

Antibodies were firstly detected by western blotting (WB) (LD Bio, Lyon, France) and few days later were detected by enzyme linked immunosorbent assay (ELISA) (Biotrin International, Lyon, France). A seventh person who shared the meal with the 6 patients but who ate the meat cooked well done, did not develop any clinical or biological symptoms. This person's serology was negative.

Microscopic examination of a sample of frozen wild boar muscle revealed the presence of rare encapsulated *Trichinella* larvae in the striated muscle tissue. Muscle peptic digestion yielded 3 larvae per gram of muscle. These larvae were identified as *T. britovi* by polymerase chain reaction analysis (PCR) carried out at the International *Trichinella* Reference Centre (Rome, Italy).

Discussion

T. britovi, already identified in a previous outbreak in France [4] and elsewhere in Europe and Asia [5,6,7,8] is a species mainly found in wild animals such as foxes and wild boars, in biotopes at 500 m above sea level [1,6]. However, an outbreak in Caceres (Spain) following the consumption of insufficiently cooked meat from a domestic pig [8], suggests a possible change in the epidemiology of trichinellosis Nowadays, because of the mandatory veterinary controls in slaughterhouses, large trichinellosis outbreaks due to horse meat consumption are rare in France, but cases in hunters and their families after raw or rare wild boar meat consumption are regularly reported, with over one hundred cases since 1975 [9].

These cases confirm the occurrence of *T. britovi* in wild boar in southern France and its relative resistance to freezing, already described by Pozio et al. [10]. Indeed, they observed that larvae from naturally infected wild boar meat frozen for three weeks at -20°C remained infectious, whereas they were not viable after four weeks. To prevent trichinellosis, an official European directive [11] recommends the freezing of meat at -25°C for at least 10 days for pieces of less of 25 cm thickness. Our patients froze their wild boar steaks at -35°C for seven days, but this freezing time appears insufficient to kill larvae, since *T. britovi* is a species relatively resistant to freezing [1]. Consequently, we recommend complete heating of wild boar meat at 80°C for 10 minutes in our area. (South of France). According to the International Commission on Trichinellosis, meat should be heated at 65°C at the core for at least 1 minute to kill *Trichinella* larvae; larvae die when the colour of the meat at the core changes from pink to brown [12].

It seems difficult, however, to bring to an end the tradition among some hunters of consuming wild boar steaks immediately after shooting and dressing the meat. Therefore, despite all the recommendations, the risk of trichinellosis is likely to continue.

Wild boar consumers should be urged to follow individual strict prophylactic rules such as freezing at -25°C for at least 10 days (or -20°C during four weeks according to Pozio et al. [10]) or sufficient heating.

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ORIGINAL ARTICLES

Outbreak report

AN OUTBREAK OF *CAMPYLOBACTER JEJUNI* ENTERITIS IN A SCHOOL OF MADRID, SPAIN

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An outbreak of gastroenteritis caused by *Campylobacter* infection was identified in May 2003 in a school in Madrid, Spain. Eighty one cases were identified in a total of 253 people studied. A retrospective cohort study showed that a custard made with ultra high temperature (UHT) milk was associated with illness (RR: 3.15; 95% CI: 1.25-7.93). The custard was probably contaminated with *Campylobacter jejuni* from a raw chicken prepared a day previously in the

same kitchen. Our recommendations were to periodically remind the school's authorities how to act if an outbreak should be suspected, to include the monitoring of a food handler's working day in each environmental investigation in order to detect any risk behaviour; to implement microbiological analysis from the surfaces and utensils of the collective kitchens and improve the sanitary education of food handlers.

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Introduction

Campylobacter organisms are the second most common cause of bacterial foodborne disease notified to public health authorities in Spain (unpublished data, Microbiological Information System). Despite this, outbreaks of *Campylobacter* illness are rare in Spain, and from 1996 to 2001 an average of 6 *Campylobacter* outbreaks (range: 2-12 outbreaks) were reported each year to the Centro Nacional de Epidemiología (unpublished data, Outbreak Surveillance System).

Campylobacter may be transmitted by food, particularly poultry [1], unpasteurised milk [2,3] and contaminated water [4,5]. The lack of standardised molecular subtyping methods for *Campylobacter* has made it difficult to recognise outbreaks and identify their sources [6].

On 22 May 2003 the Public Health Authority of Madrid was informed that *Campylobacter jejuni* had been isolated from three stool specimens of children who attended the same school. The school had 293 pupils aged between 3 and 12 years (distributed between 13 classrooms) and 26 adults. Preliminary investigations identified an increased request for diet menus in the same week. Case finding conducted among paediatricians and microbiologists did not detect any increase in gastroenteritis or isolation of *Campylobacter jejuni* in the previous 30 days.

On the 23 May the Programa de Epidemiología Aplicada de Campo (Field Epidemiology Training Programme) started an investigation to assess the extent of the outbreak, identify the mode and vehicle of transmission, and initiate appropriate control measures in the school.

Methods

Case finding

Names and telephone numbers of all students, and the menus for meals served in the school were supplied by the principal.

A case was defined as a person (student or staff) who attended school between 12 and 14 May, and developed diarrhoea (loose stools, at least 3 times in 24 hours) or stomach pain and fever during the 10 days after 14 May.

The suspected period of exposure was delimited 12, 13 and 14 May, because the school was closed during the remainder of that week.

Analytical study

A retrospective cohort study was conducted. A questionnaire was designed to obtain demographic details, symptoms of gastrointestinal illness during the ten days after 14 May, the time of onset and the duration of the symptoms, contact with other ill persons in their homes, whether the family doctor was contacted because of the illness, food and water consumed from 12 to 14 May in the school, and contact with animals.

A personal interview was conducted with children older than 7 years and adults. In children younger than six years, food information was collected through a routine daily report filled in by the teachers and the clinical information was collected from the parents by phone. Children aged six years were not interviewed because they could not remember the food they had consumed.

The data were analysed using Epi Info software, version 2002. Variables were examined for association with illness in single variable analysis, and variables for which 95% confidence interval did not include the null value were put into a logistic regression model. A trend analysis in proportions was also done to assess a possible dose-response relation.

Environmental investigation

The kitchen and dining hall of the school were inspected by the local environmental health officers on 26 and 27 May, and food and water samples were taken and submitted for culture. Water and food samples from the suspected menus were not available when the inspection started. The investigations examined transport, storage and preparation processes for the food served at the school.

The cook was interviewed to determine any recent illness and for food handling practices.

Information on brands of the raw materials was collected.

Results

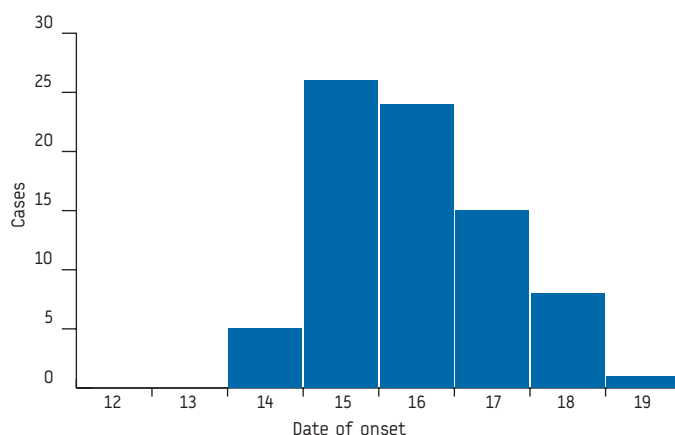
The school had 293 children (aged between 3 and 12 years, distributed between 13 classrooms) and 26 adults. From 253 valid interviews obtained we identified 81 persons who met the case definition (overall attack rate in the school = 32%). No differences were found between the attack rates (AR) by sex (31.7% in males versus 32.5% in females). The median AR by classroom was 30% (range: 12.5% - 60%).

All cases ate in the school and all of them were children. Children aged 12 years showed the highest AR (50%) and children aged 9 years showed the lowest (24%). The commonest symptoms were diarrhoea (93.6%), abdominal cramps (89.6%), fever (61.5%), nausea (29.7%) and vomiting (28%). The mean duration of the illness was 5.2 days. Of 81 cases interviewed, 31 (38.3%) consulted a physician. We are aware that 5 cases had a stool culture prescribed and 3 of these were confirmed as *Campylobacter* infection.

Date of onset of symptoms ranged from 14 to 19 May [FIGURE]. The rapid increase and decline in the number of cases and the single peak in the epidemic curve and the fact that all the cases ate in the school (children who usually ate in the dining hall of the school had a risk of illness 22.55 times higher than children who did not) suggested a foodborne point source outbreak among the students. Regarding minimum and maximum incubation periods of 1 and 7 days respectively [7] and the onset dates of the first and last cases, we estimated that the exposure day was 13 May. The median of the incubation period was 73 hours.

FIGURE

Date of onset of illness of 81 cases of *Campylobacter* infection in a school, Madrid, Spain, May 2003



Therefore, we limited additional analyses to a cohort of 199 people who ate in the school on 12, 13 and 14 May (although the most likely exposure day was 13 May, 12 and 14 were included because of the variability of the incubation period of *Campylobacter* infection).

After univariate analysis of food consumed, two food items, custard and milk, showed statistically significant relative risk estimates. Forty five percent of those who ate custard developed illness compared with 14% of those who did not, and children who drank milk had a 1.79 times higher risk [TABLE 1].

TABLE 1

Food specific attack rates for *Campylobacter* infection among students of a school, Madrid, Spain, May 2003

Food items	Food eaten			Food not eaten			RR	95 % C.I.
	Cases	Total	AR %	Cases	Total	AR %		
Monday	75	186	40.31	6	13	46.15	0.87	0.47-1.61
Paella with chicken	72	179	40.22	7	16	35.29	1.07	0.56-2.07
Fish	67	173	38.72	11	21	52.38	0.74	0.47-1.16
Salad	68	172	39.53	12	25	48.00	0.82	0.53-1.29
Fruit	71	173	41.04	7	23	30.43	1.35	0.71-2.57
Tuesday	81	192	42.18	0	7	00.00	3.80	0.60-24.30
Macaroni	79	187	42.24	1	10	10.00	4.22	0.65-27.33
Pork	75	181	41.44	4	16	25.00	1.66	0.70-3.94
Custard	77	171	45.02	4	28	14.28	3.15	1.25-7.93
Wednesday	76	187	40.64	5	12	41.66	0.97	0.49-1.95
Soup	73	181	40.33	6	16	37.50	1.07	0.56-2.07
Chicken	72	183	39.34	8	15	55.33	0.73	0.44-1.22
Fruit	72	171	42.10	7	26	26.92	1.56	0.81-3.00
Milk	61	129	47.28	18	68	26.47	1.79	1.15-2.76

In the multivariate analysis, only eating custard remained significantly associated with illness (p=0.03).

TABLE 2

Multivariate analysis. Outbreak of *Campylobacter* infection in a school, Madrid, Spain, May 2003

Food items	OR	C.I 95%	p-value
Custard	34 451	1.08-10.98	0.036
Milk	18 583	0.93-3.68	0.076

Also an analysis for trend in proportions was used for dose-response relation. This analysis showed an increase of risk of disease if the amount of custard consumed increased.

TABLE 3

Attack rates of *Campylobacter* infection by amount of custard consumed by students of a school of Madrid, Spain, May 2003

Custard	Total	Cases	RR
No exposure	28	4	1.00
Half a plateful	6	2	2.33
Whole plateful	162	73	3.15
Two platefuls	3	2	4.67

χ²: 10.34; P <0.005.

A private company supplied the staff of the kitchen and the raw materials for cooking the daily menus. Raw chicken was prepared for cooking the paella served on Monday.

Inspection of the kitchen indicated that food preparation areas for uncooked meats and ready-to-eat foods were not separated. The custard was made with ultra high temperature (UHT) milk and powder (without eggs) and kept at room temperature.

Campylobacter species were not isolated from the food samples from the kitchen or from water samples and there was no evidence of coliform contamination.

No faecal samples from children or food handlers were taken because of the delay in the start of the investigation.

Discussion

The epidemiological data from this investigation indicate that the contaminated custard was the most likely vehicle of *Campylobacter* infection in this outbreak. However, we believe that cross-contamination in the kitchen was a more likely cause of this outbreak than the purchase of contaminated custard because (1) custard was made with pasteurised milk and powder, both of which products were widely available in Madrid, (2) no outbreak caused by these products and *Campylobacter jejuni* was reported to the Regional Health Authorities of Madrid during 2003 and (3) on 12 May a paella was made with chicken which is a common food associated with *Campylobacter* infection. Similar outbreaks of *Campylobacter* have occurred in United States and Australia after cooked food became cross-contaminated by uncooked meat and poultry during preparation [8,9]. *Campylobacter jejuni* cannot long withstand drying or freezing temperatures, which are characteristics that limit its transmission. However, *C. jejuni* survives in milk, other foods, or water kept at 4°C for several weeks [7]. Also, the infectious dose of *Campylobacter* is low; ingestion of only 500 organisms, easily present in one drop of raw chicken juice, can result in human illness. Therefore, contamination of foods by raw chicken is an efficient mechanism for transmission of this organism [10].

This investigation was subject to a number of limitations. The delay between onset of illness and the cohort study was over two weeks for some cases, and this may have affected the accuracy of recall for food and water consumed. This was, to some extent, mitigated by the use of food lists when the questionnaire was administered. The fact that neither water nor food samples from the suspected days were available in order to detect *Campylobacter* species was also problematic. Also, the food handlers could not be ruled out as the source of infection because no faecal samples were taken from them.

As the results of this investigation the following measures were recommended: 1) that the the school authorities be periodically reminded how to act if an outbreak should be suspected; 2) that the working day of a food handler be included in each environmental investigation to detect any risk behaviour; 3) that microbiological analysis from the surfaces and utensils of the collective kitchens be implemented; and 4) that the sanitary education of food handlers be improved. For example, food handlers should be aware that pathogens can be present on raw poultry and meat and that foodborne disease can be prevented by adhering to the following measures: 1) raw poultry

and meat should be prepared on a separate counter or cutting board from other food items; 2) all utensils, cutting boards, and counters should be cleaned with hot water and soap after preparing other foods; 3) hands should be washed thoroughly with soap and running water after handling raw poultry or meat; and 4) poultry should be cooked thoroughly to an internal temperature of 82°C or until the meat is no longer pink and juices run clear [8].

In summary, custard cross-contaminated by chicken served in a school appears to have been the source of infection in this outbreak of *Campylobacter* enteritis. Sanitary education of the food handlers continues to be the main control measure in foodborne outbreaks.

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ORIGINAL ARTICLES

Outbreak report

OUTBREAKS CAUSED BY PARVOVIRUS B19 IN THREE PORTUGUESE SCHOOLS

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This paper reports the study of outbreaks of an acute exanthematous disease among children of three schools in the municipality of Braga (Portugal). Laboratory tests were performed for five cases, showing that the disease was not due to infection by measles or rubella virus, and infection with parvovirus B19 was confirmed. There were 41 cases in children: 12 in the kindergarten, 17 in the secondary school and 12 in the primary school. There was only one case in a staff member, who worked in the kindergarten. Eight cases were identified among household contacts; two of them were brothers, one from the kindergarten and another from the secondary school, where the outbreak occurred after the kindergarten outbreak. The estimated values of the basic reproduction number R_0 were very low and it is very likely that asymptomatic infectious cases have occurred. The local health authority produced written documents and met with staff members and parents. Primary healthcare facilities and the obstetric department of the local hospital were also informed. As we are approaching the elimination of measles in Portugal and the rest of Europe, with very high vaccine coverage, it is very likely that a high proportion of infectious non-vesicular exanthemas will be due to B19 infections. This is to be taken into account in the design and conduct of surveillance activities, in the context of measles and rubella elimination programmes.

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Introduction

Erythema infectiosum (EI), or 'slapped cheek' disease, is the most common clinical manifestation of infection with parvovirus B19 (B19) in children [1]. Several diseases, like measles, rubella, scarlet fever, erythema multiforme and this 'fifth disease' can cause similar acute non-vesicular exanthematous rashes and differential diagnosis is often necessary, especially in the context of measles elimination programmes in Europe [2] and worldwide [3]. Parvovirus B19 infections are usually benign and self-limiting [4] and commonly asymptomatic [1]; nevertheless, they can have important adverse effects among specific risk groups [1], namely pregnant women, immunocompromised individuals and patients suffering from chronic haemolytic anaemia. Thus, some authors have recommended the use of ELISA and PCR tests to confirm the aetiology of outbreaks [4].

In Portugal, EI and other B19 infections are not reportable to the local health authority (LHA), unlike measles and rubella. There are written recommendations on how to investigate suspected outbreaks (and isolated suspected cases) of measles [5]. We do not know of written reports of B19 outbreaks previously studied in Portugal but some serological data were published: in a blood transfusion department of a Portuguese hospital, 66.2% of health adults and 83.3% of haemophiliacs were found to be positive for B19 IgG antibodies [6].

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