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# ASSESSMENT OF THE EXPOSURE OF SWIMMERS TO MICROBIOLOGICAL CONTAMINANTS IN FRESH WATERS

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## ABSTRACT

As part of a prospective cohort study among triathletes to determine a relationship between the microbiological quality of fresh bathing water and the risk of acquiring an intestinal infection, the exposure of the triathletes to microbiological contaminants was assessed. Waters were collected at seven triathlons (swimming course 1-1.5km) held in the summer of 1993 and 1994 to have a range of water qualities. All were influenced by sewage effluents, most also by agricultural run-off. Samples were collected several weeks before the event to establish a sampling programme (1993) and during the actual exposure of the triathletes (1993 and 1994) and examined for thermotolerant coliforms alone (samples preceding the event) and for *E. coli*, faecal enterococci, *Staphylococcus aureus*, F-specific RNAphages, enteroviruses (1993 and 1994) and for thermophilic *Campylobacter, Salmonella, Aeromonas, Plesiomonas shigelloides* and *Pseudomonas aeruginosa* (1993). The samples taken in the weeks before the exposure showed significant differences in thermotolerant coliform concentration between locations, depths and times. Also during swimmer exposure, significant differences occurred in microorganism levels at the different sampling points over the swimming course. As the triathletes swam as a group, they were exposed to approximately the same water at the same time. The geometric mean concentration was used to characterise each site. In the epidemiological study, the risk of an intestinal infection correlated with the concentration of thermotolerant coliforms and *E. coli* but not with the other parameters. The geometric mean concentration of thermotolerant coliforms at the triathlons ranged from 11-330/100mL and 54-1,200/100mL *E. coli*. Ranking of the seven sites by faecal pollution level, based on the geometric mean concentration of a faecal indicator, resulted in a different ranking for each indicator. At the fresh water sites studied, only the ratio between the geometric mean density of *E. coli* and thermotolerant coliforms was constant. The ratio between the other parameters related to faecal pollution (faecal enterococci, F-specific RNA phages, enteroviruses) varied considerably. Water quality standards relating to faecal pollution can only be based on parameters that show a significant correlation with risk of intestinal illness. © 1997 IAWQ. Published by Elsevier Science Ltd

## KEYWORDS

Bathing water; health risk; indicator bacteria; F-specific RNA bacteriophages; enteroviruses.

## INTRODUCTION

Many studies have shown that bathers have a higher probability of acquiring health complaints than non-bathers (Stevenson, 1953; Cabelli *et al.*, 1982; El Sharlaki and Hassan, 1982; Foulon *et al.*, 1983; Dufour, 1984a; Seyfried *et al.*, 1985; Fattal *et al.*, 1987; Cheung *et al.*, 1990; Balarajan *et al.*, 1991; Fewtrell *et al.*, 1992; Medema *et al.*, 1993; Corbett *et al.*, 1993; Kay *et al.*, 1994). Reported health effects are many:

gastrointestinal and respiratory illnesses, and infections of the skin, ear and the CNS. Microbiological water quality standards, that are established to prevent the bather from negative health effects, are primarily directed towards the level of faecal contamination. A causal link between the level of faecal contamination of bathing waters and the probability of getting gastrointestinal infections is likely to exist, since the probability of enteropathogens being present in the bathing water increases with increasing faecal contamination. This assumption underlies the current bathing water standards for indicator bacteria. Several US studies have shown that the level of faecal contamination, expressed by the density of indicator bacteria, is correlated with the probability of gastroenteritis (Cabelli *et al.*, 1982; Dufour, 1984a; Cheung *et al.*, 1990). Fleisher *et al.* (1993) has criticised the exposure assessment in these studies since this was based on daily or seasonal averages of water quality data. A recent prospective cohort study in the UK, where individual exposure was assessed, showed a positive correlation between enterococci densities in the water and the risk of acquiring gastroenteritis (Kay *et al.*, 1994). As survival of indicator bacteria and pathogens in seawater and in fresh water differs (Dufour, 1984b), and the Netherlands has many fresh water recreation sites, we carried out a prospective cohort study to determine the health risk of bathing in fresh water (Asperen *et al.*, 1996). The aims of the prospective cohort study were to (1) determine if swimmers have a higher risk of gastroenteritis (and possibly other symptoms) than non-swimmers, (2) determine if a threshold level of microbiological water quality parameter(s) can be established above which this increased risk occurs and (3) determine if a dose-response relation can be established between microbiological water quality parameter(s) and the risk of gastroenteritis. These aims required a population of swimmers among which subpopulations are exposed to different water qualities. In our study, participants in quarter triathlons served as the exposed swimmer population. This population had several advantages over recreational swimmers as exposure was relatively homogeneous because all athletes swam at the same time in the same body of water. The duration of the exposure was 15-40min and exposure was relatively intense; 75% of the triathletes reported ingestion of surface water during swimming. Study logistics and planning were relatively easy. Duathletes were used as non-swimming controls. The aim of this part of the study was to determine the exposure of triathletes to microbiological contaminants in the swimming course water, both to indicator organisms (*Escherichia coli*, thermotolerant coliforms, faecal enterococci, F-specific RNA bacteriophages) or enteropathogens (enteroviruses, *Campylobacter, Salmonella, Aeromonas, Plesiomonas shigelloides, Pseudomonas aeruginosa*). Enteroviruses may be regarded both as enteropathogens and as indicators of contamination with more enteropathogenic viruses (e.g. SRSV)

## MATERIALS AND METHODS

***Study sites*** - Many quarter triathlons are held in fresh water in the summer season each year. Study sites where the swimming course was (potentially) under the influence of sewage effluents and agricultural run-off were selected from these triathlons. In addition, the number of participants had to be high to obtain sufficient power in the epidemiological study. As the exposure to microbiological contaminants in the water on one study day at one site was relatively homogeneous, triathlons at seven different fresh water sites and on different days were incorporated in the study. In 1993, four study sites were selected and a further three in 1994 (Table 1). At all triathlons, two separate races were held, one for competition participants and one for recreative participants, except in Leiden where only a recreative race was run.

***Microbiological water analysis*** - several weeks before the 1993 triathlons, each study site was sampled to establish the variation of the faecal contamination over the swimming course using thermotolerant coliform (TC) density (= preparatory analysis). Samples were taken at 0, 30 and 60min at three locations: start (0m), halfway (750m) and finish (1500m) at two depths (0 and 30cm) and examined for TC (membrane filtration with lauryl sulphate agar incubated for 4±1h at 25±1°C followed by 18±2h at 44±0.5°C). On the study day, 10L samples were taken and analysed within 28h for *E. coli* (tryptone soy agar/tryptone bile agar TSA/TBA method of Havelaar and During, 1988), TC (as above, confirmation on brilliant green lactose broth 48±4h at 44±0.5°C), faecal enterococci (FE, Kenner faecal agar, confirmation on bile aesculin azide agar for 48±4h at 37±1°C) *Aeromonas* (ADA agar; *Pseudomonas aeruginosa* on mPA-b agar) thermophilic *Campylobacter* (filtration through 0.2µm membrane filters, enrichment in Preston broth for 48±4h at 42±0.5°C under microaerobic conditions, followed by mCCDA agar at 48±4h at 42±0.5°C under microaerobic conditions) - all Dutch standard methods. In addition, the following parameters were analysed: *Salmonella* (ISO DIS

6340), F-specific RNA phages (ISO DIS 10705), *Staphylococcus aureus* (Havelaar and During, 1985) and *Plesiomonas shigelloides* (Medema and Schets, 1993). For virological analysis, one or two samples of approximately 120L and 3-5 10L samples were taken, processed and analysed for enteroviruses on BGM cells by the agar overlay method (Havelaar *et al.*, 1993). Part of the eluate of the virus samples was also analysed for F-specific RNA phages. As the analysis for thermophilic *Campylobacter* and *Salmonella* gave only semi-quantitative results and the accurate enumeration of *P. aeruginosa* and *P. shigelloides* was hampered by heavy background growth, these parameters were omitted from the water quality analysis in 1994. The number of samples per triathlon analysed for *E. coli*, TC, FE and *Aeromonas* were increased from 5-6 in 1993 to 8-13 in 1994.

Table 1. Details of the study sites

| Date | Location | No. participants | Details of swimming stage of event |
|---|---|---|---|
| 29 May 1993 | Gorkum | 264 | In de Linge, a small river contaminated by sewage effluents and agricultural run-off |
| 12 June 1993 | Strijen | 68 | In Binnenmaas, a branch of the Meuse contaminated by agricultural run-off and sewage effluents |
| 19 June 1993 | Hellevoetsluis | 296 | In Kanaal door Voorne, a channel contaminated by a sewage effluent and agricultural run-off (only if drainage pumps are in operation, not or just before study day) |
| 26 June 1993 | Hoorn | 389 | In Lake Ijssel contaminated by sewage effluents and agricultural run-off, not in the vicinity of the study site |
| 12 June 1994 | Schagen | 77 | In harbour contaminated with sewage effluent, agricultural run-off and boats |
| 25 June 1994 | Leiden | 77 | In de Vliet, a canal contaminated by nearby and distant sewage effluents and agricultural run-off |
| 2 July 1994 | Driebruggen | 142 | In Dubbele Wiericke, a canal contaminated by agricultural run-off and remote sewage effluent |

***Data analysis*** - the differences between samples within a triathlon, between the samples taken along the recreative and competitive race and between triathlons were tested with a variance analysis on the log-transformed densities. For all parameters geometric mean densities (GMD) were calculated, only *S. aureus* densities were calculated as arithmetic means (AMD).

## RESULTS AND DISCUSSION

The preparatory analysis showed that the differences caused by sampling time and sampling location and at two sites sampling depth were significantly greater than differences between duplicates. Sampling location yielded the majority (56-90%) of the variation, sampling time 5-32% and depth 0.01-10% of the variation. Variation between duplicates caused <1% of the total variation. On the study days in 1993, samples were taken at the three locations (start, halfway and finish) the moment the triathletes passed each point and by moving the sampling bottle up and down between 0 and 30cm depth. In 1994, sampling frequency was increased and samples were taken from boats accompanying the swimmers, just before the first swimmer and just after the last swimmer at 8-13 locations over the swimming course. Figure 1 shows the variation of a range of microorganisms. The variation of the indicator bacteria density is highest at Hoorn (triathlon 1), the site with the lowest densities of all bacterial indicators in this study. In this lake, the variation was caused by variation between sampling locations: the offshore samples had consistently lower densities than the near-shore samples. For thermotolerant coliforms, the triathlon sites provided a range of different water qualities. Ranking of the sites by *E. coli* densities gave approximately the same order as ranking by TC densities, but ranking by FE or F-specfic RNA phage density was clearly different (Figure 1, Table 2). The geometric mean densities of thermotolerant coliforms and of *E. coli* were correlated with the risk of gastroenteritis (Asperen *et al.*, 1996. No correlation was found with FE, F-specific RNA phages, enteroviruses and *Aeromonas*. Testing for correlation with the gastroenteritis risk on the basis of the arithmetic mean density, median density or maximum density gave similar results.

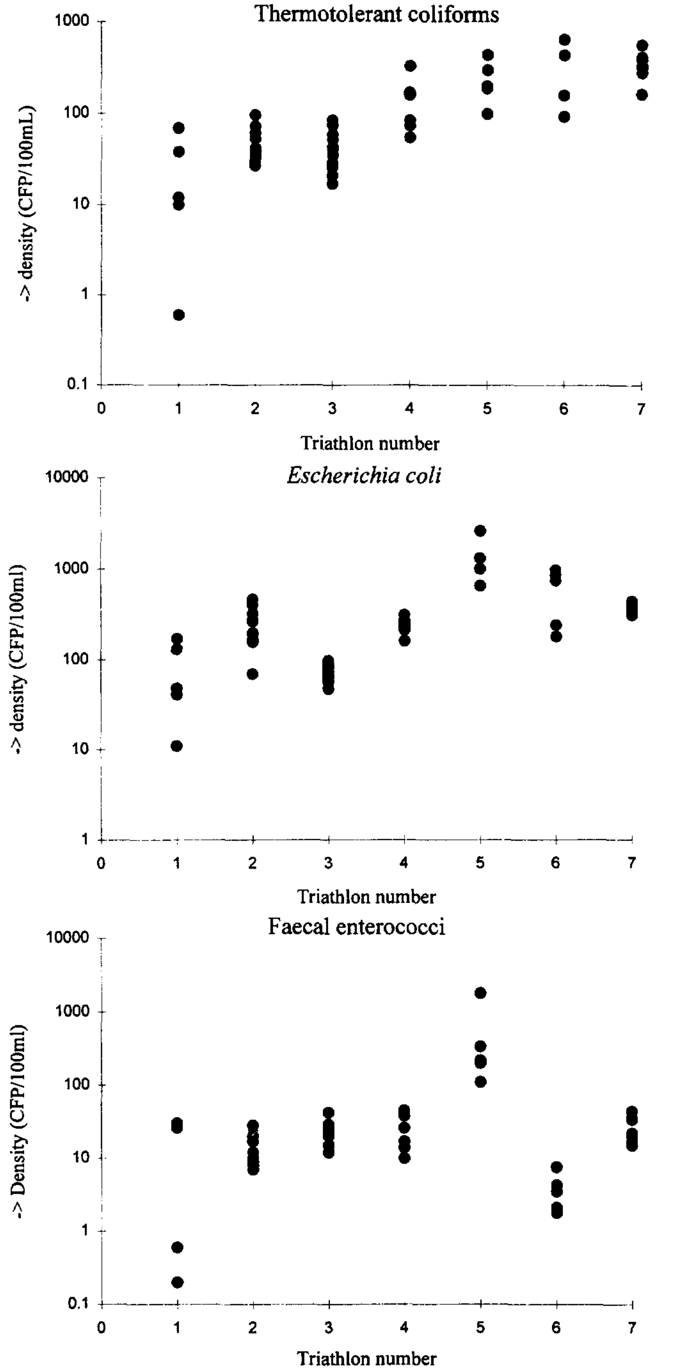


Figure 1. Variation in the microbial densities at the seven triathlons. [1 = Hoorn; 2 = Driebruggen; 3 = Schagen; 4 = Hellevoetsluis; 5 = Strijen; 6 = Gorkum; 7 = Leiden. The sites are ranked according to increasing TC densities. F-specific RNA phage densities of 0.01/100mL are densities below the detection limit].

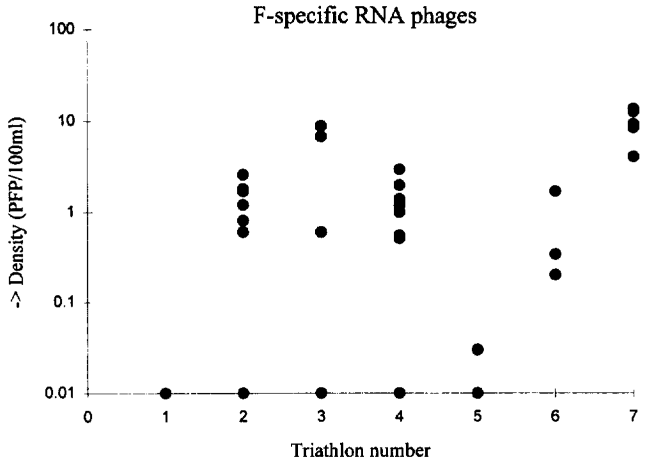


Figure 1 (contd). Variation in the microbial densities at the seven triathlons. [1 = Hoorn; 2 = Driebruggen; 3 = Schagen; 4 = Hellevoetsluis; 5 = Strijen; 6 = Gorkum; 7 = Leiden. The sites are ranked according to increasing TC densities. F-specific RNA phage densities of 0.01/100mL are densities below the detection limit].

Table 2. Geometeric mean concentration of microbiological contaminants in the water at the triathlons (in brackets: ranking of triathlons sites according to water quality)

| | *E coli* /100mL | TTC[1] /100mL | FE[2] /100mL | F+ RNA[3] /100mL | EV[4] /100L | *Aeromonas* /mL | *S aureus* /100mL[5] |
|---|---|---|---|---|---|---|---|
| Leiden | 360 (3) | 330 (1) | 23 (2) | 8.9 (1) | 9 (2) | 190 (4) | 0.006 (4) |
| Gorkum | 480 (2) | 310 (2) | 3.4 (6) | 5.2 (3) | 2.5 (4) | 90 (6) | 3.6 (1) |
| Strijen | 1,200 (1) | 220 (3) | 310 (1) | 0.2 (6) | <1 (7) | 330 (1) | <0.04 (6) |
| Hellevoetsluis | 230 (5) | 120 (4) | 22 (3) | 7.9 (2) | 0.7 (5) | 290 (3) | 0.3 (3) |
| Schagen | 71 (6) | 39 (5) | 21 (4) | 1.2 (4) | 6 (3) | 94 (5) | 0.002 (5) |
| Driebruggen | 240 (4) | 37 (6) | 13 (5) | 1.1 (5) | <1 (6) | 320 (2) | 2.7 (2) |
| Hoorn | 54 (7) | 11 (7) | 1.8 (7) | <0.1 (7) | 11 (1) | 72 (7) | <0.04 (6) |

[1]TTC = total thermotolerant coliforms; [2]FE = faecal enterococci; [3]phage; [4]enteroviruses; [5]arithmetic mean

***Thermotolerant coliforms and E. coli*** - the GMD of the triathlons in Strijen, Leiden and Gorkum was above the EC bathing water directive (76/160/EEC, 1975) guideline value of 100/100mL, but below the EC imperative value of 2,000/100mL. The GMD of *E. coli* was higher than the TC GMD at all sites. Although the TC analysis on LSA at 44°C aims to enumerate *E. coli*, this method gave, on average, 3x lower counts than the TSA/TBA method for *E. coli*. The resuscitation step in the TSA/TBA method on TSA without selective supplements at 37°C probably recovers more injured *E. coli* than resuscitation on LSA at 25°C. Because 10 characteristic colonies on LSA per sample were subcultured in BGLB for confirmation, the accuracy of this method is lower than the TSA/TBA method where all colonies are tested. As the average confirmation rate from characteristic colonies from LSA was 83%, this cannot account for the observed differences between the thermotolerant coliform and *E. coli* analysis.

***Faecal enterococci*** - the geometric mean densities of FE were ≤23/100mL at 6/7 triathlons and little variation in GMD was observed between these six triathlons. In similar epidemiological studies in sea water, a positive correlation between enterococci density and the risk of gastroenteritis was found (Kay *et al.*, 1994). In the UK study, the "no-effect" enterococci density was 32/100mL which is above the GMD in 6/7 triathlons. Since little variation existed in enterococci densities between the different triathlons, the triathlete population could only be subdivided in a few categories in terms of exposure to faecal enterococci. This may have accounted for the absence of a correlation with gastroenteritis risk.

***F-specific RNA phages and enteroviruses*** - the triathlon at Strijen had the highest geometric mean *E. coli* density, together with the lowest enterovirus density and next lowest F-specific RNA phage density. In contrast, Hoorn had the highest enterovirus density together with the lowest density of all other faecal parameters. The high mean enterovirus density at Hoorn was determined by an extremely high enterovirus count (1,700/100L) in one of the three samples taken for virus analysis. This sample was taken at the start of the race. 30min before the start, triathletes had entered the water and stood waiting at the starting line. This may have caused a resuspension of sediment, containing viruses, in the water. An excretion by a triathlete is unlikely since no increase of other faecal indicators was observed. Apart from this sample, the ratio between the enterovirus GMD and F-specific RNA phage GMD is within the range Havelaar *et al.* (1993) have reported for enteroviruses and F-specific RNA phages in rivers and lakes. The mean enterovirus densities in Hoorn and the maximum enterovirus densities in Hoorn, Schagen and Leiden were higher than the imperative value of the EC directive (<1/10L).

***Correlations between water quality parameters*** - the ratio between *E. coli* and TC was 1:0.4 (Table 3) being much more constant than between other parameters. When correlations between microbiological parameters were tested by their GMD (or AMD), only between FE and *E. coli* did a correlation exist. Correlations between TC, *E. coli* and FE densities were also tested by the densities in individual samples. When data from all triathlons were pooled, *E. coli* and FE correlated well ($r = 0.83$, $P = <0.001$); *E. coli* and TC correlated well ($r = 0.48$, $P = <0.001$); correlation between TC and FE was borderline significant ($r = 0.26$, $P = \sim0.05$). At the level of individual triathlons, only in Hoorn and Strijen were significant correlations between all three indicators observed. In Leiden, Gorkum and Driebruggen no correlations existed; at Schagen only TC and FE densities were correlated and in Hellevoetsluis TC and *E.coli* densities were correlated.

Table 3. Geometric mean ratio between geometric mean densities of faecal parameters

| | Mean (range) | | | |
|---|---|---|---|---|
| | TTC | *E coli* | Enterococci | F+ RNA phages |
| *E coli* | 0.4<br>(0.2-0.9) | | | |
| Enterococci | 5.6<br>(0.7-91) | 15<br>(3.4-140) | | |
| F+ RNA phages | 59<br>(15-1,100) | 140<br>(29-6,000) | 10<br>(0.7-1,600) | |
| Enteroviruses | 2,200<br>(1,000-170,000 | 43,000<br>(4,900-330,000) | 2,300<br>(160-31,000) | 1,500<br>(200-11,000) |

## CONCLUSIONS

This study design allowed assessment of the exposure of subpopulations of triathletes to microbiological contaminants in water. At the fresh water sites studied, only the ratio between the geometric mean densities of *E. coli* and TC was constant. The ratio between the other microbiological parameters that were related to faecal pollution (FE, F-specific RNA phages, enteroviruses) varied considerably. Water quality standards relating to faecal pollution can, thus, only be based on parameters that show a significant correlation with risk of intestinal illness. Enumerating *E. coli* with the TSA/TBA method consistently yielded higher *E. coli* densities than the LSA method. The latter method is likely to underestimate the *E. coli* density, but replacement by the TSA/TBA method will result in less compliance with the EC Directive.

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