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Risk of gastroenteritis among triathletes in relation to faecal pollution of fresh waters

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Background We conducted a prospective cohort study among endurance athletes to investigate the effects of microbiological water quality on the risk of gastroenteritis after bathing in fresh waters that meet current water quality standards. We aimed to establish quantitative relationships, in order to evaluate current bathing water standards.

Methods The study was spread over two summers, during which 827 triathletes (swimmers) in seven Olympic distance triathlons and 773 participants in 15 run-bike-runs (non-swimming controls) participated. Intensive water quality monitoring was used to assess exposure to faecal indicator organisms and detailed questionnaires were used to collect data on the occurrence of health complaints and potential confounding factors.

Results The microbiological water quality at the time of the triathlons met current Dutch and European bathing water standards. Dependent on the case definition studied, gastroenteritis developed in 0.4–5.2% of swimmers and 0.1–2.1% of non-swimmers in the week following exposure (odds ratio [OR] = 1.6–2.3). Attack rates and burden of disease varied with the case definition used. Among swimmers, the attack rate of gastroenteritis was significantly increased when the geometric mean concentration of thermotolerant coliforms in the water at the time of exposure was ≥220/100 ml or the geometric mean concentration of *Escherichia coli* was ≥355/100 ml (OR comparing high versus low exposure 2.9–4.7 dependent on the case definition studied). Thermotolerant coliform concentrations at these triathlons ranged from 100/100 ml (the EU guide level) to 960/100 ml (the EU imperative level is 2000/100 ml). Below the threshold levels attack rates were comparable with attack rates among non-swimmers. A relation with other indicators of faecal pollution was not observed.

Conclusions We observed that swimming in fresh waters that met the European imperative level for thermotolerant coliforms but failed the European guide level was associated with a significant risk of gastroenteritis among triathletes. This means that the current European imperative level for thermotolerant coliforms provides insufficient protection to gastrointestinal illness for those who are comparable with triathletes.

Keywords Cohort studies, faecal pollution, fresh water, gastroenteritis, swimming, triathletes

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Current water quality standards have been established to protect bathers from illness and are primarily directed towards the level of faecal contamination of the water. Bathing in waters meeting these standards, however, has been found to be associated with gastrointestinal illness, respiratory complaints and symptoms of the skin, ears and eyes.[1–13]

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The extent to which health risks among bathers are quantitatively associated with faecal pollution of the water has been debated for decades. Such information is essential to evaluate and, if necessary, to adjust current standards. Bathing in faecally polluted waters is most likely to be associated with an increased risk of gastroenteritis. Such an increased risk was shown in relation to concentrations of faecal indicator organisms like faecal streptococci and *Escherichia coli* in sea[2,3,9] and fresh waters.[10] However, the findings are inconsistent and have been criticised,[13,14] with the exception of a UK study in sea water which

has found a consistent relation between concentrations of faecal streptococci and risk of gastroenteritis.[9] The survival of indicator bacteria and enteropathogens in sea and fresh waters differs,[15] and these results cannot be applied to the many fresh water sites in the Netherlands and other inland European bathing waters. We present a study among endurance athletes which was carried out to: (1) estimate the risk of gastroenteritis, if any, after bathing in fresh waters that meet current water quality standards, and (2) relate the risk of gastroenteritis among bathers to the faecal pollution of the water.

Methods

Study design

Participants in Olympic distance triathlons in fresh waters were studied as swimmers and participants in run-bike-runs were the non-swimming controls. Both groups were compared with respect to exposure status, confounding factors and occurrence of gastroenteritis in the week following exposure. An Olympic distance triathlon is an athletic contest of 1 or 1.5 km swimming along a well defined swimming course, 40 km cycling and 10 km running. A run-bike-run is an athletic contest of 7 km running, 30 km cycling and 3.5 km running. We had two reasons to study endurance athletes instead of the general population. Firstly, exposure to swimming water during a triathlon is relatively homogeneous for all participants and can be determined with precision during the race. All athletes in one race swim at the same time in the same body of water for 15–40 minutes. Secondly, the study could be incorporated into the national race series of the Dutch Triathlon Federation, which facilitated study logistics. Olympic distance triathlons and run-bike-runs were organised weekly between April and September and participant numbers and location of swimming were known in advance. All triathlons were held in recreational fresh waters of which one was a designated recreational water, all others were not designated waters but were frequently used by local swimmers. Study sites had to have expected thermotolerant coliform levels $\leq$2000 per 100 ml (EU imperative level) with domestic (treated) sewage being the main source of faecal contamination. To get a range of different water qualities, in order to study exposure-response relations, a number of triathlons were studied. The study design had been pre-tested in 1992 and this showed that the design was feasible and that triathletes and run-bike-runners were comparable with respect to baseline characteristics and potential confounders.[16]

The main study was conducted among participants in seven Olympic distance triathlons and 15 run-bike-runs between May 1993 and July 1994. Six triathlons consisted of two series, one for recreative participants (who swam 1 km) and one for participants in the Olympic Distance circuit (who swam 1.5 km). One triathlon enrolled only recreative participants. All athletes who were on the contests' participants list in the week before the race were invited for the study. They received a postal questionnaire to collect information on age, sex, general health, training history (i.e. number of performed endurance races in the previous year, number of weekly training hours in the previous 2 months), exposure to surface waters in the week before and after the contest and the occurrence of gastrointestinal complaints during the contest. Triathletes were also asked whether they used wetsuits or swimming goggles during the race and if they ingested water while swimming. All athletes were further asked whether they developed gastroenteritis in the 2 days before the contest and the 6 days thereafter. Nausea, vomiting, stomach ache, diarrhoea and fever were assumed to be the relevant symptoms related to exposure to water. The occurrence of other health complaints was also collected, but will not be discussed in this paper. All subjects were asked to tick on a diary sheet the presence of each of the complaints during night, morning, afternoon and/or evening on the days within the observation period. Disability was estimated by asking whether the respondents discontinued daily activities, remained in bed, sought medical advice or used any drug. On the day of the race, all athletes were weighed just before and immediately after the exercise in underwear on inter-calibrated scales to measure weight loss during the race as a measure of dehydration. Severe dehydration (>4%) may increase risk of gastroenteritis.[17] Weight measures were carried out in 1993 and 1994 among both triathletes and run-bike-runners, and in 1994 among triathletes only.

Water quality analysis

To determine the sampling programme during the race a preparatory analysis was carried out a few weeks before each contest in the first year to establish the variation in concentrations of thermotolerant coliforms over place and time. As the variation tended to be substantial, this analysis was omitted in the second year. On the exposure day, water samples were collected along the swimming course from a boat that accompanied the swimmers and from dikes along the course. Samples were taken during both the recreative and the competitive runs. All samples were taken at time of swimming at a depth ranging from 0 to 30 cm below the surface, stored on ice and transported to the laboratory within 4 hours. At one moment in time the water temperature was measured. Water samples were analysed in duplicate within 28 hours for thermotolerant coliforms on Lauryl Sulphate Agar (4 h at 25°C and 18 h at 44°C) with confirmation on Brilliant Green Lactose Broth (48 h at 44°C), *E. coli* according to Havelaar and During,[18] faecal streptococci on Kenner Faecal agar (48 h at 37°C) with confirmation on Bile Esculin Azide agar (48 h at 37°C), enteroviruses according to Havelaar *et al.*[19] and F-specific RNA bacteriophages according to ISO DIS 10705–1.[20] Other water quality indicators were also analysed but are not discussed in this paper as they did not show a relation with the risk of gastroenteritis. Further details can be found in Medema *et al.*[21]

Data analysis

Ten subjects who did not finish the contest and nine subjects who had very incomplete questionnaire data were excluded from further analysis. Data from participants in the recreative triathlon series and from participants to the Olympic Distance circuit were, for each triathlon, pooled because in each series the swimming courses overlapped considerably and the water quality did not differ significantly. To compare the two study groups (triathletes and run-bike-runners), baseline characteristics were computed. For this, data from all the athletes who participated in at least one triathlon were pooled (*triathletes*) and the same was done for all athletes who participated in at least one run-bike-run (*run-bike-runners*). Subjects who participated in more than one contest (n = 224) were included repeatedly as

Table 1 Gastroenteritis case definitions used in the study

Case definition	Ref.	Description
Case definition NL-1	25	diarrhoea (≥2 loose stools in 24 h) accompanied by at least two of the symptoms—fever, nausea, vomiting, stomach ache or gripes. All complaints present for at least one part of the day (night, morning, afternoon, evening).
Case definition NL-2	–	diarrhoea; or nausea; or vomiting; or stomach ache; or gripes. All complaints present for at least two parts of the day (night, morning, afternoon, evening) or on two parts of the day within successive 24 h.
Case definition US	2, 10	vomiting; or diarrhoea accompanied by a fever or resulting in discontinuation of daily activities;[a] or nausea or stomach ache accompanied by a fever. All complaints present for at least one part of the day (night, morning, afternoon, evening).
Case definition UK	9	vomiting; or diarrhoea (≥3 loose stools in 24 h); or nausea accompanied by a fever. All complaints present for at least one part of the day (night, morning, noon, evening).

[a] Differs slightly from case definition used in the US studies; they defined 'diarrhoea accompanied by a fever or that was disabling' Disabling was defined as 'home because of symptoms; or in bed because of symptoms; or medical help because of symptoms'.

each event was assumed to pose an independent risk of gastroenteritis. Even so, using only the first response of each subject or using all responses produced the same results.

Many case definitions of gastroenteritis are used in the literature, each of which may give rise to a certain incidence rate or exposure-response relation. We decided to study four case definitions simultaneously (Table 1). Subjects with gastroenteritis in the 2 days before the contest were excluded from the calculation of attack rates.

The significance of differences in medians of baseline characteristics between triathletes and run-bike-runners was tested with a median test, and of differences in proportions with a χ^2 test with continuity correction or a Fisher's exact test when appropriate, using the SAS programme.[22] To study associations between faecal indicator organism concentrations and incidence of gastroenteritis, triathlons were ranked according to the geometric mean concentration measured during the race. Associations based on categorization of medians, arithmetic means, or maximum concentrations gave similar findings. The Mantel-Haenszel χ^2 test for linear trend was used to test the significance of the association. Crude and adjusted odds ratios (OR) and 95% confidence intervals (CI) for the risk of gastroenteritis according to exposure status were calculated using logistic regression analysis. To check whether the association between exposure status and risk of gastroenteritis was modified by other variables, interaction terms were included in the models. Such interactions were not found. All *P*-values were two sided.

Results

Of 1493 triathletes and 1628 run-bike-runners who participated in the events studied, 1326 and 1247, respectively, received a questionnaire in the week before the contest. Completed questionnaires were returned by 827 triathletes and 773 run-bike-runners. The overall response rate was 62% in both groups; among the run-bike-runners the response rate substantially decreased in the second year (from 67% to 54%).

Baseline characteristics of triathletes and run-bike-runners are shown in Table 2. Both groups were comparable with respect to age, sex, current health and years of participating in endurance sports. Triathletes, however, had a better training history and were more likely to have swum in surface waters in the week before and after the contest than run-bike-runners. Weight loss among triathletes was smaller than among run-bike-runners. The occurrence of upper gastrointestinal tract complaints during the contest was reported more frequently during the triathlons than during the run-bike-runs. Of these, the major complaint was belching and this was associated with swallowing water while swimming. Of the triathletes, 72% (571/793) reported having swallowed water during swimming.

The attack rate of gastroenteritis in the week after the contest was higher among triathletes than among run-bike-runners, and this was observed for all four case definitions studied (Table 3). The attack rate was highest for the NL-2 case definition and lowest for the NL-1 case definition. Triathletes who reported swallowing water during swimming reported gastroenteritis more frequently (6.8%, according to any one of the four case definitions) than those who did not swallow water (3.8%), although this association was not significant ($P = 0.11$). Of subjects with gastroenteritis, (according to any one of the four case definitions), 19% (14/72) reported discontinuation of daily activities for 0.5–2.5 days (mean 1.3 days): 15% (11/72) had to rest in bed for 1/4–2 days (mean 1 day), 3% (2/72) consulted a general practitioner and 3% (2/72) used anti-diarrhoeal drugs. Disability rates may be increased by coexistence of other health complaints but they were all substantially higher among swimmers than among non-swimmers. The highest disability occurred in gastroenteritis cases as determined by the US case definition, followed by the NL-1 case definition, the UK case definition and the NL-2 case definition.

At all locations thermotolerant coliform concentrations were below the Dutch and European Union's imperative levels (Table 4). At six triathlons concentrations of faecal streptococci were below the European guide level of 100/100 ml and in all but one triathlon the mean enterovirus concentration was <1/10 l; at four triathlons the maximum concentration was also below this level. The water temperatures ranged from 16 to 22°C.

The attack rate of gastroenteritis among swimmers was associated with geometric mean concentration of thermotolerant coliforms and *E. coli* in the water during the race. As shown for the NL-2 case definition in Figures 1 and 2, a threshold level exists beyond which increased attack rates were observed for each indicator. For thermotolerant coliforms this was a geometric mean of 220/100 ml and for *E. coli* a geometric mean of 355/100 ml. Below these levels attack rates were comparable with that among non-swimmers. A similar pattern was observed for the UK and the US case definition (data not shown). Thermotolerant coliform concentrations in all individual samples taken

Table 2 Baseline characteristics of triathletes and run-bike-runners. Values are numbers (percentage) unless stated otherwise

Risk factor	Triathletes	Run-bike-runners
Sex (male)	757/827 (92)	723/773 (94)
Regular complaints of		
gastrointestinal tract	54/808 (7)	53/754 (7)
respiratory tract	96/808 (12)	77/754 (10)
skin	40/808 (5)	37/754 (5)
eyes	32/808 (4)	30/754 (4)
ears	16/808 (2)	10/754 (1)
head (migraine)	16/808 (2)	16/754 (2)
Exposure to surface waters in week before contest	374/818 (46)	158/762 (21)***
Exposure to surface waters in week after contest	282/706 (40)	140/620 (23)**
Complaints of upper gastrointestinal tract[a] during contest	202/764 (26)	103/725 (14)***
Complaints of lower gastrointestinal tract[b] during contest	188/764 (25)	154/725 (21)
	Median (5^{th};95^{th} centile)	
Age (years)	33 (21;48)	34 (21;49)
Experience as endurance athlete (years)	5 (1;16)	6 (1;15)
Training history (h x n)[c]	60 (5;247)	42 (5;200)***
Weight loss during the race (% of body weight)[d]	1.8 (0.7;3.5)	2.0 (0.7;3.5)*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[a] Belching, stomach ache, nausea, vomiting.

[b] Side aches, gripes, flatulence, urge to discharge, diarrhoea.

[c] Expressed as number of performed races in the 12 months before the contest times number of weekly training hours in the 2 months before the contest.

[d] Calculated among 340 triathletes and 372 run-bike-runners.

Table 3 Attack rates of gastroenteritis[a] in the week following exposure to fresh water (triathletes) or physical exercise (run-bike-runners). Values are numbers (percentage) unless stated otherwise

Case definition	Triathletes		Run-bike-runners		Crude odds ratio[c]	Adjusted odds ratio (95% confidence interval)[c,d]
	n/N[b]	(%)	n/N[b]	(%)		
Case definition NL-1	3/827	(0.4)	0/773		NA[e]	NA
Case definition NL-2	42/802	(5.2)	16/756	(2.1)	2.4	1.9 (1.1–3.5)
Case definition UK	29/818	(3.6)	13/764	(1.7)	2.0	1.6 (0.8–3.2)
Case definition US	18/824	(2.2)	6/771	(0.8)	2.7	2.3 (0.9–5.9)

[a] See Table 1 for case definitions.

[b] n: Numbers with gastroenteritis in week after contest, N: Numbers without gastroenteritis in 2 days before contest.

[c] Among subjects without missing values for selected risk factors (n = 1503).

[d] Adjusted for age, complaints of upper gastrointestinal tract during contest and training history.

[e] Not applicable.

Table 4 Concentrations (per 100 ml unless stated otherwise) of indicators of faecal pollution at the seven triathlons

Indicator	Range	Geometric mean	log10 SD	No. of samples taken	EU guide level[a]	EU imperative level[a]
Thermotolerant coliforms	0.6–650	78	0.56	54	≤100/100 ml	≤2000/100 ml
Escherichia coli	11–2600	204	0.44	54	–	–
Faecal streptococci	0.2–1800[b]	16	0.66	54	≤100/100 ml	–[c]
Enteroviruses (/l)	0.007–17	0.04	0.81	18	–	0/10 l
F-specific RNA bacteriophages (/l)	<0.01–13.6	0.7	0.83	31	–	–

[a] 95% centile.

[b] Concentration in one sample; concentrations in all other samples <340/100 ml

[c] An imperative level of ≤400/100 ml has recently been proposed in the revised text of the present guidelines.[22]

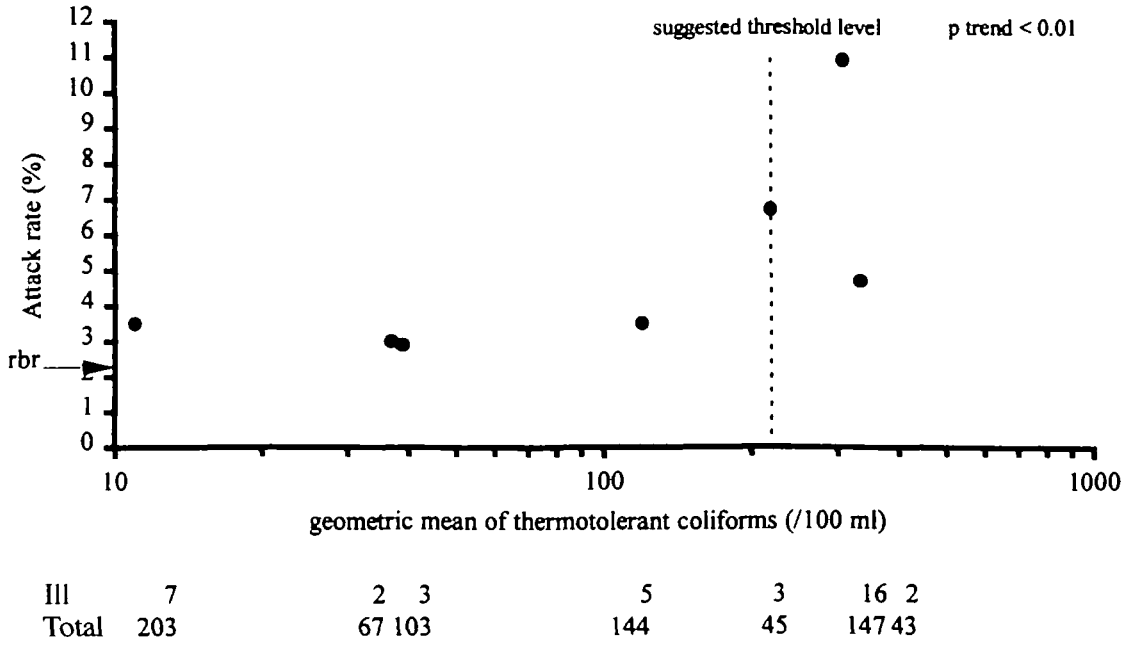

Figure 1 Gastroenteritis attack rates for NL-2 case definition in week after triathlon according to geometric mean concentration of thermotolerant coliforms in water during race

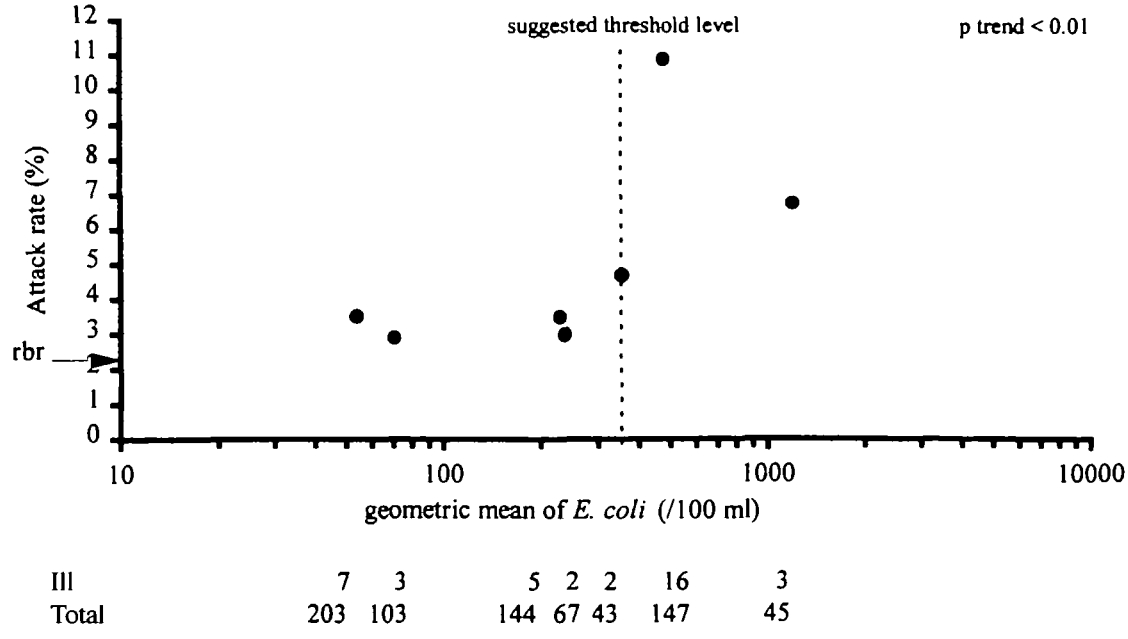

Figure 2 Gastroenteritis attack rates for NL-2 case definition in week after triathlon according to geometric mean concentration of *Escherichia coli* in water during race

during the four triathlons above the threshold level ranged from 100/100 ml to 960/100 ml. The adjusted relative risk of gastroenteritis after participating in these triathlons, as compared with participating in triathlons with lower concentrations, was 2.9–4.7 dependent on the case definition studied (Table 5). An exposure-response relation with faecal streptococci, enteroviruses and F-specific RNA bacteriophages was not observed.

Discussion

Our study among endurance athletes showed that triathletes who swam in fresh waters that met current European bathing standards were twice as likely to develop gastroenteritis in the week following exposure than non-swimmers (run-bike-runners). Occurrence of gastroenteritis resulted in discontinuation of daily activities and bed rest among some of the subjects.

Table 5 Attack rates of gastroenteritis among triathletes and run-bike-runners according to level of faecal pollution of the water measured during the race. Values are numbers (percentage) unless stated otherwise

Exposure category	Case definition NL-2			Case definition UK			Case definition US		
	n/N[a] (%)	Crude odds ratio[b]	Adjusted odds ratio (95% CI)[b,c]	n/N (%)	Crude odds ratio[b]	Adjusted odds ratio (95% CI)[b,c]	n/N (%)	Crude odds ratio[b]	Adjusted odds ratio (95% CI)[b,c]
Unexposed (run-bike-runners)	16/719 (2.2)			13/726 (1 8)			6/733 (0.8)		
Low exposure[d]	17/517 (3.3)	1 0	1.0	9/527 (1 7)	1 0	1.0	6/530 (1.1)	1.0	1.0
High exposure[d]	21/235 (8 9)	2.9	2.9	18/241 (7.5)	4.6	4.7	11/243 (4 5)	4.1	3.9
			(1.5–5.8)			(2.1–10.7)			(1.4–10.8)

[a] n. Number with gastroenteritis in week after contest, N: Among subjects without missing values for selected risk factors (n = 1511) and who were without gastroenteritis in the 2 days before the contest.

[b] Lowest exposure category as reference.

[c] Adjusted for age, complaints of upper gastrointestinal tract during contest and known with regularly complaints of the gastrointestinal tract.

[d] Low exposure: geometric mean concentration in water of triathlon for thermotolerant coliforms ≤120/100 ml (i.e. concentrations in most samples ≤100/100 ml) and for *E coli* ≤238/100 ml; high exposure: geometric mean concentration in water of triathlon for thermotolerant coliforms ≥220/100 ml (i.e. concentrations in all samples >100/100 ml) and for *E. coli* ≥355/100 ml

In waters that meet current standards concentrations of thermotolerant coliforms should not exceed 2000/100 ml. Waters having concentrations below 100/100 ml (i.e. the EU guide level) are considered to be of 'excellent quality'. We observed that the risk of gastroenteritis was significantly increased among triathletes who swam in waters with a geometric mean thermotolerant coliform concentration of ≥220/100 ml. For *E. coli* this was ≥355/100 ml. At these triathlons concentrations of thermotolerant coliforms ranged from 100/100 ml to 960/100 ml. Above the threshold levels we could not ascertain whether the risk increased with increasing concentrations, as the three triathlons involved could not be distinguished from each other (with respect to concentrations of thermotolerant coliforms) or had too small numbers to produce precise results (with respect to *E. coli*). At triathlons with a geometric mean thermotolerant coliform concentration of ≤120/100 ml or *E. coli* of ≤238/100 ml attack rates were comparable with that in non-swimmers. In these waters most concentrations of thermotolerant coliforms were <100/100 ml (i.e. the EU guide level), suggesting that bathing in such waters does not produce an additional risk of gastroenteritis.

Although *E. coli* is the main constituent of the thermotolerant coliforms, *E. coli* concentrations in the samples taken in the study were higher than concentrations of thermotolerant coliforms. The ratio of geometric means (2.5, range 1.1–5.0) was more consistent than with other indicators. A better recovery of the analysis method for *E. coli*[18] as compared with the method for thermotolerant coliforms is probably at the basis of this. As our method for *E. coli* analysis is different from that required by the EU, the observed threshold level for *E. coli* cannot be generalized unless the same method of analysis is used. Both thermotolerant coliforms and *E. coli* are indicators of faecal pollution and not the primary pathogens that caused disease. This study shows that both indicators can be good predictors of risk of gastroenteritis after bathing in fresh waters. As *E. coli* is the predominant organism amongst the thermotolerant coliforms and the only true faecal coliform, this should be the indicator of choice.

It is not likely that our findings are strongly biased. Although the overall response rate during the 2 years of study was rather low (62%) we do not consider non-respondent bias as substantial. Many athletes participated in several contests under study and most managed to complete a questionnaire only once. Among those who responded more than once there was no evidence that illness induced participation. If respondents were more likely to have health complaints than non-respondents we assume that this was equal for both swimmers and non-swimmers as they had similar response rates. Although the attack rate of gastroenteritis would be overestimated in that case, risk estimates and exposure-response relations would not because triathletes had no prior knowledge of the level of faecal pollution of the water. In addition, we consider the effect of knowledge of the study aims (can swimming cause illness?) small, as the information we gave included several factors that might cause health complaints after participation in a triathlon or a run-bike-run, one of which was swimming.

Previous studies have tried to establish relations between indicators of faecal pollution in fresh waters and risk of gastroenteritis.[10–13] Most of them had negative results;[11–13] others, as we did, reported a relation with *E. coli* but not with thermotolerant coliforms.[10] The design and analysis of most of these studies have been criticised.[13,14] In previous fresh water studies exposure-response relations were based on a mean daily concentration of indicator organisms assigned to all bathers, independent of their time, place and intensity of exposure. Since concentrations of indicator organisms may show considerable variation in time and place, assignment of a mean level to all bathers will lead to substantial misclassification bias. In contrast to these studies, the triathletes in our study were exposed simultaneously to the same body of water of a fixed swimming course. Assignment of the same level of indicator organisms to all swimmers is then more justified. In addition, in contrast to earlier studies, our risk estimates were adjusted for other factors that may have confounded the relation between water quality and risk of gastroenteritis.

In a study in the UK in sea water, faecal streptococci were the only indicator that correlated with risk of gastroenteritis.[9] Most faecal streptococci concentrations in our study were below the level at which an increased risk occurred in the UK study (i.e. 32/100 ml) and this might explain why we did not observe such a relation. In addition, indicators that correlate best with health complaints may be different for sea and fresh waters due to different survival kinetics in these types of water.[15]

In the literature, gastroenteritis has been defined by a range of case definitions. The use of different definitions impairs the comparability of studies and, even more, may result in different acceptable risk levels if they give rise to different exposure-response relations. An acceptable risk level is necessary for the definition of new water quality standards and should be based on both attack rate and severity of a combination of symptoms. We compared four case definitions of gastroenteritis, two of which have been used in bathing water studies in the UK and the US,[2,9,10] and observed that both attack rate and burden of disease varied with the case definition used. In contrast with the US definition, the UK definition yielded a higher attack rate but a lower disability for each case. The NL-2 case definition yielded the highest attack rates and the lowest burden of disease. In the UK study relatively high attack rates were found[9] and this may be partly due to the case definition used. Higher 'no-effect' levels of faecal streptococci would probably have been found in the UK study if the US case definition had been used.

Our results are not directly applicable to recreative bathers or to sea waters. Triathletes may be either more susceptible (due to intensive exposure to swimming water or a temporal decrease in immune response associated with exercise) or less susceptible (due to good health and habituation to swimming in surface waters) to infections than the general population. The results, however, show that the current European standards provide insufficient protection to those who are comparable with triathletes, such as surfers, divers and long-distance swimmers. They further show that the proposed replacement in the EU bathing water standards of thermotolerant coliforms with *E coli* without a modification of the limit values[23] will still result in a substantial risk of mild gastrointestinal illness among such bathers. This replacement may also result in less compliance if current methods are replaced by methods with higher recovery. To verify the findings of the present study in a general population, prospective investigations are currently being undertaken among primary school children in the Netherlands.[24]

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