	1.02–5.08
Occupation relative to animals	26 (27)	0 (0)	1.78	0.8–4.09
Animal breeding	22 (22)	0 (0)	1.54	0.69–3.56
Fresh cheese consumption from a local producer	85 (87)	3 (5)	105.7	30.0–447.3

Variables were inserted in the multivariable analysis model before backwards elimination.

explain 100% of the cases. Based on this assumption, if the control group had also included breeders, the total number of controls who were not exposed to the cheese of the specific breeder could have actually been greater, leading to an even higher odds ratio.

Limited ability of performing bacteria cultures or more specific serological tests at the local level (such as Enzyme-linked immunosorbent assay (ELISA) or complement fixation test) and the unavailability of samples from the raw cheese leftovers, hampered the microbiological confirmation of the apparent epidemiological association.

Data from the mandatory notification system show that the last two cases of human brucellosis among residents of the island were notified in the beginning of 2009. There have not been any human cases identified ever since (until the end of October 2011) and the routine serological testing of local herds by the competent veterinary authorities also demonstrates the effectiveness of the implemented measures. The vaccination programme on the island is still ongoing. However, it is still soon to say that the island is free of brucellosis again, based on the data provided by the local veterinary authorities.

This outbreak has clearly demonstrated that control and eradication of brucellosis is not only a question of designing a strategy, but rather of ensuring its continuous, strict implementation through well organised policies and programmes. Political will, commitment for inter-sectoral collaboration between all involved parties and close monitoring and evaluation of the measures implemented are unquestionable prerequisites for disease control and eradication [16].

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