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An outbreak of viral meningitis associated with a public swimming pond

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SUMMARY

From July to October 2001, 215 cases of aseptic meningitis occurred among the inhabitants of the German city of Kassel and neighbouring counties. A matched case-control study identified bathing in a public, nature-like pond during the beginning of the outbreak as a risk factor for disease [matched odds ratio (mOR) 44·8, 95% confidence interval (CI) 3·9–515·6]. Among bathers, patients with meningitis spent more time in the water (mOR 18·8, 95% CI 2·0–174·1) and swallowed water more frequently (mOR = 7·3, 95% CI 0·7–81·8). Of 30 cerebrospinal fluid samples tested, echovirus 30 was cultured from 16, and echovirus 13 from seven. An echovirus 30 sequence obtained from one pond water sample showed a 99% nucleotide and 100% amino-acid homology with patient isolates. This outbreak demonstrates the potential of nature-like swimming ponds to cause widespread community infection with substantial public health impact.

INTRODUCTION

Although most cases of enterovirus infection are asymptomatic, enteroviruses are the most common cause of 'aseptic' meningitis [1, 2]. Echovirus 30 (E30) and echovirus 13 (E13) have been associated with outbreaks of aseptic meningitis [3–6]. Humans are their only known reservoir, and close human contact appears to be the primary route of spread of infection [7]. Only few enterovirus outbreaks have been traced back to a point source. Echovirus outbreaks associated with exposure to contaminated drinking water [8], water from a swimming pool [9, 10], and contact with a body of water [11] have been reported previously. We report here the results of the investigation of a community outbreak of aseptic meningitis which identified swimming in a public, nature-like pond as a risk factor for disease.

Between 13 and 15 August 2001, approximately 10 children and 15 young adults were reported to the health department of the city of Kassel with clinically suspected aseptic meningitis. All had been admitted to local hospitals. Following these reports an outbreak investigation was initiated to identify the pathogen, describe the outbreak, and identify risk factors and possible control measures.

Kassel is a German city with a population of 195 000 inhabitants. Neighbouring counties are the semi-rural counties of Kassel (246 000 inhabitants) and Göttingen (265 000 inhabitants). During the hot summer of 2001 bathing facilities and lakes were heavily frequented. In Kassel and neighbouring areas

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there are two bathing lakes, several public swimming pools and a swimming pond (pond A).

Pond A, originally built as a chlorinated swimming pool, was converted into a nature-like swimming pond and reopened in 2001. The rebuilt pond has a total water volume of 3680 m³. Water disinfection with oxidizing substances (e.g. chlorine), as required for public swimming pools, cannot be implemented in nature-like ponds. Organic pollutants are supposed to be degraded through natural processes, mainly through re-circulation of pond water through a planted regeneration area. The pond also included a wading area for small children, including toddlers. During the summer holidays, which ended on 5 August 2001, up to an estimated 1500 persons visited the pond each day.

METHODS

Case finding and case definition

To establish the actual number of cases, area hospitals, resident physicians and local health departments of neighbouring counties were contacted and questioned about cases of aseptic meningitis. Clinicians were asked to report additional cases. Persons hospitalized in the city of Kassel between 15 July and 31 October 2001 were considered as cases if they had (1) a lumbar puncture because of clinical meningitis, (2) a negative bacteriological cerebrospinal fluid (CSF) culture, (3) a moderate CSF pleocytosis, and (4) no alternative diagnosis. On 16 and 17 August 2001, a pilot study was conducted for hypothesis generation: 13 patients with aseptic meningitis were interviewed about personal contacts and activities during their incubation period.

Case-control study

To determine risk factors for the outbreak and to help suggest possible control measures a matched case-control study was conducted. We included in the case-control study only cases with aseptic meningitis who fell ill and were reported to the health department between 31 July and 28 August 2001, and were resident in Kassel or neighbouring counties (case persons). Control persons were selected by systematic random sampling from community registries. For every case seven controls were selected ('pair-matching'). Seven controls per case were chosen to (1) increase the statistical power by obtaining four controls per case, assuming a response rate of 60% and (2) reduce the probability that cases will be lost for the matched analysis because of missing controls. To be eligible for inclusion a control had to be the same age ± 1 year, the same sex, and to be resident in the same municipality as the matched case.

Starting on 24 August 2001 self-administered questionnaires were mailed to cases and controls. The questionnaire gathered information about the onset, duration and characteristics of symptoms, demographics, medical care, and exposures to risk factors from 22 July to 24 August 2001. Risk factors investigated included contact with persons suffering from severe headache, fever, vomiting and nausea; visits to childcare centres, schools and holiday camps; bathing in pools and lakes; number of persons living in the same household, and age of the youngest household member. Starting on 18 September 2001 a second questionnaire was sent to non-responders.

Statistical analysis

Statistical analyses were conducted using Epi-Info Version 6.04d (Centers for Disease Control, Atlanta, GA, USA). In the main analysis matched odds ratios (mOR), their associated P values (assessed with the Mantel–Haenszel χ^2 test for matched data) and 95% confidence intervals (CI) were calculated. To determine whether risk factors changed over time, we calculated mOR for three different time intervals. Period I included cases with symptom onset between 31 July and 9 August 2001, period II those with symptom onset between 10 and 19 August 2001, and period III those with symptom onset between 20 and 28 August 2001. These periods were chosen to subdivide the period 31 July to 28 August 2001 into equal parts and still retain a sufficiently high number of cases in each period for statistical analysis.

In a subgroup analysis among controls we determined whether exposure to a risk factor influenced the risk of 'influenza-like/gastrointestinal illness'. A case of 'influenza-like/gastrointestinal illness' was defined as a control who, from 31 July to 28 August 2001, had at least three of the following symptoms: headache, fever, nausea, vomiting, abdominal pain and stiff neck. We calculated relative risks (RR), Mantel–Haenszel χ^2s and 95% CIs.

Environmental investigation

Laboratory reports of the routine investigation of water samples from pond A were reviewed. Samples

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had been taken at weekly intervals from 31 May to 5 September 2001 at three sampling points and tested for bacterial contamination. No water samples collected for routine investigation were available for a retrospective analysis.

On 22 August 2001 water samples from the two swimming lakes and pond A were drawn. Ten-litre samples from three different sampling points from the two lakes and five sampling points from pond A were taken, frozen the same day and further processed as described below.

Laboratory methods

Water samples were thawed at room temperature and then concentrated as follows [12]: 10 l of surface water were mixed with 20 ml of 10% $Al_2(SO_4)_3$, and the pH adjusted to 5·5–5·8 to form a precipitate. When the precipitate had sedimented after 2–12 h, the supernatant was discarded. The sediment was centrifuged at 2000 ***g*** for 30 min, and the pellet resuspended in 10 ml of 0·1 M sodium citrate, pH 4·7. This suspension was shaken for 2 h at room temperature, and left overnight at 4 °C. Afterwards, it was centrifuged at 2000 ***g*** for 30 min, the pellet discarded, and the supernatant centrifuged again at 100 000 ***g*** for 5 h to pellet the viruses. The pellet was resuspended in 1 ml PBS for viral testing.

Virus isolation and enterovirus PCR in two genomic regions (5′-NCR and VP1) was performed on CSF specimens from a convenience sample of 30 patients with aseptic meningitis and all water samples.

Virus isolation was performed by conventional cell culture methods using human rhabdo-myosarcoma cells. Cultures with an enterovirus cytopathic effect were typed by micro-neutralization with the use of WHO pool sera as well as monospecific in-house rabbit antiserum [13].

Enterovirus RNA-positive water samples, four CSF isolates and corresponding original CSF were included in molecular studies. RNA was extracted from the supernatant fluid of infected cell cultures, CSF or concentrated water samples by the spin-column technique (Qiagen GmbH, Hilden, Germany). Primers used for PCR and sequencing are listed in Table 1. cDNA synthesis was performed with MMLV reverse transcriptase at 42 °C for 1 h. Based on the general primer-mediated 5′-NCR-PCR (primers 1 and 2) described by Zoll et al. [14] a nested diagnostic PCR detecting all human enterovirus serotypes (primers 5 and 8) was used.

Table 1. *Primers used for PCR and sequencing, aseptic meningitis outbreak, Germany, 2001*

Primer	Sequence[a]	Position[b]
1	CAAGCACTTCTGTTTCCCCGG	168–191
2	ATTGTCACCATAAGCAGCCA	609–581
5	TACTTCGAGAAACCYAGTA	248–267
8	AACACGGACACCCAAAGTA	566–547
187	ACIGCIGYIGARACIGGNCA	2554–2573
222	CICCIGGIGGIAYRWACAT	2914–2896
10	AGGTGTGTGTTGAACCGACA	2611–2630
11	CTGATGAGTGAGGACAGGTGC	2888–2868
12	CGACACGTGGTCAACTWCCA	2611–2630
13	TGCGTTAGTGGAGGGGAGTC	2888–2869

[a] Sequences are shown 5′ to 3′, using standard IUB nucleotide ambiguity codes (I = deoxyinosine).
[b] Nucleotide sequence coordinates are given relative to the sequence of ECHO 30 Bastianni (GenBank accession no. AJ131523).

Sequence data encoding the capsid protein VP1 (~300 nt) was used to determine the molecular relationship between outbreak strains. The first round of amplification of VP1 was performed as described by Oberste et al. (primers 187 and 222) [15]. For the second PCR round nested primers for specific amplification of E30 and E13 from CSF and water were designed on the basis of sequences of recently isolated viruses in Germany (primers 10 and 11 for E13; primers 12 and 13 for E30). All amplifications were carried out in 35 cycles consisting of 30 s at 94 °C, 30 s at 42 °C, and 45 s at 72 °C. PCR products were analysed by electrophoresis in 1·5% agarose gel with ethidium bromide staining. Both strands of the nested PCR products were sequenced directly, using a dye terminator cycle sequencing kit (PerkinElmer, Rodgau-Jügesheim, Germany) and ABI Prism 377 DNA sequencer (Applied Biosystems, Darmstadt, Germany).

RESULTS

In exploratory interviews 13 patients with aseptic meningitis could be interviewed. Nine (70%) had been bathing in various swimming pools and lakes during their incubation period, four (31%) in pond A. No other common risk factor could be identified. Thus, bathing in a public pool, pond or lake was used as working hypothesis.

Descriptive epidemiology

Between 15 July and 31 October 2001, 215 cases of aseptic meningitis were reported (Fig. 1). The first

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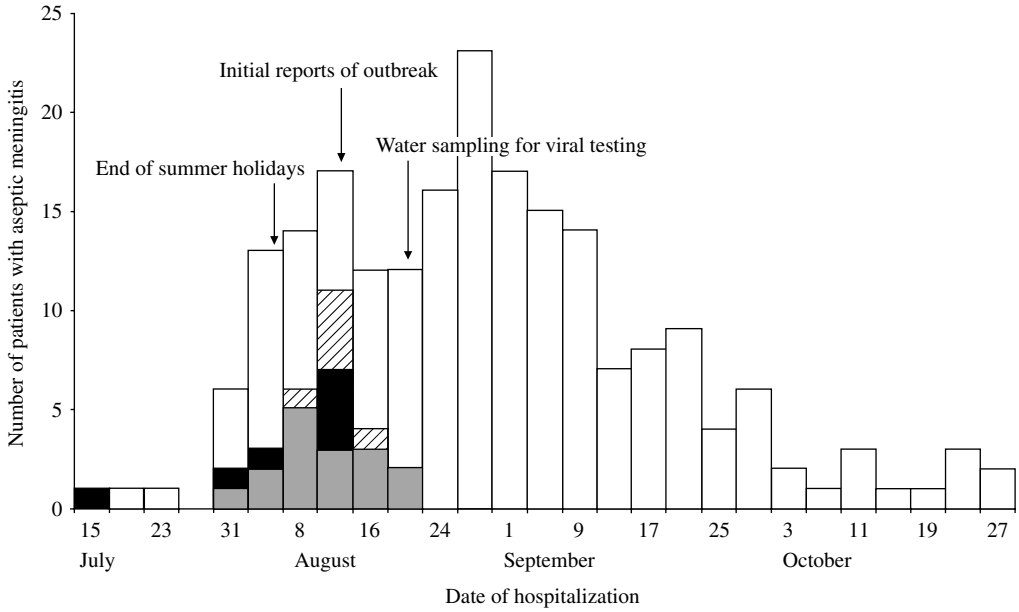

Fig. 1. Number of patients with aseptic meningitis, by date of hospitalization and virological testing, Germany, July–October 2001. ▒, Echo 30 virus ($n=16$); ■, Echo 13 virus ($n=7$); ▨, Viral isolation negative, PCR positive ($n=6$); □, No virological tests ($n=180$).

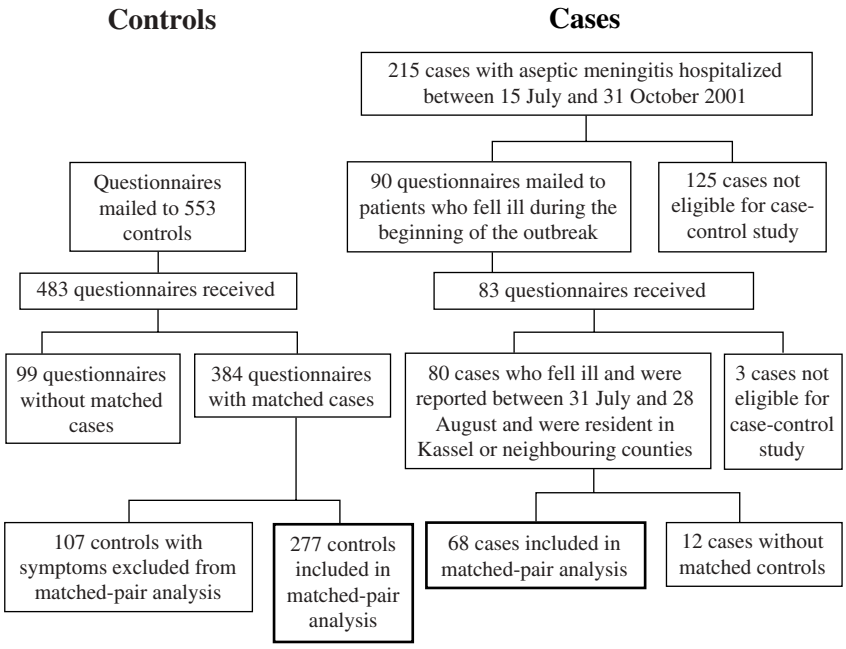

Fig. 2. Flow diagram of the selection of the study population.

patient was hospitalized on 15 July 2001, the last on 28 October 2001. 31 July 2001 was the first day when two cases of aseptic meningitis were hospitalized. The outbreak peaked on 31 August 2001 with 10 admissions. Forty-three per cent (92/215) of cases of aseptic meningitis were resident in Kassel, 56% (121/215) in neighbouring counties. The median age was 10 years (range 3–70 years), 60% (128) were children between 5 and 14 years, and 63% (136) were male. It was not possible to compare the cases of aseptic meningitis in 2001 with previous years as there is no routine reporting system.

Questionnaires were returned from 92% (83) of the 90 contacted patients with aseptic meningitis (Fig. 2). Headache (98%), fever (82%), nausea (81%), stiff neck (80%), vomiting (78%), and ocular pain (57%)

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Table 2. *Potential risk factors for aseptic meningitis, by period of symptom onset, Germany, July–August 2001*

Risk factor (exposure between 22 July and 24 August 2001)	Period I[a]						Period II[b]						Period III[c]					
	Cases[d]		Controls[d]				Cases[d]		Controls[d]				Cases[d]		Controls[d]			
	Exp.[e]	%	Exp.	%	mOR[f]	95% CI[g]	Exp.	%	Exp.	%	mOR	95% CI	Exp.	%	Exp.	%	mOR	95% CI
Bathing in pond A	15	54	22	20	44·8	3·9–515·6	2	13	8	13	1·8	0·1–28·2	2	9	7	8	1·3	0·1–11·7
Bathing, but not in pond A	8	62	45	51	2·9	0·5–13·8	11	79	37	67	2·2	0·4–13·9	15	75	57	67	1·4	0·4–4·7
Contact with a sick person outside household	5	22	12	16	1·3	0·4–4·5	4	31	13	27	2·3	0·5–9·5	8	47	10	15	6·8	1·6–30·0
Contact with a sick person inside household	12	44	10	9	4·9	1·9–12·5	6	38	7	11	2·6	0·8–8·9	8	36	10	11	9·0	2·0–39·8
Attended childcare centre	2	12	12	11	0·2	0·0–3·0	3	25	11	17	—	—	5	26	26	29	0·9	0·2–4·3
Attended school	11	39	62	56	0·4	0·1–1·1	7	44	35	56	0·3	0·0–1·7	12	55	54	59	0·7	0·2–2·5
Attended holiday camps	2	7	5	5	4·2	0·9–20·6	1	6	2	3	1·8	0·2–18·8	2	9	4	4	2·8	0·4–19·4
Bathing time >2 h[h]	8	53	3	14	18·8	2·0–174·1	0	0	1	13	—	—	0	0	2	29	—	—
Frequent water swallowing while bathing[h]	8	67	5	33	7·3	0·7–81·8	0	0	1	25	—	—	0	0	4	67	—	—

Exp, Exposed.
[a] Between 31 July and 9 August 2001; [b] between 10 and 19 August 2001; [c] between 20 and 28 August 2001; [d] denominator varies slightly due to missing information or due to analysis of subgroups; [e] exposed; [f] matched odds ratio; [g] 95% confidence interval; [h] among persons who reported bathing in pond A.

were the most frequently reported symptoms. Symptoms lasted a median of 5 days (range 1–28 days). Twenty-five per cent (21/83) of patients with aseptic meningitis reported bathing in pond A. Seventy-one per cent (15/21) of persons who reported bathing in pond A compared to 21% (13/62) of those who reported not bathing in pond A fell ill during period I, i.e. between 31 July and 9 August 2001 ($P=0{\cdot}004$).

Case-control study

Data from 68 out of 80 (85%) cases eligible for inclusion in the case-control study could be analysed in the matched-pair analysis (Fig. 2). For nine cases no addresses for controls were available from community registers and in three instances controls were mismatched for age or sex.

Questionnaires were received from 483 controls. These 483 questionnaires included questionnaires received from controls initially selected for cases who subsequently proved not to fulfil the inclusion criteria for the case-control study, e.g. for the presence of an alternative diagnosis. For the 68 cases included in the case-control study, questionnaires were mailed to 476 controls, of whom 81% (384) responded. Controls were only included in the matched-pair analysis if, between 31 July and 28 August 2001, they did not suffer from headache or fever and at least one of the following: abdominal pain, nausea, stiff neck, ocular pain, sore throat, or vomiting. There were 277 controls included in the matched-pair analysis.

Fifty-five (81%) of 68 cases compared to 181 (65%) of 277 controls reported bathing in a pool or lake from 22 July to 24 August 2001 (mOR 2·95, $P=0{\cdot}006$, 95% CI 1·4–6·3). Nineteen (28%) of 68 cases compared to 37 (13%) of 277 controls reported bathing in pond A (mOR 7·6, $P=0{\cdot}0002$, 95% CI 2·4–24·3). For all other swimming pools and lakes there was no association between bathing and aseptic meningitis (data not shown).

Fifteen of 28 cases with symptom onset during period I reported bathing in pond A compared to 22 of 111 matched controls (mOR 44·8, 95% CI 3·9–515·6, $P<0{\cdot}0001$) (Table 2). Thirteen of the 15 cases reported bathing in pond A during the 10 days preceding their illness, and a further two cases reported bathing in pond A from 22 July to 24 August 2001 but did not respond to the questions which asked about the 10 days before symptom onset. Among those who reported bathing in pond A, cases were

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more likely to report bathing for more than 2 h (mOR 18·8, $P=0·01$) and to report swallowing water occasionally or frequently while bathing (mOR 7·33, $P=0·3$) than their matched controls. In period II, which included 16 cases and 63 controls, none of the risk factors investigated were significantly associated with aseptic meningitis. In period III, which included 22 cases and 91 controls, contact with a sick person inside or outside the household was more common among cases than among controls.

Ninety-four per cent of cases (64/68) and 86% of controls (239/277) lived in households where the youngest household member was less than 15 years of age (mOR 4·1, 95% CI 1·0–17·1).

Thirteen per cent (63/483) of controls fulfilled the case-definition 'influenza-like/gastrointestinal illness'. Twenty-seven per cent (22/83) of controls who reported bathing in pond A and 10% (41/397) of controls who reported not bathing in pond A had an 'influenza-like/gastrointestinal illness' (RR 2·6, 95% CI 1·6–4·1). Among controls who reported bathing in pond A, 43% (9/21) of those who bathed for >2 h and 20% (12/61) of those who bathed for $\leqslant 2$ h had an 'influenza-like/gastrointestinal illness' (RR 2·2, 95% CI 1·1–4·4). Among bathers in pond A, 38% (12/32) of those who reported swallowing water occasionally or frequently, and 7% (2/27) of those who reported swallowing water never or rarely, had an 'influenza-like/gastrointestinal illness' (RR 5·1, 95% CI 1·2–20·7).

Laboratory findings

Twenty-eight of the 30 CSF samples were 5′-NCR-PCR positive. Viral isolation yielded E30 from 16 CSF samples and E13 from seven CSF samples. Only one sample was negative for PCR and virus isolation.

All water samples were negative in cell culture. Only three water samples from pond A were positive with 5′-NCR-PCR. From one of the three positive water samples the VP1 region could be amplified with specific E30 primers. There was no amplification with nested primers specific for E13.

VP1 sequences obtained from cell cultures and the CSF of two patients with E30 infection were compared to each other as well as to the E30 sequence obtained from the water sample. The sequences from patients were identical in both genomic regions. Homology of VP1 from patients and water was 99·2% and 100% on nucleotide and amino-acid level respectively.

Environmental investigation

Weekly testing of pond water for bacterial counts at 20 °C and 36 °C, total coliforms, faecal coliforms, enterococci, *Staphylococcus aureus*, pH, and temperature never indicated an excess of European Union (EU) bathing water guideline limits.

Control measures

The identification of the outbreak coincided with the end of the bathing season in 2001. In 2002 the following control measures were implemented: (1) Access to pond A was limited to 750 persons per day, approximately half the maximum number of daily visitors in 2001. (2) Warning signs informed visitors about the increased risk of infection associated with bathing in non-chlorinated water. (3) Children under the age of 4 years were forbidden to use pond A. They were referred to a nearby chlorinated wading pool. It was not possible to demonstrate the effectiveness of control measures except that the number of cases of aseptic meningitis remained low during the 2002 bathing season and no further incidents suggesting transmission of infection at pond A were reported to local health departments.

DISCUSSION

The aetiological agents identified in this outbreak of aseptic meningitis were E13 and E30. Co-circulation of different enterovirus serotypes during outbreaks of aseptic meningitis has been described [11, 16]. E13 was isolated infrequently throughout the world until 2000, when isolation rates increased dramatically in several countries in association with aseptic meningitis outbreaks, e.g. in England and Wales, Scotland, Ireland, Germany, France, and The Netherlands [17–20].

Although echoviruses were not isolated from pond water samples until around 3 weeks after the outbreak began, the molecular studies were highly suggestive of E30 contamination and swimming in pond A was associated with echovirus infection in patients who fell ill during the beginning of the outbreak. The magnitude of this association, the consistency of these findings with other studies [8–11], the results of viral studies of bathing water, and biological plausibility (high capacity of enteroviruses to survive in non-chlorinated water [21, 22] and the absence of efficient disinfection procedures for pond A) support a causal

association between bathing in pond A and developing illness. Moreover, the fact that persons with more intensive water contact fell ill more frequently, suggesting a dose–response relationship, further strengthens the association.

Cases used in a pilot study should normally not be included in the main study. However, excluding these cases from the case-control study would not have left enough cases for statistical analysis. We, therefore, decided to include them in the case-control study.

Although recall bias could have led to an over-reporting of more intensive contact with pond water by cases, it is unlikely to account for the strong association between exposure to pond water and illness during the beginning of the outbreak. Further, information on several risk factors and recreational bathing facilities and lakes was collected, but none was publicly highlighted during the investigation. However, recall bias could have led to cases reporting contacts with sick persons more frequently than controls.

Echovirus infections are frequently asymptomatic or associated with influenza-like illness. In E30 outbreaks the relation between clinical and subclinical cases has been described as 1:1 or 1:2 [23, 24]. In our outbreak cases of aseptic meningitis were probably only a small proportion of all echovirus infections. Asymptomatic infection of controls is possible and may be common. The resulting non-differential misclassification may have led to an under-estimation of the association between exposure and outcome.

We could not definitively determine how the pond became contaminated. Bathing ponds have to be refilled with water of drinking-water quality. Given the small size of the pond, the low infective dose of $\leqslant 100$ virus particles, the potentially high concentrations of virus particles in stool, the absence of efficient disinfection procedures and the high number of visitors, water contamination by faeces of a single person has the potential to cause a high number of echovirus infections.

The E30-PCR positive water sample from pond A was drawn on 22 August 2001, approximately 2 weeks after the end of the period for which we showed an epidemiological association between bathing in pond A and aseptic meningitis. Several points may be relevant in explaining this finding: (1) The positive E30-PCR finding in pond water does not indicate the presence of infectious virus particles and water infectivity may have decreased over time. (2) At the end of the hot summer period and of the summer holidays on 5 August 2001 the number of first-time visitors to pond A decreased, leading to decreasing numbers of susceptible persons bathing in pond A. (3) Epidemiological evidence suggests increasing person-to-person transmission in later periods of the outbreak and (4) the cases could have contaminated the pool, rather than the other way round.

In Germany and Austria an increasing number of public chlorinated swimming pools are being rebuilt into nature-like bathing ponds. Bathing ponds generally have an appealing ambiance and offer considerable economic advantages to communities with limited financial resources. However, while in conventional swimming pools chlorination assures a rapid inactivation of introduced pathogens, in bathing ponds water purification is left to natural degrading processes. In swimming lakes dilution effects and water exchange with ground water quickly reduce infection risks to co-bathers following a point contamination. Water volume in bathing ponds is considerably lower.

Weekly testing of bacterial indicator organisms in pond A never rose above EU bathing water guideline limits. Indicator bacteria are useful markers of faecal contamination only if the faecal contamination results of water influx into a body of water with the water influx containing an average load of pathogens (e.g. from depuration plants, agricultural land wash). If water is contaminated directly by a bather, the relation between pathogens and faecal indicators is much higher and faecal indicators fail.

This outbreak demonstrates the potential of nature-like swimming ponds to cause widespread community infection with substantial public health impact. A whole host of other organisms, including hepatitis A, Norovirus and polioviruses are potentially waterborne [25–27]. There is an urgent need to reconsider the popular tendency to convert chlorinated swimming pools into nature-like swimming ponds. Existing ponds need to be promoted with care and require careful maintenance.

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