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Clostridium perfringens in London, July 2009: two weddings and an outbreak

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Food poisoning outbreaks caused by *Clostridium perfringens* enterotoxin occur occasionally in Europe but have become less common in recent years. This paper presents the microbiological and epidemiological results of a large *C. perfringens* outbreak occurring simultaneously at two weddings that used the same caterer. The outbreak involved several London locations and required coordination across multiple agencies. A case-control study (n=134) was carried out to analyse possible associations between the food consumed and becoming ill. Food, environmental and stool samples were tested for common causative agents, including enterotoxigenic *C. perfringens*. The clinical presentation and the epidemiological findings were compatible with *C. perfringens* food poisoning and *C. perfringens* enterotoxin was detected in stool samples from two cases. The case-control study found statistically significant associations between becoming ill and eating either a specific chicken or lamb dish prepared by the same food handler of the implicated catering company. A rapid outbreak investigation with preliminary real-time results and the successful collaboration between the agencies and the caterer led to timely identification and rectification of the failures in the food handling practices.

Background

Food poisoning caused by *C. perfringens* is quite common [1]. Occasional outbreaks occur in Britain [2]; however, due to symptoms often being mild and of short duration, outbreaks are often not reported. We report the unusual occurrence of a simultaneous outbreak of *C. perfringens* at two large venues in London in July 2009.

Gastrointestinal illness caused by *C. perfringens* is characterised by sudden onset of abdominal pain followed by diarrhoea, and less commonly by vomiting and fever. Severe illness and fatal outcomes are rare. A short incubation period is usual (median 10-12 hours, range 6-24 hours [3]). Disease symptoms are caused by an enterotoxin produced by *C. perfringens* type A strains. Sufficient heat inactivates *C. perfringens* vegetative cells, however, its spores can survive and germinate in contaminated food under circumstances of poor temperature control, particularly a lack of cooling and insufficient reheating [4]. If food containing high numbers (>10^5 cfu/g) of *C. perfringens* vegetative cells is consumed, the bacterial cells can sporulate and produce enterotoxin in the human small intestine. Most *C. perfringens* food poisoning outbreaks are caused by a failure of adequate food preparation procedures. Recent evidence has also shown that healthy human food handlers can carry enterotoxigenic *C. perfringens* indicating that poor personal hygiene in catering staff is a risk factor for this foodborne illness [5].

In July 2009, the North West London Health Protection Unit (NWL HPU) was notified of a number of cases of gastroenteritis, which appeared to have been contracted at two different weddings on the same day in different London boroughs (administrative districts). The food at both these events had been supplied by the same caterer from a third borough.

The outbreak investigation and control was led by the local Health Protection Unit (HPU) in collaboration with the environmental health departments of three London boroughs. This team was supported by several divisions of the Health Protection Agency (HPA), including the regional Food, Water and Environmental laboratory, the National Reference Laboratory at the Centre for Infections, and the Regional Press Officers.

This paper adds to the current evidence base on *C. perfringens* outbreaks and reports on the findings of the microbiological and epidemiological investigations, as well as the logistics of investigating such outbreaks.

Methods

Case definitions

Probable cases were defined as persons who fell ill with one or more of the following symptoms: abdominal pain, diarrhoea or vomiting within 24 hours of attending a wedding in either of the affected venues. Confirmed cases were defined as persons fulfilling the case definition with microbiological confirmation of a gastroenteritis-causing organism in their stool sample. Controls were defined as persons who attended either of the two wedding receptions but did not develop any of the above symptoms within the following 24 hours.

The case-control study

Case-control methodology was used to investigate the outbreaks. A cohort study could not be conducted because complete guest lists were not available from either of the two events. Verbal consent was obtained from adults and young people. Children under the age of 12 years were excluded from the study, as their food histories were unlikely to be accurate. Many symptomatic people notified themselves, but active case finding was performed through the wedding hosts and environmental health officers. Controls were nominated by cases. Ninety-three cases and 41 controls were identified.

A single standardised questionnaire including questions on food consumption was administered through telephone interviews (n=124) or in person (n=10) between day 2 and day 9 after the wedding. The questionnaire had been developed, piloted and tested for validity in other outbreaks prior to this incident. Some common food items were served at the two weddings and the same questionnaire was used with the food items adapted for the specific venues. The data was entered into a secure database, cleaned and cross-checked for inconsistencies. Data analysis was performed with Intercooled STATA 9.2. Odds ratios (OR) and confidence intervals (CI) were calculated for all food items and chi-square and Fisher's exact tests were used for single variable analysis. Multivariable analysis was performed using logistic regression. The model was built in a forward fashion, and items which were significantly associated with illness in the single variable analysis ($p<0.05$) were included stepwise according to their *a priori* plausibility. At each step, a likelihood ratio (LR) test was performed to test whether the new variable significantly added to the explanatory power of the model. Only variables achieving an LR test of $p<0.05$ were kept in the model. Continuous variables were compared with two-sided t-tests, using natural logarithms to transform skewed distributions if appropriate.

Single- and multivariable analysis of all served food and drink items was performed separately for both venues. In addition the data from the two venues was merged for an analysis of all food items, treating the two wedding receptions as one large outbreak. In this analysis common food items, served at both venues and according to the caterer prepared together, were analysed as a single common variable. The two separate analyses had similar results in the final model and only results of the common analysis are shown in this paper, as these are based on a larger sample.

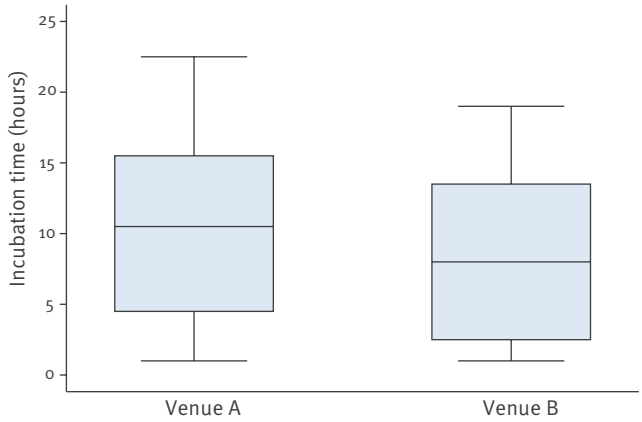
The environmental health officers collected detailed information on the preparation, storage, and transportation processes for the food catered at the two events.

Microbiology

Stool samples from eight symptomatic patients were collected and tested for a range of routine organisms, including *Campylobacter, Salmonella and Shigella*, and norovirus. Five of these were also tested for the presence of *C. perfringens* enterotoxin by Techlab ELISA at the Health Protection Agency (HPA) Laboratory of

Figure 1

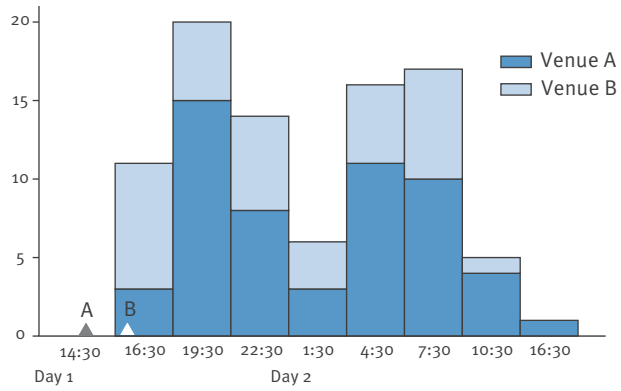
Incubation period, gastroenteritis outbreak at two weddings (venues A and B), London, July 2009 (n=91[a])



[a] For two cases onset time was not known.

Figure 2

Gastroenteritis outbreak at two weddings (venues A and B), London, July 2009 (n=91[a])



Scale: three-hourly.

The triangles indicate the start of the meals in venue A (A) and venue B (B), respectively.

[a] For two cases onset time was not known.

Gastrointestinal Pathogens, Centre for Infections. Samples from leftover food and environmental swabs from the catering company, in addition to stool samples and hand swabs from the catering staff were sent and tested at the HPA's Food Water and Environmental Laboratory, Centre for Infections.

Results

A total of 134 individuals were interviewed from the two wedding receptions (referred to as venue A and venue B), of whom 93 were cases (57 and 36 from venue A and venue B, respectively) and 41 were controls (16 and 25 from venue A and venue B, respectively). An estimated 150 guests attended venue A and about 400 attended venue B.

Descriptive epidemiology

The median age of cases was 28 years (mean: 31.5 years; range: 12–74 years). Males and females were almost equally distributed (55% and 45% respectively). The majority of cases had a rapid onset of symptoms and the median incubation period was 9.5 hours, (mean: 10 hours; range: 1–22.5 hours), however, the incubation period in venue B was significantly shorter than in venue A (8 hours versus 10.5 hours, respectively, p=0.033, Figure 1). The epidemiological curve is shown in Figure 2. The median duration of illness was two days (mean: 2.3 days; range: 1–10 days).

Overall, the majority of the 93 interviewed cases experienced symptoms of diarrhoea (95%) and abdominal pain (89%), followed by nausea (51%), headaches (38%), vomiting (24%) and fever (18%). Three patients reported to have had blood in their stools, an uncommon event in *C. perfringens* gastroenteritis. All three had attended venue A. Significantly more cases in venue B experienced vomiting compared to venue A (39% versus 14%, Fisher's exact test: p=0.001). There were no other significant differences in the epidemiological characteristics or the symptoms among the cases at the two venues.

The single variable analysis of all food and drinks items in both venues is shown in Table 1. However, many people will have chosen more than one dish in this buffet-style menu. In order to adjust for confounding, a logistic regression analysis was performed. Table 2 shows the final logistic regression model and all candidate variables which were excluded because they did not add to the explanatory power of the model.

After adjusting for all other food or drinks items, only the Jeera chicken (OR: 11.5; 95% CI: 3.7–35.9) and the Lamb karahi (OR: 2.71; 95% CI: 1.11–6.58) remained in the final model and were significantly associated with illness. These two dishes accounted for 88.2% of illnesses. In all, 92% (n=48) of persons who ate the Jeera chicken and 75% (n=69) of those who ate the Lamb karahi became ill. Thirty-five of the 37 who consumed both of these dishes became ill. There was no statistical interaction between the lamb and the chicken dish (p=0.995).

Microbiology

Stool samples from cases

All eight stool samples available for testing were negative for all routinely tested organisms, i.e. *Salmonella, Shigella, Campylobacter* and enteric viruses. Five of these samples were tested for *C. perfringens* enterotoxin and it was detected in two.

Leftover food samples

A small amount of uneaten food was collected from take-home portions of attendees from both weddings. From venue B, only samples of the Daal tarka (a lentil dish) and an unspecified lamb dish (several lamb dishes were served at venue B) remained, and in both of them *Enterobacteriaceae* and *Bacillus cereus* were detected at levels exceeding the acceptable thresholds ($>10^5$ cfu/g) as detailed in the European regulations [6]. From venue A, samples from seven different dishes (none of them were chicken or lamb dishes) were analysed, and in three of these *Enterobacteriaceae* and *B. cereus* were detected at levels exceeding acceptable thresholds [7]. However, these findings remain inconclusive, as these items were collected more than 72 hours after the weddings. No *C. perfringens* was isolated in any leftover food examined.

Environmental samples

High levels of *Enterobacteriaceae* and *B. cereus* were also found in environmental samples taken at the caterer's preparation premises. In addition, one out of three hand swabs, from the chef who had prepared the Jeera chicken and Lamb karahi for the weddings, tested positive for enterococci, *Enterobactericeae* and *E. coli*. The hand swabs were not tested for *C. perfringens*.

Stool samples from chefs

Stool samples were available for four of the kitchen chefs. None of them had gastrointestinal symptoms and all tested negative for oocytes, parasites, *Salmonella, Shigella, Campylobacter, C. perfringens*, and *Staphylococcus aureus* using standard isolation methods.

Kitchen inspection

The environmental health officers visited the caterer's premises on numerous occasions during the outbreak investigation. In addition to the samples mentioned above, some samples of food stored in the kitchen (but not used in the food for the two weddings) were taken: A garlic and ginger paste and a not fully processed paneer cheese were both found to have above threshold levels of *Enterobactericeae* and *E. coli*. In addition to the poor kitchen hygiene, the environmental health officers found that none of the staff were adequately trained in food hygiene and that temperature control during food handling was inadequate.

Table 1

Single-variable analysis of all food and drinks items consumed at the two venues of the outbreak, London, July 2009 (merged analysis)

	Cases		Controls				
	Exposed	Not exposed	Exposed	Not exposed	OR	95% CI	P value
Chicken tikka[a]	79	14	28	13	2.62	1.11–6.18	0.027
Lamb karahi[a]	69	22	23	18	2.45	1.13–5.33	0.022
Fish pakora[a]	67	24	28	13	1.3	0.58–2.88	0.528
Vegetable Pakora	43	49	6	35	5.12	2.01–12.99	<0.0001
Shish kebab	43	49	8	33	3.62	1.53–8.52	0.003
Jeera chicken	48	43	4	37	10.33	3.53–29.96	<0.0001
Sag paneer	35	56	6	35	3.65	1.42–9.29	0.006
Chicken Biryani	50	42	11	30	3.25	1.47–7.17	0.003
Onion kucha	9	74	2	37	2.25	0.52–infinite	0.304
Lamb tikka	35	57	18	23	0.78	0.37–1.64	0.524
Samosa	27	65	16	25	0.65	0.30–1.39	0.271
Aloo tikki	28	63	15	24	0.71	0.33–1.54	0.393
Chicken karahi	25	66	15	26	0.66	0.30–1.43	0.292
Mixed vegetable curry	19	72	7	34	1.28	0.50–3.26	0.611
Bombay aloo	19	73	12	29	0.63	0.27–1.44	0.278
Daal tarka	24	66	14	27	0.7	0.32–1.54	0.382
Lamb biryani	32	60	17	24	0.75	0.36–1.59	0.461
Green seasoning sauce[a]	20	70	6	35	1.67	0.63–4.39	0.313
Red seasoning sauce[a]	24	66	4	37	3.36	1.13–9.95	0.029
Plain roti	46	46	9	32	3.56	1.55–8.15	0.002
Cucumber raita[a]	22	70	8	33	1.3	0.53–3.15	0.575
Salads and pickles[a]	31	61	20	21	0.53	0.25–1.12	0.098
Ras malai[a]	45	48	13	28	2.02	0.94–4.33	0.073
Gajar halwa[a]	36	56	14	27	1.24	0.58–2.65	0.584
Milk-based drinks	3	78	1	35	1.35	0.18–infinite	0.799
Tap water	17	63	9	27	0.81	0.33–2.00	0.654
Fruit juice	22	60	14	22	0.58	0.25–1.31	0.19
Iced drinks	13	67	6	30	0.97	0.35–2.70	0.955

CI: confidence interval; OR: odds ratio.

[a] Items which were served in both venues.

Note that the number of exposed persons may not add up to 100% because of missing data.

Table 2

Logistic regression model of the implicated food items consumes at the two venues of the outbreak, London, July 2009 (merged analysis)

	OR	95% CI	p (Wald test)	p (LR test)
Jeera chicken	11.52	3.70–35.86	<0.0001	<0.00001
Lamb karahi[a]	2.71	1.11–6.58	0.03	0.03
Variables not included in the model				
Chicken tikka[a]				0.08
Vegetable pakora				0.08
Shish kabab				0.35
Sag paneer				0.95
Chicken biryani				0.13
Plain roti				0.62
Red seasoning sauce[a]				0.63

CI: confidence interval; LR: likelihood ratio; OR: odds ratio.

[a] Items which were served in both venues.

Variables were included in the model if $p<0.05$ in the LR test.

Discussion

This paper presents the findings of a point-source outbreak linking two weddings and one caterer in three London boroughs. There is strong evidence that a meal at either of the two venues was associated with becoming ill with diarrhoea and vomiting. There is no evidence that this outbreak was the result of human-to-human transmission. The epidemiological analyses as well as the biological plausibility (e.g. incubation time, clinical picture and duration of illness) suggest that *C. perfringens* was the likeliest causative agent [3], and the detection of *C. perfringens* enterotoxin in stool samples of two of the cases supports this conclusion. It should be noted that few stool specimens from the cases were available for testing and that *C. perfringens* enterotoxin is only detectable in stools up to two days after illness onset [8]. Although outbreaks related to *C. perfringens* are occasionally reported in the UK, these have become increasingly rare in developed countries, often attributed to improved temperature control in kitchens, but also due to mild symptoms and subsequent underreporting of illness [9].

The multivariate analysis of food items demonstrated a significant association between the consumption of Jeera chicken or Lamb karahi and illness. Although we cannot exclude that other dishes may have been contaminated with *C. perfringens*, it is likely that these two dishes will have contained high numbers of enterotoxigenic *C. perfringens*. Both are curry-based dishes that were prepared together in one common process by the same chef. The high levels of contamination with faecal organisms isolated from the hand swabs of this chef raise the possibility of insufficient personal hygiene as a risk factor for this outbreak. Although no *C. perfringens* was detected in the stool samples from the chefs, it should be noted that *C. perfringens* carrying the enterotoxin gene has been found in healthy food handlers, but only with specialist isolation methods and not routine methods as were used here [5].

Of those exposed to Jeera chicken or Lamb karahi, 92% and 75%, respectively, became ill, but 95% of those who consumed both of these dishes became ill; it is possible that this reflects a dose response.

A blast chiller is normally used for cooling large quantities of food quickly by this particular caterer; however it was not being used appropriately at the time of the incident. Temperature control of foods during preparation, cooling, transportation and reheating was poor. Furthermore, the vans used for food transport had no refrigeration and these events took place in July. The evidence of insufficient hygiene, cooling and reheating at the catering company during transport and at both venues (according to environmental health department inspections) are in keeping with a toxin-related gastroenteritis outbreak, including *C. perfringens* enterotoxin [4,9,10]. As the distance from the caterer to venue B was substantially longer than to venue A, the lack of adequate temperature control during transport may have led to a higher infective dose in venue B, which could explain the shorter incubation time and higher proportion of cases with vomiting.

We present the results of a pragmatic outbreak investigation. Its strengths and weaknesses are defined by the rapid investigation required for the public health response, the need for coordination across multiple organisations and the difficulties in contacting attendees at large functions.

The study would have benefited from more controls, and this would have increased the statistical power to detect rare risk exposures. The absence of guest lists, the need for rapid investigations, and the high attack rate made it difficult to recruit additional controls. It is possible that a larger sample size with more controls would have led to increased effect sizes of associations between food items and illness, but it is unlikely that more controls would have altered the main results, because of the high effect sizes observed.

One of the main challenges in this investigation was the lack of appropriate food samples from the weddings and the difficulty in obtaining stool samples from cases, who came from all over the UK. The scarcity of, and delay in obtaining, microbiological samples, including food, stool and environmental samples, illustrates some of the challenges to coordinate actions across multiple organisations in several districts or regions. The decentralised testing of microbiological samples with environmental health officers from different boroughs ordering different tests led to delays. Despite these limitations the epidemiological and microbiological evidence is sufficient to establish a strong association between consumption of the aforementioned food items and subsequent gastrointestinal illness.

The outbreak investigation was conducted rapidly and in a timely fashion, enabling quick implementation of control measures and also minimising recall bias in the interviews. Real-time updates on the results were presented to the outbreak control team and informed further sampling and public health measures. This was partly due to an already prepared outbreak investigation tool kit, including previously tested questionnaires that were available at the North West London Health Protection Unit. The caterer was served with a Hygiene Emergency Prohibition Notice. This required the production of a food safety risk assessment according to specific Hazard Analysis and Critical Control Points (HACCP) before any catering could take place. The caterer complied with the control measures, employed a food hygiene consultant to oversee the food preparation, and produced specific HACCPs for each food product. The company was therefore allowed to continue catering for events despite the prohibition order.

In summary, we report the results of the microbiological and epidemiological investigation of a large point-source *C. perfringens* enterotoxin outbreak occurring

simultaneously at two weddings. The outbreaks were associated with consumption of specific curry-based dishes provided by the same caterer. The preparedness and collaboration between different stakeholders enabled real-time availability of investigation results and helped to control this outbreak quickly with a proportionate response.

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Investigation of a large outbreak of Clostridium difficile PCR-ribotype 027 infections in northern France, 2006-2007 and associated clusters in 2008-2009

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In 2006 and 2007, a large outbreak of *Clostridium difficile* infections (CDIs) with PCR-ribotype 027 was identified in northern France. Overall, 38 healthcare facilities notified 529 CDIs over a 22-month period, including 281 laboratory-confirmed CDI 027 and 248 non-confirmed CDI 027 cases (incidence rate per 10,000 elective bed days: 1.63, range: 0.07 to 7.94). The cases occurred mainly in long-term care hospital facilities and nursing homes, near the border between France and Belgium. An active surveillance and prevention campaign was launched at the first epidemic peak including hygiene precautions for healthcare professionals, which supported healthcare facilities to improve care organisation. The outbreak was controlled at the end of 2007, but sporadic cases were identified until the end of 2009. A bundle of appropriate control measures may halt the spread of such outbreaks, provided that substantial human resources and financial support are available.

Background

Clostridium difficile is an anaerobic Gram-positive, spore-forming bacterium, which is responsible for 15–25% of antibiotic-associated diarrhoea and virtually all cases of pseudomembranous colitis [1]. Since 2003, outbreaks of severe *C. difficile*-infection (CDI) have been increasingly reported in Canada and the United States (US) [2,3]. These outbreaks were associated with the emergence and rapid spread of a specific strain of *C. difficile* belonging to PCR-ribotype 027 or pulsotype NAP1 (North American Pulsotype 1). Some of the characteristics of this strain are higher *in vitro* production of toxins A and B and presence of a third toxin named binary toxin [2,4]. The epidemic strain has begun to spread for the last five years in northern Europe (United Kingdom (UK), Belgium and the Netherlands) [5-7].

The first cases of the PCR-ribotype 027 epidemic strain in France were reported by a healthcare facility through the national mandatory notification system for nosocomial infections to the regional infection control coordinating centre (CCLIN) on 2 February 2006 [8,9]. All healthcare facilities in the region were alerted and urged to send *C. difficile* strains to the national reference centre to confirm whether they belonged to the 027 epidemic strain. An epidemiological investigation was then launched to evaluate the magnitude of the outbreak. In addition, a nationwide prevention and information campaign was implemented by the national institute for health surveillance (Institut de Veille Sanitaire, InVS) and the Ministry of Health to identify and control the potential spread of the outbreak.

Methods

C. difficile toxins were detected from stools using enzyme immunoassays or by cytotoxicity assay according to each local standard procedure. Culture of *C. difficile* was performed on selective media (cefoxitin-cycloserine fructose agar plates). After incubation at 35 °C for 48 hours under anaerobic conditions, suspected colonies (based on Gram staining, typical odour and chartreuse fluorescence under ultraviolet light) were confirmed using biochemical gallery (RapID 32A, Biomérieux). *C. difficile* isolates were then sent to the national reference laboratory for typing. Strains were characterised by PCR-ribotyping by previously described techniques [12].

The study area included two administrative regions (Nord Pas-de-Calais and Picardie) with 26,800 hospital beds in 145 healthcare facilities and with approximately 450 nursing homes. The study covered the period from the beginning of 2006 to the end of 2009. Among healthcare facilities, 55% were acute care hospitals and 27% were long-term care hospitals or rehabilitation centres. The term 'outbreak' is used here to

denominate the overall epidemic situation and a group of affected healthcare facilities. The term 'cluster of cases' is used here to denominate a local epidemic situation in one healthcare facility after the outbreak period. CDI was suspected in all patients presenting with unexplained diarrhoea and were tested for *C. difficile* toxin A and B using standard technique. Diarrhoea was defined as three or more liquid stools per day and pseudomembranous colitis was diagnosed based on colon videoscopy. A CDI was considered as severe if a patient presented with at least one of the following criteria: CDI requiring hospitalisation in intensive care, white cell count higher than 20,000/mm^3, need for digestive surgery, or fatal outcome within 30 days after CDI diagnosis.

To describe the outbreak, the case definition was based on standard clinical and microbiological criteria given in the guidelines from the European Centre for Disease Prevention and Control [10]. Confirmed cases were CDI cases PCR-positive for ribotype 027. Non-confirmed CDI 027 cases were CDI cases with a positive toxin assay and one of the following criteria: (i) a nosocomial case acquired in a healthcare facility where at least one confirmed case was staying at the time, or (ii) a case imported from a healthcare facility where at least one confirmed case was identified, or (iii) a recurrence in a patient from whom a 027 strain had been isolated in the past. All healthcare facilities having reported at least one confirmed or non-confirmed CDI 027 case were included in the study. Criteria for hospital-acquired infections were those established by the US Centers for Disease Control and Prevention [11].

In each participating healthcare facility, data were collected by the infection control team using a standardised questionnaire including information on age, sex, date of admission, CDI clinical characteristics (diarrhoea or colitis) and severity, date of CDI onset and outcome (death, hospital stay or not at the time of the study), date of the first positive toxin assay, and result of laboratory culture. Data were sent every week to the regional coordinating centre for nosocomial infection control for tracing the progression of the outbreak.

Data analysis was performed using Stata release 8.0 (Stata Corp LP). Incidence rates were the ratio of the number of cases per 10,000 bed days. Comparison of characteristics of confirmed versus non-confirmed cases was made using Student's t-test or Pearson's chi-square test. All tests were considered significant at $p<0.05$.

Table

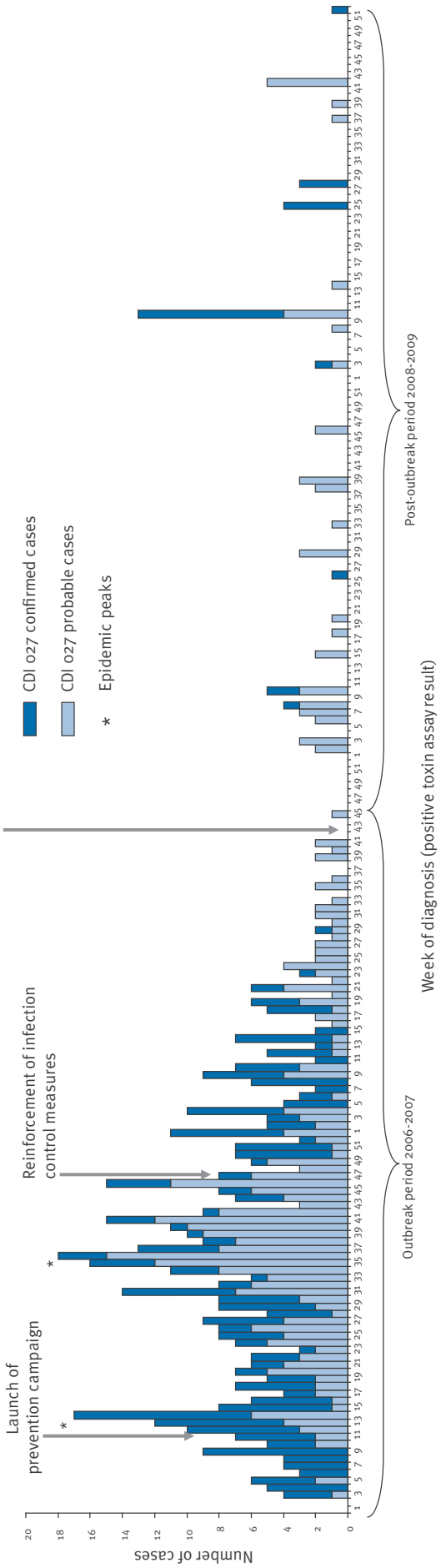
Characteristics of patients with *Clostridium difficile* 027 infection, northern France, outbreak period 2006-2007 (n=529)

Characteristics	Confirmed CDI 027 cases (n=281)	Non-confirmed CDI 027 cases (n=248)
Personal data		
Mean age (years)	79.8	77.6
Sex ratio male/female	0.53	0.48
Origin		
Acute care	68 (24.2%)	67 (27.0%)
Long-term care	130 (46.3%)	104 (41.9%)
Nursing home	25 (8.9%)	25 (10.1%)
Other hospital	21 (7.5%)	19 (7.7%)
Community-acquired	21 (7.5%)	14 (5.6%)
Unknown	16 (5.7%)	19 (7.7%)
Clinical data		
Diarrhoea	260 (92.5%)	233 (93.9%)
Pseudomembranous colitis	15 (5.3%)	14 (5.6%)
Unknown	6 (2.1%)	1 (0.4%)
Severity of CDI		
Severe	34 (12.1%)	33 (13.3%)
Mild	242 (86.1%)	214 (86.3%)
Unknown	5 (1.8%)	1 (0.4%)
Outcome		
Death	82 (29.2%)	82 (33.1%)
Hospital discharge	120 (42.7%)	91 (36.7%)
Transfer to another hospital	68 (24.2%)	54 (21.8%)
Unknown	11 (3.9%)	21 (8.5%)

CDI: *Clostridium difficile* infection.
No statistical significant difference between groups.

Figure 1

Confirmed and non-confirmed *Clostridium difficile* infection with ribotype 027 in northern France, 2006-2009 (n=602 cases)



Results

Outbreak period 2006-2007

From 1 January 2006 to 31 December 2007, 38 healthcare facilities (20% of healthcare facilities in the region) notified at least one confirmed or non-confirmed CDI 027 case, including 31 hospitals with more than one case. In addition, 27 (6% of nursing homes in the region) nursing homes reported community-acquired cases. Among 529 CDIs, 281 were confirmed cases and 248 non-confirmed. The number of confirmed and non-confirmed CDI 027 cases varied between the healthcare facilities, ranging from one to 126. The mean incidence rate of total CDIs per 10,000 hospitalised days was 1.63 (range: 0.07 to 7.94), with 1.19 cases per 10,000 days of hospitalisation in acute care facilities (range: 0.1 to 4.5) and 2.39 in long-term or rehabilitation facilities (range: 0.15 to 19.8).

Most cases were over 80 years-old (mean age: 79.8 years) and the male/female sex ratio was 0.53). Cases occurred more often in healthcare facilities, but a substantial number were detected in nursing homes. Diarrhoea was the main symptom (92.5%), whereas pseudo-membranous colitis was infrequent (5.3%). Comparison between confirmed and non-confirmed CDI 027 cases did not show any statistical differences (Table).

The epidemic curve is displayed in Figure 1, showing the timing of the prevention campaign. Overall, the outbreak developed over a period of 22 months. The index case was identified in week 4 in 2006. The epidemic curve presents two major peaks: the first from February to April 2006 with the highest number of cases in week 14 (17 cases), the second from September to December 2006 with the highest number of cases in week 36 (18 cases).

In April 2006, a prevention campaign was launched at the regional level in order to help infection control and medical staff to detect CDI cases early and promptly implement barrier precautions. Enhanced control measures and specific disinfection procedures against CDI were recommended including isolation precautions according to standards, reinforcement of hand hygiene using alcohol-based hand rub solutions following hand washing with liquid soap, wearing gloves, dedicating equipment, environmental cleaning with hypochlorite solutions (0.5%), and a specific process for waste management [13]. As cases were still occurring after the first bundle of measures, the campaign was reinforced with a focus on the implementation of cohorting units with isolation in private rooms and dedicated staff personnel. This second bundle of measures was maintained until the outbreak was considered to be under control at the end of 2007, when no healthcare facility had reported a new major cluster of cases in three months.

Spatial analysis of the reported cases highlighted two geographical outbreaks (Figure 2). During the epidemic

period 2006-2007, the main outbreak spread near the Belgian border, including 447 cases identified in 25 healthcare facilities (of which 56.1% were confirmed cases). Among them, 10 episodes included between six and 51 confirmed cases, 26 less than six confirmed cases and two clusters consisted only of non-confirmed CDI 027 cases. The index case of this outbreak was located in an area with a high density of hospital beds and frequent patient transfers among healthcare facilities. The second major outbreak spread near the Somme estuary, including 25 cases (of whom 21 were confirmed CDI 027 cases) identified in two healthcare facilities. A further 11 healthcare facilities with episodes of CDI 027 were distributed throughout the region and were not part of the two main geographical outbreak areas.

Post-outbreak period 2008-2009

After a two-month period with no cases, new cases were identified. Overall, 73 cases of CDI were notified in 2008 and 2009, 29 confirmed CDI 027 cases and 44 non-confirmed CDI 027 cases. These cases belonged to 15 notified clusters of CDI 027 with between two and 13 cases each, and to 22 sporadic cases in several healthcare facilities that had already been affected during the outbreak period. In 2009, 10 cases of CDI 027 occurred in the Paris area. The typing results showed that the patients were infected with the epidemic *C. difficile* 027 strain and were therefore considered as a consequence of the outbreak in northern France.

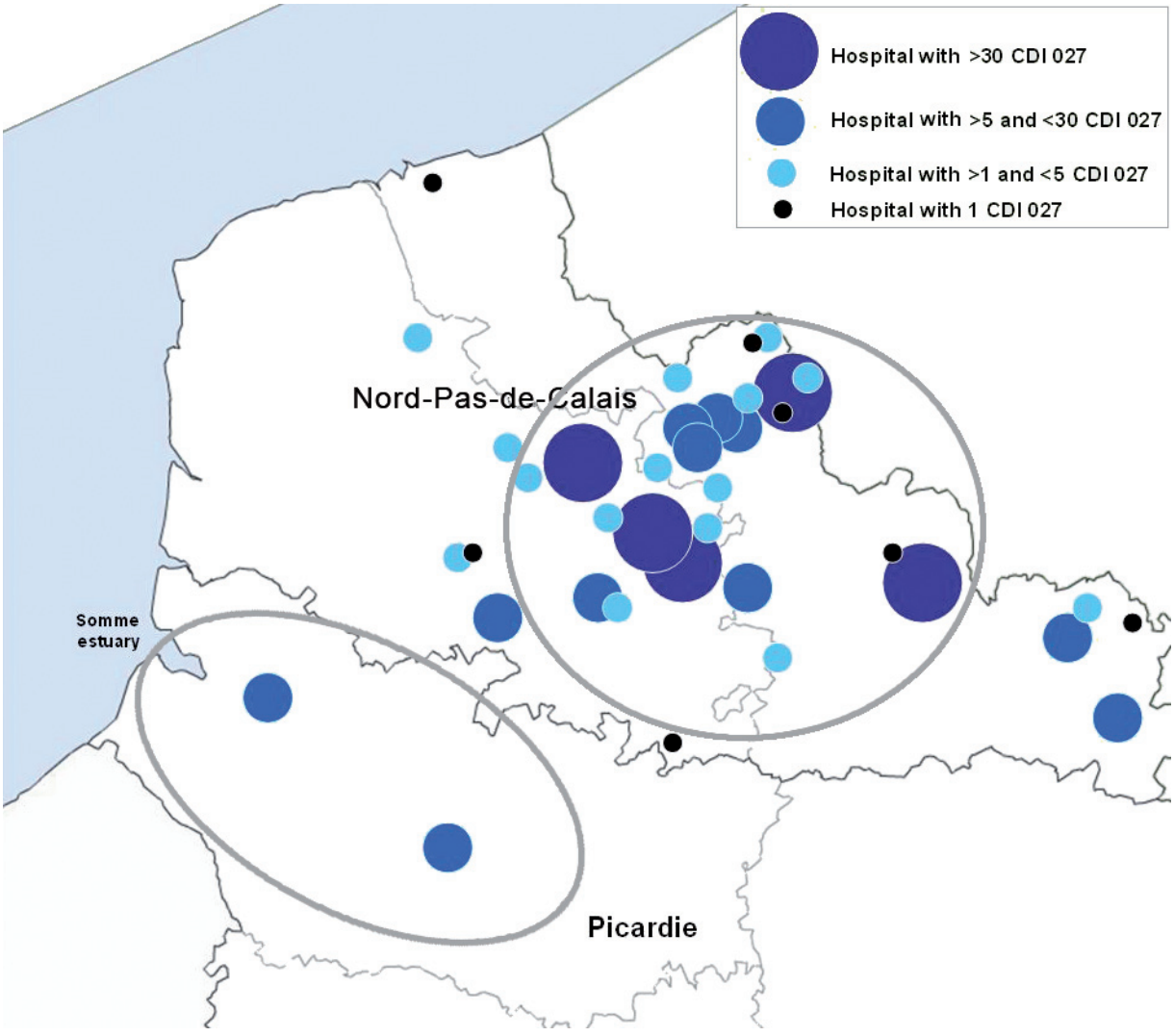
The proportion of confirmed cases was higher in the period 2008-2009 (83.3%) than in the period 2006-2007 (35.3%). The ratio confirmed/non-confirmed cases varied from 0 to 10 according to week of diagnosis.

Discussion

Since the emergence of the *C. difficile* 027 epidemic in North America and Europe, this is the first time that a large outbreak of CDI with PCR-ribotype 027 is described in France. This outbreak seems to be under control now in this country, although sporadic cases

Figure 2

Spatial distribution of healthcare settings with clusters of cases of *Clostridium difficile*-027 associated disease in northern France, 2006-2007 (n=38 healthcare facilities)



Grey circles indicate two geographically separate outbreaks.

are still occurring. Accordingly, surveillance data in Canada and the US show a similar increasing incidence of CDI directly associated with the emergence of *C. difficile* PCR-ribotype 027. In 2005, this organism represented 80% and 50%, respectively, of strains isolated in Canada and the US [14,15]. This strain then spread through northern Europe, especially the UK and the Netherlands, and in Belgium bordering the epidemic area in northern France with higher than usual incidence rates [6,10,16,17].

The time period between the occurrence of the first case and the first notification to health authorities which launched the prevention campaign was about three months. This raises the question of why there was such a delay although an effective mandatory notification system was in place in France for early detection of outbreaks in general or unusual healthcare-associated infections. According to national guidelines promoted by the Ministry of Health, the notification should be made by a hospital infection control practitioner according to defined criteria. During the outbreak, a large information campaign on CDI 027 was held in northern France. This campaign has increased the awareness among medical and paramedical teams of the notification and of why, when and how to notify a case of CDI. Furthermore, microbiologists have been informed on and trained in methods of toxin assay and stool culture for isolation of *C. difficile*. In consequence, the number and the quality of microbiological analyses and notifications have increased following the outbreak period.

However, most epidemic cases of CDI in our study could not be notified promptly because they occurred in long-term care facilities or nursing homes that had few healthcare personnel and often no infection control specialist. In addition, there are no defined criteria for diarrhoea or associated gastroenteric diseases in the current mandatory notification system for nosocomial diseases. Extended notification criteria or a new targeted surveillance system focused on acute enteric diseases in healthcare facilities should further improve the effectiveness of outbreak detection.

As already demonstrated, isolation of symptomatic patients with CDI is a key measure to control *C. difficile* outbreaks [18-20]. Indeed, environmental contamination occurs as a result of CDI, especially when patients have large amounts of liquid stool or stool incontinence. Our study suggests that the incidence of CDI decreases if a bundle of measures such as strict enteric contact precautions, double hand hygiene washing off spores with soap before using alcohol-based hand rub, and appropriate cleaning of the environment surrounding cases are performed. Better hygiene practices should be combined with a better organisation of care including cohort nursing, i.e. gathering cases in a designated ward, movement restrictions on staff and patients, and intensive education of staff. Whether *C. difficile* PCR-ribotype 027 is more easily cross-transmissible than non-027 strains remains questionable. Akerlund *et al*. demonstrated that the epidemic (027/NAP1) strain in Sweden sporulated more effectively (60%, $p<0.001$) than others. They conclude that this contributes to its survival and facilitates cross-transmission and spread despite standard hygiene precautions [21]. Antibiotics treatments and particularly the use of fluoroquinolones have certainly had an influence in the occurrence of this outbreak [22].

Detection of asymptomatic *C. difficile* carriers is an additional possible control measure, although it remains controversial. Riggs *et al*. demonstrated that more than half of the patients surrounding epidemic cases were asymptomatic carriers and should be actively screened [23]. Additionally, colonisation of the skin and airborne transmission may play an important role in the epidemiology of CDI [23]. The isolation of asymptomatic carriers may contribute to combatting outbreaks. On the other hand, systematic screening of patients (on admission and weekly or monthly), especially in nursing homes or long-term care is costly and hard to implement. In an epidemic context, the screening would be more cost-effective when focussed on newly admitted patients. In our study, only a small proportion (10%) of cases came from other care facilities, suggesting that systematic screening would have been feasible.

The outbreak mostly affected elderly patients and was therefore characterised by significant mortality and severe disease. The mortality rate given in the Table is a crude rate and does not consider comorbidity, medical history or exposure to antibiotics of the patients, which can be confounding factors. The mortality rate would need to be adjusted for these confounding factors to avoid potential bias. The high severity of CDI 027 is assumed to be associated with higher amounts of toxin production of this strain [2,24]. However, implementation of control measures was highly time-consuming with heavy financial consequences for the healthcare system. Strong efforts were required from both personnel working in healthcare facilities and the infection control specialists who help implement control measures with the support of the public health authorities. Based on a subset of healthcare facilities, we estimated the extra-cost of such an outbreak including only charges due to additional personnel, material and products to be about EUR 31,000 per patient-case and EUR 1,000 per day. This estimate is consistent with those previously reported [25].

The CDI 027 notified in 2008 and 2009 were mostly sporadic cases or part of small clusters. This observation could be explained by the spread of the epidemic strain in the community. A recent article has estimated the proportion of community-acquired CDI in North Carolina, US, at 20% [26]. Elderly patients (the main population affected during the outbreak period was over 80-years-old) are sometimes transferred to nursing homes after their hospitalisation. As a step between the hospital

and the patient's home, nursing homes could facilitate the spread of *C.difficile* strains from hospitals to the community. Transmission of the epidemic strain from an infected patient to other people living in the same nursing home could create human reservoirs of *C. difficile* in this population. Conversely, the life of people in nursing homes often being disrupted by hospital stays, the hospitalisation of a patient coming from home or nursing home and infected or colonised with *C. difficile* 027 could provoke an outbreak in the hospital, if the infection control precautions are not quickly implemented, even more so if this happens in a region never affected by the epidemic strain before. To prevent such a scenario, sustained efforts of detection and control are warranted to prevent the re-emergence of a new epidemic wave. A crucial point is informing healthcare workers about the infection control measures against *C. difficile* transmission.

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Congenital toxoplasmosis in France in 2007: first results from a national surveillance system

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When immunocompetent people become infected with the parasite *Toxoplasma gondii*, the disease is generally asymptomatic. However, transplacental transmission of *T. gondii* may lead to severe congenital infection including *in utero* abortion, foetal death, or neurological or ocular damage of the foetus. France has had a national programme to prevent congenital toxoplasmosis since 1978. However, although estimated seroprevalence in pregnant women has fallen from 84% in the 1960s to 44% in 2003, no reliable data have been available on the annual number of cases of congenital toxoplasmosis or the severity of infection. In 2006, the French National Institute for Public Health Surveillance (Institut de Veille Sanitaire) and the National Reference Centre for Toxoplasmosis recommended that a national laboratory-based surveillance system be used for the surveillance of the disease. In 2007, 31 laboratories reported at least one congenital case through the surveillance system, giving a total of 272 cases. A total of 11 terminations of pregnancy were reported (six abortions and five foetal deaths). Of the live-born cases, 206 were asymptomatic, 28 were symptomatic and seven had a severe form of the disease. As there were 818,700 births in France and French overseas departments in 2007, the overall prevalence of congenital toxoplasmosis observed that year was 3.3 (95% confidence interval (CI): 2.9 to 3.7) per 10,000 live births and the incidence rate of the disease at birth was 2.9 (95% CI: 2.5 to 3.2) per 10,000 live births; the estimated incidence rate of symptomatic congenital toxoplasmosis was 0.34 (95% CI: 0.2 to 0.5) cases per 10,000 live births.

Introduction

Toxoplasmosis is caused by the protozoan parasite *Toxoplasma gondii*, which is widely distributed in the environment. When immunocompetent people become infected, the disease is generally asymptomatic. However, transplacental transmission of *T. gondii* may lead to severe congenital infection including *in utero* abortion, foetal death, or neurological or ocular damage of the foetus [1]. In France, estimated seroprevalence among pregnant women fell from 84% in the 1960s to 54% in 1995 to 44% in 2003. Models estimating the incidence of toxoplasmosis by age showed that between 1995 and 2003 the incidence rate fell by 17.6% for 20-year-old women and by 8.3% for women aged 40 years [2,3]. The number of women who seroconverted during pregnancy was estimated in 1995 to be approximately 2,700 per year [4]. The risk of transplacental transmission increases with gestational age at the time of maternal infection: in 1999, Dunn estimated an overall global transmission rate of 29% during pregnancy [5].

Clinical signs of toxoplasmosis are very diverse and can be serious (foetal death). Prognosis of infection is principally dependent on the time of maternal infection and genotype of the *Toxoplasma* strain (greater virulence being associated with atypical genotypes) [6]. Congenital infection can lead to severe sequelae for the foetus and newborn, with neurological lesions or visual impairments often documented [7,8].

A national programme to prevent congenital toxoplasmosis has existed in France since 1978. In addition, since 1992, pregnant women who are not immune to toxoplasmosis have been tested monthly until delivery. Despite this, there have been no reliable data on the annual number of cases of congenital toxoplasmosis or the severity of infection. In 2006, a working group on congenital toxoplasmosis recommended that a laboratory-based system would be most appropriate for surveillance of this disease. The French National Institute of Public Health Surveillance (Institut de Veille Sanitaire, InVS) and the National Reference Centre for

Toxoplasmosis are responsible for this system, which has been active since June 2007 [9]. The system aims to collect information on cases of congenital toxoplasmosis diagnosed during pregnancy by amniocentesis, or diagnosed in newborns and infants under one year whose mother had seroconverted during pregnancy. The objectives of the surveillance are to estimate overall prevalence of the disease in France, monitor prevalence trends and estimate the proportion of cases with severe forms of infection (hydrocephalus, microcephalus and macular chorioretinitis).

Several preliminary surveys were undertaken to identify laboratories able to diagnose the infection in newborns or infants, in order to optimise surveillance of the disease [9]. A surveillance system was set up, ToxoSurv, based on a network of 35 specialised laboratories that are certified in prenatal and neonatal diagnosis of toxoplasmosis and 74 additional medical biology laboratories that occasionally carry out diagnosis. In this report, we present the results of the surveillance from 1 June to 31 December 2007, with retrospective data collection for the first six months of that year.

Methods

Case definitions

A case of congenital toxoplasmosis was defined as a foetus, newborn or infant aged under one year with at least one of the following [10]:

- *T. gondii* in body tissues or fluids by polymerase chain reaction (PCR), inoculation of mice, cell culture or immunocytochemistry
- specific IgM or IgA antibodies
- specific IgG antibodies within the first 12 months of life
- persistent IgG positivity until one year of age.

Case diagnosis and notification

Cases of congenital toxoplasmosis are reported to the National Reference Centre for Toxoplasmosis in two ways: firstly, via internet data entry for the 35 specialised laboratories (through http://www.chu-reims.fr/professionnels), using specifically developed software, Voozanoo (EpiConcept). Secondly, the 74 additional laboratories send paper forms to the National Reference Centre for Toxoplasmosis, where the notifications are entered through the internet data entry system. Two notification forms were created: one for cases diagnosed antenatally, the other for postnatal diagnoses.

As described in 2008 [9], patient data are reported, such as estimated gestational age at the time of maternal infection and age of mother), pregnancy outcome (abortion, foetal death or living newborn) and clinical status of the newborn or child (particularly neurological lesions and visual impairments, e.g. chorioretinitis, with localisation).

Demographic data on the distribution of births by region were obtained from the National Institute for Statistics and Economic Studies (INSEE).

Cases diagnosed between 1 January and 31 December 2007 in France and French overseas departments were included in our analysis.

Definitions of prevalence and incidence regions

Overall prevalence of congenital toxoplasmosis is defined as follows: (LB + FD + IA) divided by the total number of live births in France in 2007, where LB is the number of live-born infants with congenital toxoplasmosis, FD is the number of deaths of foetuses with congenital toxoplasmosis (from 20 weeks' gestation) and IA is the number of induced abortions or terminations of pregnancy after prenatal diagnosis of congenital toxoplasmosis (at any gestational age).

The incidence of congenital toxoplasmosis is defined as the number of live-born infants with congenital toxoplasmosis divided by the total number of live births in France in 2007.

Results

Epidemiology

During 2007, 31 laboratories reported at least one congenital toxoplasmosis case through the surveillance system (29 specialised diagnostic laboratories and two laboratories occasionally carrying out diagnosis). A total of 272 cases were notified for 2007: 38 (14%) were notified in antenatally, 74 (27%) in ante- and postnatal periods and 160 (59%) in the neonatal and postnatal periods.

The distribution of congenital toxoplasmosis according to gestational age at the time of maternal infection was variable. Estimates of the age were available for 235 of the 272 cases: 17 (7%) occurred after maternal infection in first trimester (0–12 amenorrhea weeks) of pregnancy, 83 (35%) after maternal infection in the second trimester (13–26 amenorrhea weeks) and 135 (58%) after maternal infection in the third trimester (27–40 amenorrhea weeks) (Figure 1). For 37 cases, the date of the infection was not determined, due to lack of information about previous serological examinations.

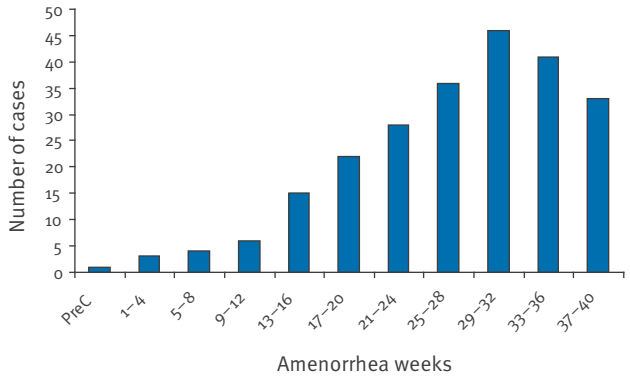
The prevalence of the disease varied according to the mothers' age at delivery: it was highest in young women aged under 20 years (Figure 2).

Geographical distribution of cases was variable, with a higher prevalence observed in the north-east and south-west of France (Figure 3). Additionally, nine cases were reported from Cayenne (French Guiana), one from Martinique and one from Réunion, but none from Guadeloupe.

As there were 818,700 live births in France and French overseas departments in 2007, the overall prevalence of congenital toxoplasmosis observed was 3.3 (95% CI:

Figure 1

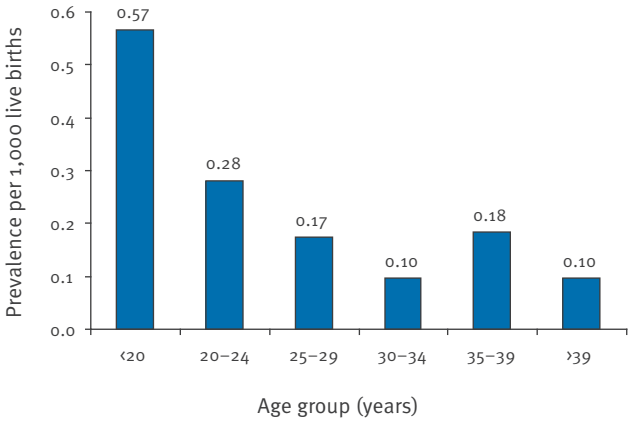
Congenital toxoplasmosis cases by gestational age at maternal infection expressed in amenorrhea weeks, France, 2007 (n=235)



PreC: preconception.

Figure 2

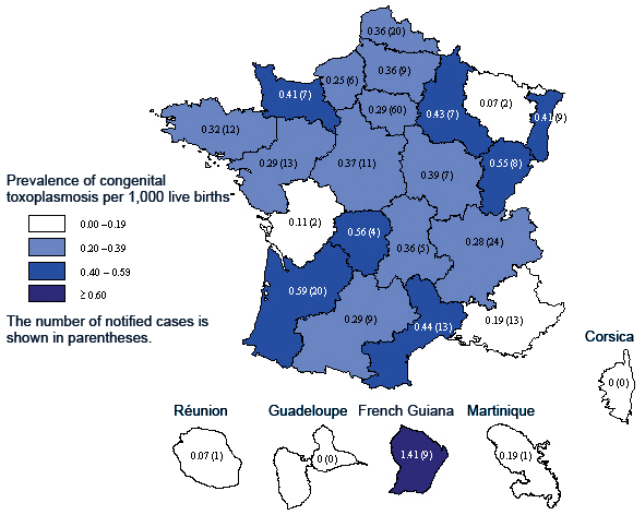
Prevalence of congenital toxoplasmosis per 1,000 live births by maternal age at delivery, France, 2007



Source: National Institute for Statistics and Economic Studies (INSEE), 2007.

Figure 3

Regional distribution of congenital toxoplasmosis prevalence per 1,000 live births, France, 2007 (N= 272)



2.9 to 3.7) per 10,000 live births and the incidence at birth was 2.9 (95% CI: 2.5 to 3.2) per 10,000 live births.

The distribution of the disease according to the month of the children's birth showed no seasonality in disease transmission (data not shown).

Practices in antenatal and postnatal diagnosis

Antenatal diagnosis: amniocentesis was performed in 112 cases: 108 were positive. The median delay between estimated date of maternal infection and amniocentesis was six weeks (range: 1–17). Half of the tests were carried out between weeks 5 and 8 of pregnancy.

Ultrasound examinations were carried out for 82 pregnant women with antenatal diagnosis (73%), among these a majority were performed during the second trimester of pregnancy. The examinations were abnormal in 13 of the women, with severe lesions observed for four cases. Magnetic resonance imaging was performed in 28 of the women.

Postnatal diagnosis: infection was diagnosed postnatally in 160 children (59%). Of these, 130 (81%) were diagnosed before the age of two months; 22 (14%) were diagnosed between the age of two months and one year.

Clinical outcomes

A total of 11 (4%) terminations of pregnancy were reported: six abortions were performed for medical reasons (cerebral lesions were detected by ultrasound examination in four cases) and there were five foetal deaths (due to cerebral lesions in four cases). In six cases, maternal infection was acquired in first trimester and in five cases, in the second trimester. No pregnancies were terminated following late maternal infection.

For 27 foetuses diagnosed in the antenatal period, the clinical outcome was unknown (Figure 4). Among the 234 live-born infants, the male–female sex ratio was 0.92. Among live-born infants, 206 (87%) were asymptomatic and 28 (13%) symptomatic: the incidence of symptomatic congenital toxoplasmosis was estimated to be 0.34 cases (95% CI: 0.2 to 0.5) per 10,000 live births. Among symptomatic children, 21 had moderate lesions (intracranial calcifications and/or peripherical chorioretinitis) and seven (3%) had a severe form of the disease (three with hydrocephalus and four with macular chorioretinitis) (Figure 4).

Discussion

The observed prevalence of toxoplasmosis has decreased by nearly 20% from 1995 to 2003 in France [2]. Previously, only retrospective or small-scale studies on prevalence were attempted in very few hospital centres in the country. We concluded that a laboratory-based surveillance system was the most adapted for surveillance of congenital toxoplasmosis, because

laboratories carry out the diagnosis of infection in mothers and then in foetuses or newborns [9].

The number of congenital toxoplasmosis cases observed in France in 2007 is considerably lower than that estimated in the 2007 report of the French Food Safety Agency (272 versus 600). This is probably because the French Food Safety Agency used the estimated number of seroconversions during pregnancy from the 1995 national perinatal survey [4]. The calculated incidence rates of congenital toxoplasmosis in 2007 are also lower than the reported rates of congenital infection during the 1980s and 1990s. The incidence rates for these decades were certainly overestimated because they were derived from mathematical models considering using and estimated incidence rates. The number of cases directly observed in 2007 is probably more robust and reliable than that of 1995 due to the exhaustive process adopted for notification (all laboratories carrying out the diagnosis in France were invited to participate in the surveillance).

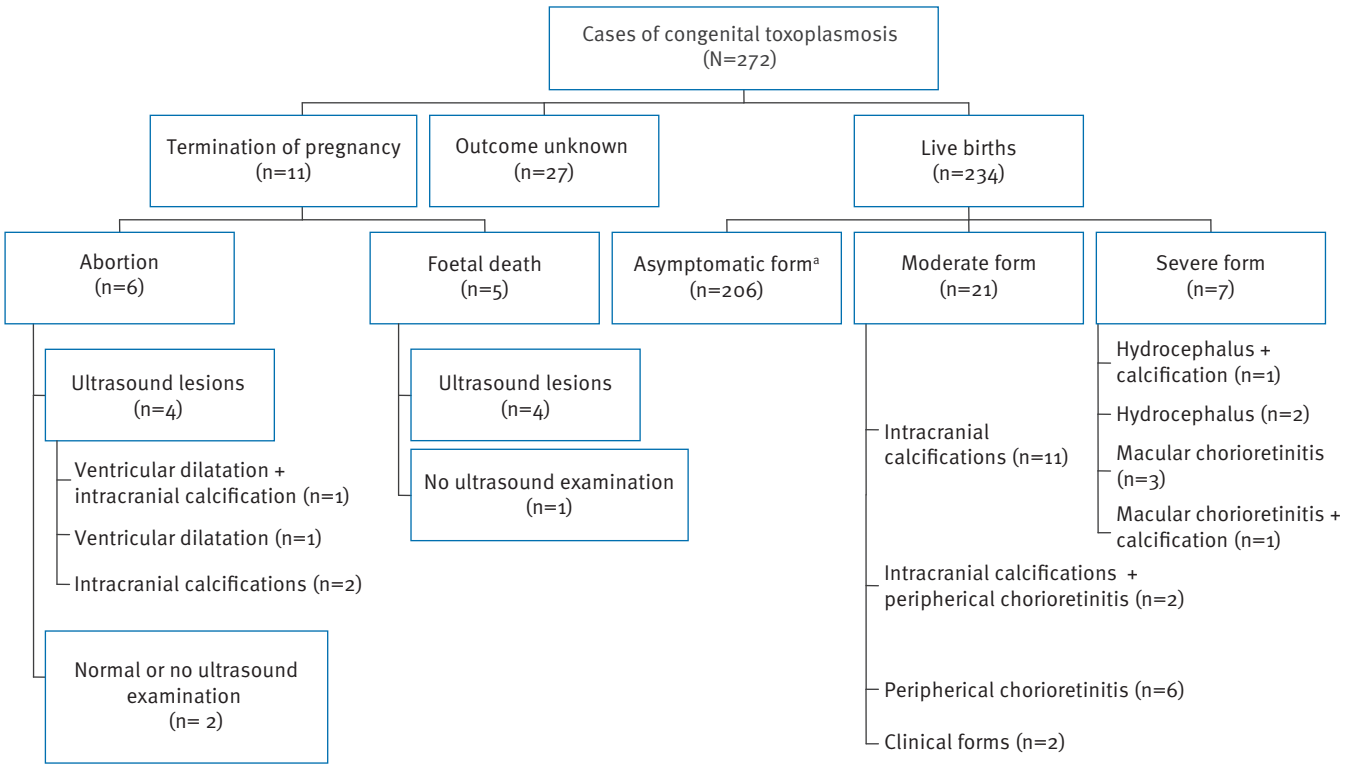
The incidence of congenital toxoplasmosis observed in France in 2007 was 2.9 (95% CI: 2.5 to 3.2) per 10,000 live births. Incidence data from other countries are very scarce and often calculated on regional data collected before 2000. The prevalence of congenital toxoplasmosis observed in France in 2007 is in the same range as incidences per 10,000 births reported in other European countries, e.g. in Poland (5.5, 95% CI: 0.2 to 29, per 10,000 live births) [11], Denmark (2.1 per 10,000 live births) [12] and Switzerland (4.3 per 10,000 live births) [13], but is much higher than that reported in Sweden (0.73, 95% CI: 0.2 to 2.1, per 10,000 live births) [14].

The incidence rate of symptomatic congenital toxoplasmosis was estimated to be 0.34 (95% CI: 0.2 to 0.5) cases per 10,000 live births in France in 2007. Although the overall seroprevalence of toxoplasmosis is much lower in England, the incidence rate of symptomatic congenital toxoplasmosis observed in a study in England and Ireland between 2002 and 2004 (0.16, 95% CI: 0.08 to 0.28 per 10,000 live births) [15] is comparable to that of France.

We observed large variations in the prevalence of the disease between geographical regions. A similar geographical distribution was observed for the seroprevalence reported in pregnant women in 2003 [2]. Only 11 (4%) of the cases were notified from French overseas departments. These regional disparities could reflect the transmission of *Toxoplasma* in the population and could be linked to climatic factors and the eating habits of women (e.g. eating raw meat). Similar climatic or eating habit disparities at regional level are observed in different European countries. However, most do not have a congenital toxoplasmosis screening programme because of lack of evidence of its cost-effectiveness. Data published from these countries concern only symptomatic forms of the disease and are thus not exhaustive.

Figure 4

Outcomes of congenital toxoplasmosis, France, 2007 (N= 272)



[a] One infected child dead at birth without clinical signs of congenital toxoplasmosis.

The distribution of congenital toxoplasmosis according to gestational age at the time of maternal infection was variable, with the majority of disease occurring when maternal infection occurred during the last trimester of pregnancy. This is in accordance with foetal transmission rates previously reported in large cohorts [5]. Congenital toxoplasmosis appears to be more frequent in children from younger mothers, especially those under 20 years. This is probably associated with the fact that young women are generally less informed about the risks of congenital toxoplasmosis in pregnancy and thus adhere less well to the antenatal screening programme and to the recommendations for avoiding contamination [16]. As cases occurred regularly during the year, there was no seasonality in transmission of congenital infection.

Some 60% of congenital cases diagnosed in 2007 were diagnosed at birth, with the majority diagnosed before the age of two months, as a result of serological examinations of newborns and babies. Sometimes diagnosis occurred late (up to the age of one year). Immunological tests must be performed regularly until definitive disappearance of maternal antibodies enables confirmation of the absence of infection.

In terms of clinical outcome, pregnancies were terminated in 11 (4%) of cases, principally because of foetal cerebral lesions. Prenatal prevention programmes can detect severe forms of congenital infection, usually by ultrasound examination. Magnetic resonance imaging is used less frequently as a first-line diagnostic tool, being usually reserved for confirmatory diagnosis. When severe lesions are diagnosed, abortions for medical reasons are recommended. This prenatal prevention policy could explain why at birth, the majority of congenital infections were asymptomatic. Only seven severe forms were observed in 2007.

Another possible explanation for the low rate of severe forms is that most maternal infections occurred during the second and third trimester of pregnancy. Infection at these stages has been shown to result in a less severe clinical presentation [5,17] It is interesting to note that the severe form leading to foetal death was observed in cases of early maternal infection, as is often reported in literature [7,8,18]. The severe forms are hydrocephalus (three cases in newborns) and macular chorioretinitis (four cases in newborns): they represented only 0.1 per 10,000 live births in France in 2007. At birth, intracranial calcifications were observed in half of symptomatic cases but without clinical consequences, while chorioretinitis appeared to be less frequent. However, ocular lesions are the major complication of congenital toxoplasmosis leading to visual impairment in long-term follow-up [8]. Eye examinations at birth only partially estimate the burden of congenital toxoplasmosis, as new lesions may be observed during the first two years of life (when the children are at high risk of developing new lesions [8]) or at any time later in life [19,20].

We observed only 28 symptomatic forms of infection at birth (12% of cases) in 2007 in France. Studies have shown that in the absence of prenatal screening and antenatal treatment, the frequency of chorioretinitis and cerebral lesions was higher [21]. In the European Multicentre Study on Congenital Toxoplasmosis (EMSCOT) study [18], 20% of infants had one or more clinical manifestations. In some European countries, however, there are few data on clinical manifestations of congenital toxoplasmosis as there are no screening programmes in place. In Denmark, where neonatal screening was performed, 12 of 47 infected children (25.5%) had clinical signs at birth [7].

In our study, 88% of infected infants were asymptomatic at birth – a figure higher than other published studies, with 81% asymptomatic in the SYROCOT study (a systematic review of congenital toxoplasmosis) [21]. These figures could suggest a positive impact of prenatal screening. Treatment may also have an impact on these figures. In France, spiramycin is prescribed when seroconversion occurs in pregnant women. All such women were treated with this antibiotic, although its impact on vertical transmission is still controversial. When amniocentesis is positive, spiramycin treatment is stopped and a pyrimethamine–sulfonamide combination is generally prescribed until delivery. This antibiotic combination is considered to be effective in reducing the risk of severe congenital sequelae.

The surveillance system in France only detects lesions evident at birth. The true burden of congenital toxoplasmosis should be evaluated by long-term follow-up of cases, as congenitally infected newborns that are asymptomatic at birth are at risk of developing ocular lesions during childhood and adolescence, leading to visual impairment [18]. However, long-term case follow-up is not the objective of this surveillance programme.

Systems for the surveillance of congenital toxoplasmosis in European countries are very variable and are principally dependent on prevalence rate. A recent investigation aimed to describe these different systems in Europe [22]. The results showed that, of the 28 countries investigated, only four had a specific surveillance system for congenital toxoplasmosis: in addition to France, the others were Denmark, with a programme based on neonatal Guthrie card adapted for testing for *Toxoplasma*-specific IgM (which was discontinued in July 2007), Germany, where cases have been notifiable since 2001, and Italy, with surveillance of live newborns (but confined to a regional programme in the Campania region since 1997).

Conclusions and public health perspectives

The surveillance system for congenital toxoplasmosis in France appears to be effective and, for the first time, provides reliable data. Surveillance needs to continue for several years in order to assess the overall prevalence of the disease and to follow its trend. Toxoplasmosis seroprevalence among women

of childbearing age is regularly estimated in France through national perinatal surveys based on cross-sectional surveys of births at a national level during a given week. With these two indicators, it will be possible to perform economic analyses – as carried out by Ancelle *et al*. in a recent study [23] – for the development of different screening strategies. Surveillance of congenital toxoplasmosis is an indispensable tool to assess the efficiency of new screening strategies that could be implemented in France in the future.

Members of the National Reference Centre for Toxoplasmosis and Toxosurv network in alphabetical order:
A Totet (Hospital and University Centre Amiens), B Cimon (Hospital and University Centre Angers); E Scherrer (Hospital and University Centre Besançon); B Couprie (Hospital and University Centre Bordeaux); G Nevez and D Quinio (Hospital and University Centre Brest); C Duhamel (Hospital and University Centre Caen), B Carme (Hospital and University Centre Cayenne); A Bonnin, B Cuisenier and F Dalle (Hospital and University Centre Dijon); MP Brenier-Pinchart, H Fricker-Hidalgo, H Pelloux (Hospital and University Centre Grenoble); S Azia (Hospital and University Centre Guadeloupe); A. Morel (Hospital Centre Le Havre); L Delhaes (Hospital and University Centre Lille); D Ajzenberg, M L Dardé (Hospital and University Centre Limoges); M. Wallon (Hospital and University Centre Lyon); J Franck and R Piarroux (Hospital and University Centre Marseille); N Desbois (Hospital and University Centre Martinique); P Bastien, F Pratlong (Hospital and University Centre Montpellier), M Machouart (Hospital and University Centre Nancy); F Gay-Andrieu (Hospital and University Centre Nantes); N Ferret, P Marty (Hospital and University Centre Nice); S Houze (Hospital and University Centre Paris Bichat), T Ancelle, H Yera (Hospital and University Centre Paris Cochin), F Derouin, J F Garin, J Menotti (Hospital and University Centre Paris St Louis); S Brun, L Paris (Hospital and University Centre Paris Salpetrière); N Godineau (Hospital and University Centre Paris St Denis); P Roux (Hospital and University Centre Paris St Antoine); M H Rodier (Hospital and University Centre Poitiers); D Aubert, C Chemla, I Villena (Hospital and University Centre Reims); F Gangneux (Hospital and University Centre Rennes); L Favennec (Hospital and University Centre Rouen); P Flori (Hospital and University Centre St Etienne); E Candolfi, D Filisetti and O Villard (Hospital and University Centre Strasbourg); MH Bessières and S Cassaing (Hospital and University Centre Toulouse); TH Duong (Hospital and University Centre Tours) and JM Costa (Laboratory Cerba, Paris), G. Denoyel (Laboratory Biomnis, Lyon).

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2009 pandemic influenza A(H1N1) outbreak in a complex of schools in Paris, France, June 2009

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An outbreak of 2009 pandemic influenza A(H1N1), involving 81 cases with symptoms of influenza-like illness, was confirmed in June 2009 in a complex of schools in Paris, France. At that time, there was no community transmission in France. The index case, a 10-year-old girl, had travelled to the United Kingdom with her school class. Of the 81 symptomatic cases, 35 were confirmed and 46 were probable; 48 of the cases were female. Three were adults and 78 were children (median age of the children was 7.9 years, range: 6 months to 12 years). Control measures were implemented as soon as a new case was confirmed in a school, which included active case finding among the pupils in the same class as the index case, setting up a dedicated influenza outpatient clinic that families were recommended to consult if necessary, prophylactic treatment of contacts and school closure. A retrospective study was conducted on all confirmed cases and all symptomatic cases who had consulted the dedicated outpatient clinic from 17 to 27 June 2009. Further work is needed to better define conditions under which the pandemic virus can be transmitted in schools and in households.

Background

In response to the appearance of the 2009 pandemic influenza A(H1N1) virus first detected in Mexico and the United States in April 2009 [1], France developed an active surveillance system for influenza-like illness [2-4]. Up to 8 July 2009, surveillance was aimed at preventing the introduction and community spread of the pandemic virus in France and was based on the identification of all possible cases among recent travellers coming from affected areas [4].

On 19 June 2009, pandemic influenza was confirmed in a 10-year-old girl, who attended a primary school in Paris, France. At that time, there was no community transmission in France. The girl had travelled to the United Kingdom (UK) with her class (n=30) and three accompanying adults, returning to Paris on 12 June. She developed influenza-like symptoms on 17 June and was hospitalised the following day, for medical supervision and in order for samples to be taken, according to the recommended procedure at that time in the country [3].

Following the girl's positive test for the pandemic influenza virus on 19 June, local health authorities were alerted and began to contact families of the other children in her class in order to assess their health and organise control measures, such as chemoprophylaxis. Between Friday 19 June and Sunday 21 June 2009, families of 27 of the 30 pupils were contacted by telephone. Eight children had developed influenza-like symptoms (two of them had already recovered). The Necker Hospital for Sick Children, located close to the school, set up a dedicated influenza outpatient clinic [5].

Setting

This pandemic influenza outbreak affected several schools. It began first in the primary school that the index case attended (School A, with 360 children aged between six and 11 years in 13 classes) and then spread to a nursery school (School B, with 253 children aged three to six years), to a day care school (School C, children aged three months to three years, total number of children unknown) and to another primary school in the neighbourhood (School D, 293 children aged six to 11 years). Siblings in the same family attended different schools, according to their age.

The children shared common spaces: children in School B shared the main entrance and other facilities (such as the canteen) with School A, a gym in School D was open to the children of School A. A playground in

a square close to all the schools was used by most of the children.

The children in the class that travelled to the UK (the index class) were aged 10–11 years. At the beginning of the outbreak, close contacts (eligible for antiviral chemoprophylaxis) were identified as the family and classmates of the index case, the adults accompanying the children to the UK and the families of probable cases. Later, as the outbreak affected other classes and schools, all the pupils in the four schools and the families of pupils with symptoms were considered to be close contacts.

In collaboration with the director of the school, the local health authorities sent information to the families of all the pupils in the school, recommending them to attend the dedicated outpatient clinic, for case management and chemoprophylaxis of contacts (all the pupils in the schools and the families of symptomatic pupils were considered close contacts at that point). Following the recommendations of the public health authorities, the primary school (School A) and a nursery school (School B) were closed by the city council from 22 to 29 June 2009.

A retrospective descriptive study was conducted on all confirmed cases and all probable cases that consulted the influenza outpatient clinic from 17 to 27 June 2009. This paper describes the epidemiological characteristics of and public health responses to this outbreak.

Methods

Case definitions

The following case definitions were used [3].

- A possible case of pandemic influenza virus infection was defined as a person with fever (≥38 °C) or asthenia or myalgia and at least one acute respiratory symptom (cough or dyspnoea) or diagnosis of influenza-like syndrome and a medical history of curative treatment (with oseltamivir for five days) for influenza.
- A probable case was defined as a person with a history of close contact with a confirmed case during the period of possible viral excretion (from 24 hours before to seven days after the onset of symptoms).

When more than one person in a school was a probable or confirmed case, all possible cases attending that school were classified as probable.

- A confirmed case was defined as a person in whom infection with the pandemic virus confirmed by real-time polymerase chain reaction (PCR).

Information about the cases (demographic details and potential exposure to the pandemic virus) was obtained by telephoning the parents or from hospital medical records. Information about the classes and schools (e.g. how the classes were distributed, the size of the school and their playgrounds and entrances) and the neighbourhood (e.g. common spaces) was obtained by telephoning the directors of the schools.

The study population consisted of children from all four schools and their close contacts.

Results

Outbreak description

The investigation team identified a total of 81 symptomatic cases (35 confirmed and 46 probable) between 17 and 27 June 2009 (Figure 1).

Nasopharyngeal swabs were taken for 44 (54%) of the symptomatic cases: the pandemic virus was detected by PCR in 35 (80%) of the samples, nine were negative. Those that were negative were classed as probable cases. The distribution of confirmed and symptomatic cases by school is shown in Table 1.

Of the symptomatic cases, 48 (59%) were female; three were adults and 78 were children. The mean age of the children was 7.5 years (standard deviation (SD): 3.1; median: 7.9; range: six months to 12 years).

All confirmed cases were children: their mean age was 8.4 years (SD: 2.8). Of these, 26 (74%) were girls. The age range for the girls was from 1 to 11 years and for boys from 4 to 11 years.

There were 11 symptomatic cases in the index class (eight confirmed and three probable): the first (a confirmed case) developed symptoms on 17 June 2009, five days after returning from the UK, where the pandemic virus was already circulating in the community. Ten classmates of the index case developed symptoms, four on 18 June and six more between 19 and 22 June (Figure 2).

In the rest of School A, there were 29 symptomatic cases, of which 18 were confirmed cases. The outbreak started on 17 June (the day the index case developed symptoms), with symptoms developing in two other confirmed cases. These cases were in the same class, which was different from the index class. The infection then spread to 10 other classes; the peak number of cases developed symptoms on 22 June. The number of cases then decreased.

The first case at School B developed symptoms on 18 June. The number of cases increased substantially from 21 June; the peak was seen on 23 June.

At School C there were four cases: the first became symptomatic on 19 June. At school D there were three cases: the first developed symptoms on 20 June.

We identified 13 family clusters (more than one person affected in the same family). Of the family members affected, 15 did not attend school or attended other schools. The first case developed symptoms on 21

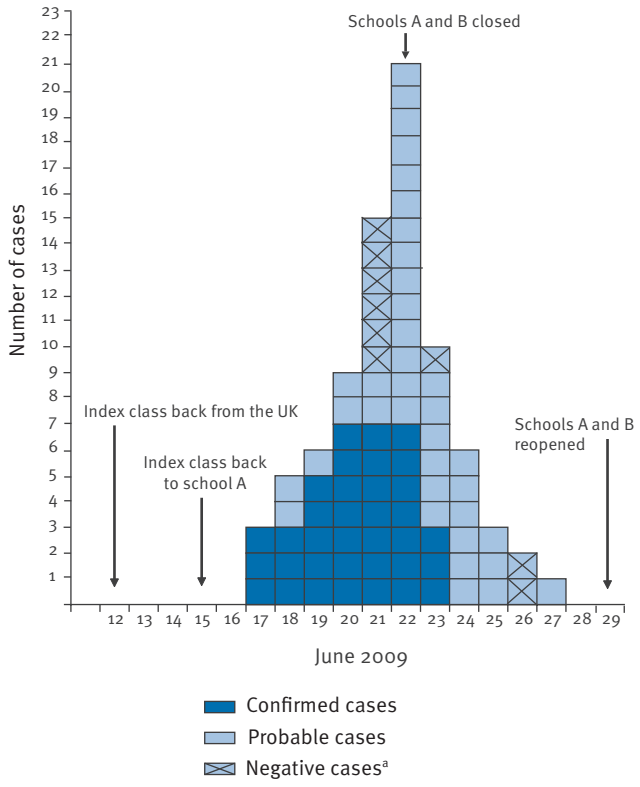
June; the peak number of cases developed symptoms on 22 June (Figure 2).

In five of the 13 family clusters, two or more affected children in the same family attended one or more of the affected schools.

- In cluster 1, three members of the same family were affected: two sisters attended School B: one developed symptoms on 18 June; her sister and father developed symptoms on 21 June.
- In clusters 2 and 5, all children attended School A and developed symptoms within a two-day interval starting 19 and 20 June.
- In cluster 3, the first child attended School A and became symptomatic on 19 June; two days later his sister in School C developed symptoms.
- In cluster 4, the first child attended School C and developed symptoms on 19 June. Her sister, who attended school D, developed symptoms the following day.
- In clusters 6-13, only the index case in the family attended an affected school. Other family members developed symptoms between zero and eight days after symptom onset in the index case.

Figure 1

Symptomatic cases of 2009 pandemic influenza A(H1N1) in Schools A–D and in family and friends by date of symptom onset, Paris, France, June 2009 (N=81)



UK: United Kingdom.

[a] Symptomatic cases whose polymerase chain reaction (PCR) test was negative (as they had a history of close contact with a confirmed case during the period of possible viral excretion, they were included as probable by definition).

Some affected children neither attended an affected school nor had siblings attending any affected school; however, they had had contact with a confirmed or probable case attending one of the affected schools. These cases were identified from the hospital's medical records.

Attack rates

Including all symptomatic (confirmed and probable) cases attending any of the affected schools (n=66), the attack rate was 37% for the index class and was 30% overall in the three classes in the same school and year group as the index class. The attack rate was 10% in School A, 7% in School B and 1% in School D (Table 1).

Including only confirmed cases (n=35), the attack rate was 27% for the index class, 20% in the three classes of the same school and school year as the index class, 7% in School A, 2% in School B and 0.3% in School D.

Clinical epidemiology

The reported symptoms of the confirmed and probable cases (n=81) were fever (n=78), cough (n=50), asthenia (n=23), headache (n=28), rhinorrhoea (n=18), sore throat (n=15), abdominal pain (n=5) and vomiting (n=3) (Table 2).

Symptoms were similar in confirmed and probable cases. All cases who tested negative by PCR had fever; seven of them also had a cough. Negative cases were tested a median of one day (range: zero to five days) after the onset of symptoms.

Seven children were hospitalised but recovered without complications.

One child received oseltamivir prophylatically for two days; however, he developed symptoms after the second day and swabs were taken. After testing positive for the pandemic virus, the child was then prescribed curative treatment.

Public health response

The local health authorities recommended that the families attend the Necker Hospital for Sick Children for examination and test and/or treatment (prophylactic or curative) if needed (if they developed symptoms or were in contact with a confirmed case).

A specific mobile paediatric emergency response team worked in a tent in front of the emergency department of the hospital in order to care for potentially infected children. Two examination rooms, a waiting room and medical equipment were installed in three hours. This outpatient clinic was open 24 hours a day, staffed by additional personnel who usually worked in the emergency department. All children and families arriving at the emergency department were evaluated by a nurse. Anyone with symptoms resembling those of influenza was taken straightaway to the tent once they had put

on a mask. A similar model has recently been described in Houston, Texas, in the United States [5].

Asymptomatic close contacts were advised to adhere to isolation measures (i.e. remain at home and avoid contact with others) until they had taken the second prophylactic dose of oseltamivir.

Schools A and B were closed for five days from 22 to 29 June 2009. A school party planned for Saturday 20 June was cancelled by the City Council of Paris; an information meeting for parents was held that Saturday morning in the school.

Staff of the local health authorities were present at the reopening of the schools on 29 June in order to answer parents' questions.

Discussion

In this report we describe an outbreak of the 2009 pandemic influenza (N=81) involving four schools in the same neighbourhood of Paris, France, which arose following the visit of one school class (in School A) to the UK. Virus transmission occurred in the school, in their families and to the other three schools. Provision of information to the families, the setting up of a mobile paediatric emergency team, mass antiviral prophylaxis and school closure were the main public health responses.

The fact that the peak of the outbreak in the rest of School A (on 22 June 2009) was reached four days after the peak in the index class suggests that a large number of the cases in the school were secondary cases resulting from person-to-person transmission within the school or their families. The peak of the outbreak in family cases was concomitant to the peak of the outbreak in all cases.

Cases started in two classes of School A at the same time; however, the infection spread more quickly in the index class. As shown in Figure 2, there was a lag in the distribution of the cases in the rest of the school and another lag for cases in the nursery (School B) and in the affected families.

Transmission of the virus to the other three schools occurred through infected pupils who were siblings of affected pupils in School A. In School B, the proximity of the two buildings and the sharing of facilities could also have helped transmission by increasing direct contact between pupils from both schools.

The source of the outbreak was assumed to be the index case, a 10-year-old girl, who had returned from a country with sustained human-to-human transmission of the pandemic virus five days before symptom onset. This case could have had a long incubation period and then spread the virus to other pupils in the school, mainly those in her class. This hypothesis is supported by the length of the incubation period of the pandemic influenza, which was estimated to be between one and seven days [6] and also by the fact that children might shed virus several days before illness onset, and that

Table 1

Distribution and attack rates for confirmed (n=35) and all symptomatic[a] (n=66) 2009 pandemic influenza A(H1N1) cases in Schools A–D , Paris, France, June 2009

School	Number of pupils	Confirmed cases		All symptomatic cases[a]	
		Number	Attack rate (%)	Number	Attack rate (%)
A					
Year group (age in years)					
6–7	70	5	7	6	10
7–8	77	4	5	8	10
8–9	79	1	1	1	1
9–10	61	1	2	3	5
10–11: all pupils	73	15	21	22	30
10–11: index class[b]	30	8	27	11	37
Total	360	26	7	40	11
B					
Year group (age in years)					
3–4	76	1	1	6	8
4–5	93	3	3	9	10
5–6	84	2	2	4	5
Total	253	6	2	19	8
C	No data	2	–	4	–
D	293	1	0.3	3	1
Total	–	**35**	–	**66**	–

[a] Confirmed and probable cases.

[b] The only class that travelled to the United Kingdom.

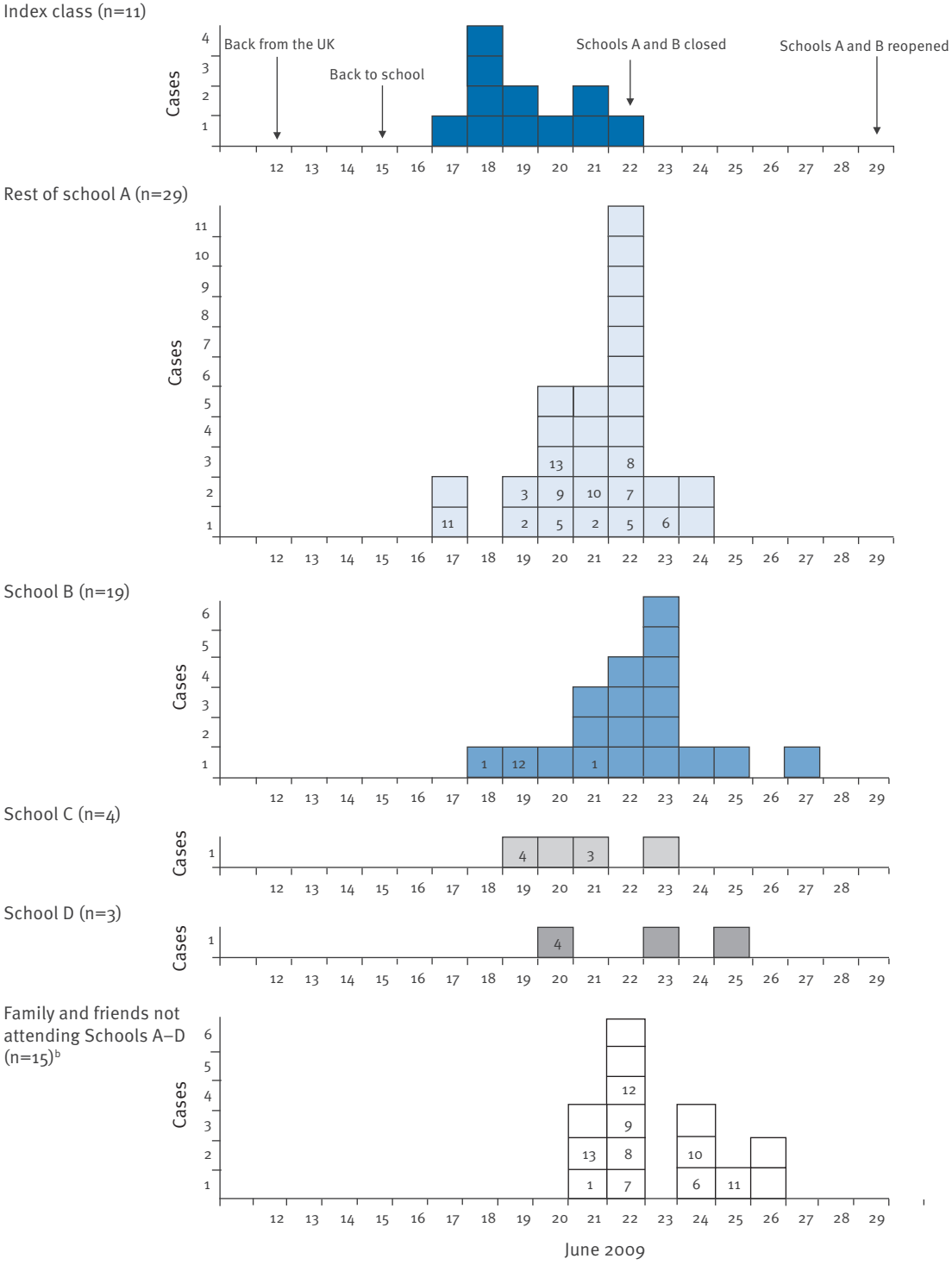
children can be infectious for 10 days or more after onset of symptoms [6].

The lag between last potential exposure in UK and the peak in the index class suggests that the whole index class was not infected in the UK. As the typical incubation period for influenza is one to four days (mean: two days), a mean incubation of six days for the whole index class is unlikely [6].

Two other confirmed cases in a different class (in the same year group) of School A also developed symptoms

Figure 2

Symptomatic cases of 2009 pandemic influenza A(H1N1) in Schools A–D by date of symptom onset and school, Paris, France, June 2009 (N=81)[a]



UK: United Kingdom.

[a] Numbers in boxes refer to cases in the same cluster of family and friends not attending Schools A–D.

[b] Blank cells in this curve represent cases who neither attended an affected school nor had siblings attending any affected school.

on 17 June 2009 (Figure 2, data on classes not shown). Therefore, exposure to a non-identified case at some point between 12 and 17 June cannot be excluded.

In fact, the teacher of the index class, who also travelled to the UK, presented general symptoms (fever and myalgia) from 16 to 18 June. However, as she presented no respiratory symptoms she did not meet the definition of possible case. She could have infected some pupils in the index class in the coach coming back from the UK or when she was back at the school on 15 June, 24 hours before the onset of her symptoms. She gave her classes on 18 and 19 June.

A party at School A on Friday 19 June, which the index class and other classes attended, and a party in the local parish church on Sunday 21 June could also have contributed to dissemination of the virus.

Family contacts probably played a role in the transmission. Several cases were siblings, so we could hypothesize transmission at home followed by the reintroduction of the virus by these secondary family cases into other classes of the school and to other schools.

In the family clusters in which there were affected siblings who did not attend any of the four schools involved in the outbreak, household transmission is the most likely explanation. However, transmission may have occurred outside the family (e.g. in playgrounds and through interfamily activities). Indeed, in order to understand the spread of the virus in this outbreak, it is important to note the intense social life in this neighbourhood. There were many activities between the families of children in the different schools. In addition, there were two after-school centres: one in one school and the other in the parish church (schools in France are closed on Wednesdays, so children attend outdoor pursuits centres). There was also a park just in front of the schools where children from the four schools and other children from the neighbourhood played.

Antiviral prophylactic treatment of contacts and school closure may have contributed to the rapid decrease in the number of cases after the weekend (20–22 June). However, we cannot exclude the possibility that some symptomatic cases may have not visited the Necker Hospital for Sick Children and could therefore have been missed.

The symptoms recorded for cases were limited by the case definition, which was not very sensitive: a patient needed only general and respiratory symptoms to be classified as a possible case. One child presented only fever, and was therefore not considered to be a case, according to our definition. However, he was tested at the outpatient clinic and turned out to be positive. The definitions used may not have been appropriate as the clinical presentation of this new virus was not well known at the beginning of the outbreak [7].

It is evident from previous reports (and unpublished data) that schools are important in transmission of the pandemic virus and that outbreaks in schools occur frequently [8]. Since the start of the 2009 pandemic, several school outbreaks have been reported around the world [7-11] and a notable proportion of household transmission has been attributed to children [12].

Previous studies suggest that the majority of contacts in school-age children are with their peers [13]. This could explain why attack rates in the year group 10–11 (which included the index class) were higher than in other year groups in the school (School A).

Conclusion

Up to early July 2009, surveillance of pandemic influenza cases in France was based on the identification of all possible cases in order to implement control

Table 2

Symptoms reported by the confirmed (n=35), negative[a] (n=6) and all symptomatic[b] (N=81) cases of 2009 pandemic influenza A(H1N1) in Schools A–D and in family and friends, Paris, June 2009

Symptoms	Confirmed cases n=35		Negative cases[a] n=9		All symptomatic cases[b] N=81	
	Number	(%)	Number	(%)	Number	(%)
Fever	34	97	9	100	78	96
Cough	23	66	7	78	50	62
Asthenia	17	49	1	11	23	28
Headache	16	46	2	22	28	35
Rhinorrhoea	10	29	1	11	18	22
Sore throat	6	17	2	22	15	19
Abdominal pain	5	14	0	0	5	6
Vomiting	2	6	1	11	3	4

[a] Classed as probable cases.

[b] Confirmed and probable cases.

measures around each of them, aimed at delaying the spread of the virus.

In this outbreak, nasopharyngeal swabs were taken from the first 44 cases. The large number of cases in this outbreak led to the adjustment of case management and to restrict biological confirmation. Every other new symptomatic case that had been in contact with a probable or confirmed case was assumed to have pandemic influenza. The global dissemination of the virus and the start of community transmission in France led to a shift to population-based surveillance [4,7]. Indications for sampling of possible cases were restricted to three cases in each suspected pandemic influenza cluster.

During the outbreak, decisions had to be made without delay and had to be adapted according to new information available and changes in management protocols. In this context, good communication and cooperation among the different people involved (healthcare authorities, the city council, clinicians, staff from schools, parents and children) were of major importance.

This epidemic shows the transmission of the pandemic virus in a school setting and in households. The measures established appeared to have stopped the transmission. The absence of transmission in the community at that time in France justified the measures taken.

Further work is needed to better define conditions under which the pandemic virus may transmit in a school setting and in households [12,13].

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European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2010 – call for abstracts

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This year's European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) will take place in Lisbon, Portugal, from 11 to 13 November 2010.

Besides sharing scientific knowledge, the conference aims to provide a forum for strengthening networks of professionals involved in applied infectious disease epidemiology. It also aims to offer a dedicated platform for the European Programme for Intervention Epidemiology Training (EPIET), and Field Epidemiology Training Programme (FETP) fellows to present their work.

The call for abstracts for the conference is now open, and abstracts can be submitted via the dedicated 'call for abstracts' portal on the ESCAIDE website (http://www.escaide.eu/). The closing date for submissions is 12 July 2010.

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