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Sanitary study of surface water and of the beach of a water sports and leisure complex

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SUMMARY

This report presents the parasitological, bacteriological, mycological and physicochemical data obtained from both surface water and beach sand of a lake used for water sports. These show that the lake is contaminated in both winter and spring by water which overflows from the River Maine, and is self-purified by a mechanism of 'lagunage'. In summer signs of pollution are at their lowest level although use of the complex is at its peak. Conversely, the amoebic flora, which is independent of the usual criteria of pollution, predominates in summer, and serves as a marker for the need for increased surveillance. The sand of the beaches does not appear to show any infectious hazard. Environmental pressure will doubtless change these data over a period of time, and it is planned to monitor this.

INTRODUCTION

Lac du Maine is a nautical centre comprising a complete sports complex (sail-boards, pedal boats, canoes and kayaks) and a bathing beach. The complex is situated a few minutes from the centre of Angers, in western France.

A parasitological, bacteriological, mycological and physicochemical study of this sports and leisure complex was carried out during 1983 to try to determine whether there was any risk of infection for the user.

MATERIALS AND METHODS

Sampling

Sampling was carried out quarterly, on 11 January, 19 April, 30 August and 8 November 1983. The sample sites were rigorously standardized in each series.

Four samples of water were taken from the lake (Fig. 1, points 1, 2, 3 and 4) on each occasion and two more (points 5 and 6) on 30 August and 8 November.

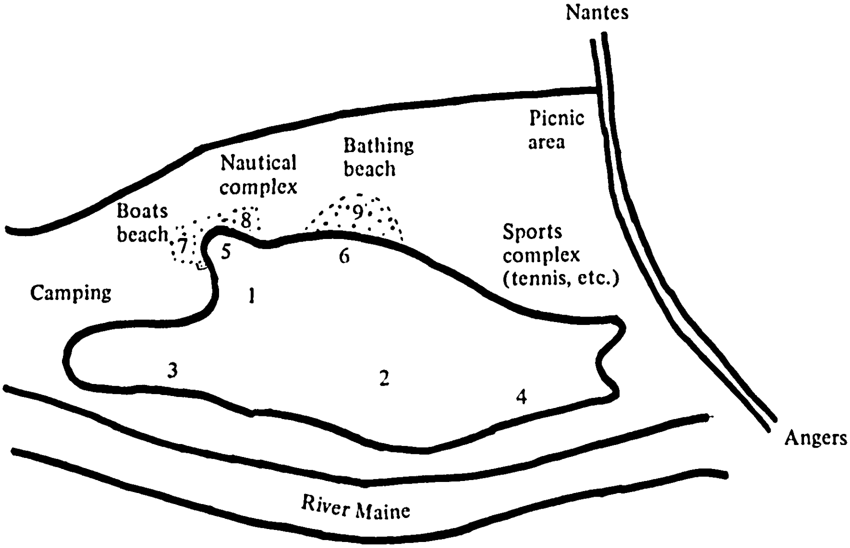

Fig. 1. Map of the Lac du Maine showing sampling points. *Samples in each season.* (1) On the edge; (2) nature reserve; (3) in the middle; (4) on the opposite edge. *Summer samples of lake water.* (5) Launching zone of sail-boards; (6) on the edge of the bathing area. *Sand samples.* (7, 8) Boats beach; (9) bathing beach.

The samples were taken at a depth of 50 cm, and the temperature of the water and of the ambient air were recorded.

Three series of superficial sand samples were collected on each occasion. Two were from the beach by the sail-boards (points 7 and 8), and one was from the bathing beach itself (point 9). Each series consisted of a central sample and four peripheral samples 1 m to the north, south, east and west of the central sample. All were taken with a sterile stainless steel soup spoon.

The five samples from each point were pooled to make a single sample of between 1·5 and 2 kg. Part of the sample was placed in a plastic bag which had been sterilized with ethylene oxide, and this was used for the parasitological and mycological study. The rest was placed in a sterile 250 ml glass container for bacteriology.

Laboratory tests were made within 2 h of collection of the samples.

Parasitological study

Lake water. The study was directed exclusively at the isolation of telluric amoebae, the methodology being constant by using a membrane filter (pore size 0·45 μ) and by incubating these membranes on an appropriate medium, as follows:

(*a*) The number of free amoebae per litre was determined by the most probable number method (MPP/litre) and incubation at 22 °C on the isolation medium; this allowed a global range of all the species of amoebae.

(*b*) Samples of 0·5, 1 and 2 ml were incubated on 2% monoxenic agar at 37, 41 and 44 °C to detect thermophilic amoebae pathogenic to man.

(*c*) Selective monoxenic agar media (Berentil) was inoculated with small amounts of water (0·1–15 ml) to detect pathogenic amoebae.

When positive, micropipettes were used under the inverted microscope for cloning, and the isolated strains were identified by following our previous method (Simitzis-le-Flohic, 1976; Simitzis-le-Flohic & Jacquemin, 1982; Simitzis-les-Flohic, Lejeuen & Chastel, 1983).

Beach Sand. The investigation involved the detection of *Toxocara* ova, a parasite of both cats and dogs.

A concentration method for the detection of *Toxocara* eggs was used on each sample of approximately 500 g after washing and riddling the sand. The technique of Laborde and his colleagues (1980) has been used by us previously (Chabasse, Bouchara & Rive, 1983), with excellent results.

Bacteriological study

This was carried out on both water and sand. On the latter, 10 g of each sample was washed under agitation in 100 ml of sterile distilled water for 2 h. The washing water was subjected to bacteriological testing: 10 ml of water was taken to represent 1 g of sand.

The study comprised: (i) the colony count at 22 and at 37 °C; (ii) colimetry and detection of coliform organisms in broth culture from lake water and also by filtration on a millipore membrane (pore size 0·045 μ) for the sand eluate; and (iii) *Staphylococcus aureus*, faecal streptococci and salmonellas were counted by a membrane filtration technique.

Mycological study

Lake water. At each sampling point 500 ml of lake water was filtered through two millipore membranes of 0·45 μ pore size. Each membrane was then transferred aseptically to a Petri dish containing standard mycology medium (Tifomycine-Sabouraud medium) with and without Actidione.

The Petri dishes were incubated at 22 °C and each yeast colony isolated was identified according to the criteria defined by Lodder (1970) and Barnett & Pankhurst (1974).

Sand. Every sample was investigated as follows: Keratinophile earth fungi (especially geophile dermatophytes) were isolated by using the trapping technique on children's hair as described by Vanbreuseghem (1952) and modified by de Clercq & de Vroey (1981).

Yeasts were isolated by shaking 10 g of sand in 100 ml of water. The supernatant was filtered through a millipore membrane which was then incubated on Sabouraud agar.

Physico-chemical study

The pH, detection of organic substances in alkaline medium and the detection of chlorides, nitrates, nitrites, sulphates, ammoniacal nitrogen and ferrous ions were all determined. This was carried out only on lake water (1 litre per sample); the methods and references are published by the French Association of Normalisation (AFNOR, 1983).

Table 1. *Physico-chemical parameters of waters of the lake (mean values)*

	Winter 12. i. 83	Spring 19. iv. 83	Summer 30. viii. 83	Autumn 8. xi. 83
The air temperature (°C)	2	15	14	14
The water temperature (°C)	6	12	21	11
pH	7·7	7·6	7·8	8
Electrical resistivity (OHMS)	3098	3216	4352	2967
Water hardness (°F)	14·9	15·6	14·2	14·3
Alkalinity (mg/l)	108	113	134	122
Organic materials (mg/l)	5	5·5	4	4·1
Chlorides (mg/l)	44·5	36·8	45·5	44·5
Nitrates (mg/l)	16	13·9	1	0·8
Nitrites (mg/l)	0·03	0·03	0·03	0·03
Ammonia (mg/l)	0	0	0·5	0·159
Sulphates (mg/l)	38·8	22	23·5	18·5
Iron (mg/l)	0·32	0·33	0·35	0·075

Table 2. *Bacterial flora of the waters and the beaches of the lake*

	Winter 12. i. 83	Spring 19. iv. 83	Summer 30. viii. 83	Autumn 8. xi. 83
Per ml of water (w) or per gram of sand (s)				
After 24 h at 37 °C	386 (w)	496 (w)	139 (w)	638 (w)
	168500 (s)	157500 (s)	46900 (s)	16866 (s)
After 72 h at 22 °C	711 (w)	1672 (w)	469 (w)	367 (w)
	220000 (s)	160000 (s)	315500 (s)	187375 (s)
Per 100 ml of water (w) or per gram of sand (s)				
Coliforms	190 (w)	6375 (w)	31 (w)	528 (w)
	66 (s)	0 (s)	4 (s)	2 (s)
Esch. coli	47 (w)	397 (w)	24 (w)	313 (w)
	0 (s)	0 (s)	0 (s)	0 (s)
Faecal streptococci	45 (w)	32 (w)	4 (w)	22 (w)
	1 (s)	0 (s)	0 (s)	0 (s)

RESULTS

The physico-chemical parameters and bacterial counts obtained are shown in Tables 1 and 2. Each figure is an average of the sample results at each sampling point on any one occasion.

Parasitology

Lake water. The amoebic population was generally constant in all samples in each season (Table 3). It was found to be low except in summer, when the water temperature reaches 21 °C. In summer the NPP/litre is as high as 4600 at 22 °C at the edge of the bathing beach, and at the launching area for the sail-boards in the shallow water at the edge of the lake, owing to the heat of the sun.

None of the species of amoebae isolated can be considered pathogenic to man (De Jonckheere, Melard & Phillippent, 1983; De Lattre & Oger, 1981; Simitzis-

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Table 3. *Free amoebas found in the waters of the lake*

	Winter 12. i. 83	Spring 19. iv. 83	Summer 30. viii. 83	Autumn 8. xi. 83
Acanthamoeba	+	+	+	+
Arcella	0	0	+	+
Glaeseria	+	+	+	0
Hartmanella	+	+	+	+
Mayorella	0	0	+	+
Naegleria	0	0	+	0
Saccamoeba	0	0	+	+
Vannella	0	0	+	+
Vahlkampfia	+	+	+	0

le-Flohic, 1984; Willaert & Stevens, 1976); *Naegleria fowleri* was not isolated, and there was no correlation between free amoebas and other hydrological data.

Sand. We particularly looked for *Toxocara* ova in the sand, but did not find any in any sample. The beaches are kept free from contamination by dog or cat faeces, and this accounts for the negative *Toxocara* findings. We were thus not able to substantiate any risk of toxocariasis from these beaches.

Bacteriology

Lake water. According to the French official standards for bathing (Anonymous, 1969), the results obtained with the different samples taken during the four seasons gave the following results: 12 January, 'water of good quality'; 14 April, 'polluted water, outside accepted limits'; 30 August, 'water of very good quality'; 8 November, 'water of average quality'.

There was no significant difference, in any season, between any of the sites sampled.

Staphylococcus aureus was not found in any sample. These organisms are, of course, not considered to be an index of human faecal pollution, but rather as human skin and mucous membrane flora. Of more interest, one species of *Salmonella* was found at sampling point 1 in the spring sample–that is, during the flood season.

Beach sand. The results show that in general the sand seems less polluted than the water. It is also apparent that, despite the mixing effect of the water, a single sample from one site does not give a true representation of the bacteriological flora of the beach.

Mycological study

Lake Water. At least 18 different species were isolated (Table 4) among which 4 belonged to the genus *Candida*: none appeared to be pathogenic. Some have previously been found in similar biotypes (Cooke, 1960; Hinzelin, 1977; Hinzelin & Lectard, 1978). Some yeasts, such as *Debaromyces hanseni*, the main species found in sea water (Hinzelin & Lectard, 1978; Kolesnitskaya & Maximova, 1982) were present in each series. Others (*Saccharomyces*, *Geotrichum candidum*) appear to be related to faecal contamination (Ekundayo, 1983; Simard, 1971). These were found in samples taken in winter and spring when the river Maine overflows into the lake.

Table 4. *Yeasts in surface waters of the lake*

	Winter 12. i. 83	Spring 19. iv. 83	Summer 30. viii. 83	Autumn 8. xi. 83
Debaryomyces hansenii	+	+	+	+
Candida boidinii	+	0	0	0
C. melibiosica	+	0	0	0
C. lipolytica	0	0	+	0
C. berthetii	0	0	0	+
Torulopsis dattila	+	0	0	0
T. cantarelli	0	0	+	0
Trichosporon capitatum	+	+	0	0
Rhodotorula sp.	0	+	0	0
Sporoboromyces	+	0	0	0
Lipomyces	+	0	0	0
Kluveromyces bulgaricus	+	0	0	0
Saccharomyces diastaticus	0	+	0	0
S. cerevisiae	+	0	0	0
Bullera alba	0	0	+	0
Geotrichum candidum	+	+	0	0
Non-identified yeasts	+	+	+	+

Table 5. *Fungal flora found in the sand of the beaches*

	Winter 12. i. 83	Spring 19. iv. 83	Summer 30. viii. 83	Autumn 8. xi. 83
Trichophyton terrestre	0	0	+	+
Chrysosporium pannorum	+	0	+	0
C. keratinophilum	+	+	+	+
Anixiopsis stercoraria	+	+	+	+
Arthroderma curreyi	0	0	0	+
Geotrichum candidum	+	+	0	0
Trichosporon capitatum	0	+	0	0

Sand. No fungal strain which could be classified as a dermatophyte was discovered (Table 5). *Trichophyton terrestre* is a banal telluric saprophyte, often isolated from sand. Among the other keratinophiles, *Chrysosporium* species are frequently found, especially *Chrysosporium keratinophilum* and its sexual form *Anixiopsis stercoraria*. At present these fungi are considered to have a very limited pathogenic role (Ghcho & Guinet, 1982; Quadripur, 1979; Rippon, Lee & McMillen, 1970; Todaro & Crisco, 1984). None of the yeasts appears to present a risk to man. In particular, *Candida albicans* and *Cryptococcus neoformans* were not isolated. The presence of *Geotrichum candidum* in samples taken in spring is due to contamination from flooding.

Hydrological study

This study was carried out on surface water of the lake. The pH of the water was between 7·62 and 8·17 (normal limits are pH 6 to pH 9). Electrical resistivity was that of good quality water.

The lake water content of organic material is not high: values outside flood seasons (summer–autumn) resemble those of drinking water (< 3 mg/l). During

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winter and spring flooding organic content and nitrates increase. This may be due to chemical pollution, as, for example, following the use of agricultural fertilizers. Despite such seasonal variations, the results are all within tolerable limits.

DISCUSSION

The study proved to be of value in assessing the possibility of infection with pathogenic organisms when frequenting a lagoon of this type. The parasitological, bacteriological, mycological and physico-chemical findings all show that both lagoon water and beach sand do not present a serious risk for users so far as known transmissible infectious disease is concerned in summer. During August, when the temperature of the water reached 21 °C and was thus subject to bacterial and fungal multiplication, official standards indicated the water to be 'of good quality' and the lowest active microbiological content was when the use of the lagoon was at its maximum. In April, however, and to a lesser degree in January, faecal pollution was shown to be present, *Salmonella infantis* being isolated on 19 April 1983. This was probably due to flooding of the River Maine into the lagoon.

The low bacterial content of the lagoon during summer may well be due to the bulk of micro-organisms finding conditions inimical to their survival: alternatively the 'Lac du Maine' may represent a model of 'lagunage' with real self-purification taking place. Walker & Leclerc, (1973) postulate the operation of two simultaneous mechanisms, both physical and biological, the former being associated with sedimentation and the depositing of mud and organic sludge in which organisms are present, the latter corresponding to the competitive balance between different forms of life. In this manner faecal organisms are eliminated from the surface of the water, the principle of 'lagunage' being similarly applied to the purification of urban biological effluent in both natural and artificially constructed basins. The efficiency of such systems is not proven (Kabler, 1959; Cody & Tisher, 1965), though Joshi, Parhad & Rao (1973) consider that some 90% of 'coliform' organisms and 99% of salmonellas are eliminated.

To achieve this balance it is essential that the microbiological pollution of the lake does not exceed its capacity for self purification. Following pollution due to flooding, natural recovery takes place so that by summer, when most aquatic activity is taking place, order has been restored. Seasonal fluctuation of this kind has little influence on the presence of free amoebae which seem to be independent of classical pollution criteria and the 'lagunage' phenomenon.

When the water is at its highest temperature in summer the presence of thermophilic organisms which are capable of survival at temperatures of 41 °C and over provide a valuable criterion during surveillance. Griffin (1972), de Jonckheere, Vandijck & Van De Voorde (1975) and Wellings *et al.* (1977) all attribute the presence of such pathogens to this thermal phenomenon.

The presence of *Naegleria fowleri* has been confirmed in water in the USA (Willaert & Stevens, 1976), Belgium (De Jonckheere, Melard & Phillipent, 1983), Czechoslovakia (Cerva, Jeona & Hyhlik, 1980), and France (Dive, De Lattre & Leclerc, 1982), the latter being discovered in a cooling water reservoir of an electrical power station near Metz: it was found to be present in July and had disappeared by November.

To date there has been no reported case in France of meningo-encephalitis caused by free-living amoebae, though surveillance must continue: environmental changes such as progressive urbanization and increasing human use of aquatic facilities like those described can lead to the intrusion of pathogenic micro-organisms where none had been isolated previously.

Indeed, it is incredible that French legislation has so far totally ignored the need for adequate standards for bathing water. Faced with the need for increasing aquatic leisure facilities near to towns, picnic areas and camp sites, due to both migration of indigenous human population from city to countryside and an influx of foreign visitors in summer, it is vital to know whether nature self-purification capacity will be adequate to deal with such sudden pollution from human sources. The enrichment of beach sand with large amounts of human keratin may well eventually lead to favourable conditions for an increase in numbers of pathogenic keratinophilic fungi. Hence such developing natural areas would seem to require microbiological surveillance, especially when they are subjected to the kind of environmental pressure described. In this way the evolution of microbiological species and populations in response to physico-chemical conditions and their transfer from human sources may be usefully followed: in particular the surveillance of amoebic fauna, so conveniently overlooked by French legislation (*Journal Officiel*, 7 avril 1981) may prove to be life saving by detecting the presence of such organisms and preventing human exposure to them.

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