

***Cercarial dermatitis* in the Netherlands caused by *Trichobilharzia* spp.**

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ABSTRACT

Outbreaks of *cercarial dermatitis* which occurred in recreational lakes in the Netherlands were studied and a method for direct rapid detection of the parasite *Trichobilharzia* in water samples was developed. A standardized questionnaire with questions on health complaints and exposure was distributed to individuals who developed symptoms of *cercarial dermatitis* after visiting fresh water lakes. Snails from the suspected lakes were examined for the presence of *Trichobilharzia* by microscopy and PCR. Water samples were concentrated by filtration and examined by PCR. Water quality was tested according to European Bathing Water Directive 76/160/EEG. *Trichobilharzia* was detected in snails and water samples from lakes which met European bathing water standards. Despite a response of 25.5%, epidemiological data suggested that longer and more frequent exposure to the water resulted in increased reporting of symptoms of *cercarial dermatitis* and confirmed the importance of exposure as a risk factor. A novel method for direct detection of *Trichobilharzia*, which includes concentration of water samples by filtration and detection of the parasite by PCR proved to be a valuable and simple tool for confirmation of presumptive outbreaks, particularly when snails could not be found in the suspected water and public health protecting measures were necessary.

Key words | *cercarial dermatitis*, recreational water, schistosomes, swimmers' itch, *Trichobilharzia*, water quality

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ABBREVIATIONS

DNA	Deoxyribo Nucleic Acid
G	gravitation force
ITS	Internal Transcribed Spacer
PCR	Polymerase Chain Reaction
rpm	rounds per minute

INTRODUCTION

Recreational or occupational exposure to microbiologically contaminated surface water may have adverse health effects. To protect public health, the European Bathing Water Directive 76/160/EEG (Anonymous 1976) has been operational since 1976. According to this Directive,

member states of the European Union have to test water quality at their official bathing sites for compliance with imperative values for total and faecal coliforms. However, several epidemiological studies (Kay *et al.* 1994; Van Asperen *et al.* 1998; Wiedenmann *et al.* 2006) have shown that these faecal indicator parameters do not optimally reflect the presence of faecal contamination of human or animal origin and that current guideline values for these parameters do not sufficiently protect public health. Responding to these new findings, the European Committee and the European Parliament have recently agreed on revised bathing water legislation including new faecal indicator parameters and more stringent guideline values (Anonymous 2006a). Compliance with the imperative values for the new parameters *Escherichia coli* and

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intestinal enterococci, which display a much better relation between faecal contamination and negative health effects in bathers, however, will not protect bathers from illness caused by micro-organisms of non-faecal origin including pathogens causing eye, ear or skin conditions. In 2003 and 2004 about 40% of the reported incidents of water related health complaints in the Netherlands were skin conditions, presumably due to schistosomes or toxic cyanobacteria (Schets & de Roda Husman 2005a, b). In 2005, 59% of the reported outbreaks were incidents of skin complaints; 55% of these outbreaks patients had symptoms of swimmers' itch (Schets & de Roda Husman 2007).

Swimmers' itch or *cercarial dermatitis* is the result of the penetration of the human skin by cercariae of non-human schistosomes. *Cercarial dermatitis* is considered an emerging disease in Europe (De Gentile *et al.* 1996). Due to high eutrophication of the water and colonization of water bodies by susceptible snails and nesting ducks the number of outbreaks of *cercarial dermatitis* has increased (De Gentile *et al.* 1996). In Europe the most frequently reported species causing swimmers' itch belong to the genus *Trichobilharzia* (De Gentile *et al.* 1996). These bird schistosomes have a two-host life cycle with freshwater snails as their intermediate host and waterfowl as their final host. Development of larvae takes place in freshwater bodies which may also be used for recreational activities.

Each summer, outbreaks of presumptive *cercarial dermatitis* in freshwater lakes are reported in the Netherlands (Schets & de Roda Husman 2005a, b). Generally the affected bathers are diagnosed solely on the basis of their health complaints consisting of reddening of the skin, an itching sensation and macular eruptions of different size and intensity. The diagnosis is seldom confirmed by detection of *Trichobilharzia spp.* in water or snails. In the summer of 2004, two outbreaks of presumptive *cercarial dermatitis* were investigated; water and snails were examined for the presence of *Trichobilharzia spp.* and bathers were questioned for exposure to the suspected water and symptoms of swimmers' itch. Water quality was tested according to European Bathing Water Directive 76/160/EEG. In 2005 and 2006 a novel method for direct detection of *Trichobilharzia* in water samples was tested at two outbreak sites.

METHODS

Site description recreational lake the Wed

The Wed is a dune lake located in the Kennemer dunes along the North-Holland coast. It is a former sand reclamation originating from the 1950s. The lake covers about 30,000 m²; the average water depth is 3–4 m, with a maximum of 9 m. Green algae (*Characeae*) are present in the water and mallards (*Anas platyrhynchos*) and European herring gulls (*Larus argentatus*) are regular visitors of the lake. During the summer months, children participating in holiday camps spend several days together playing in the dunes and swimming in the Wed or one of the other dune lakes nearby. The Wed has a history of swimmers' itch.

Site description recreational lake the IJzeren Man

The IJzeren Man is a recreational lake within the city boundaries of Eindhoven. The isolated lake is a former sand reclamation originating from the 1930s. It covers about 40,000 m² and has a maximum water depth of 3–4 m. Heavy growth of waterweed (*Elodea canadensis*), particularly in the deeper parts of the lake, has been reported. Before the start of the bathing season, the waterweed is mechanically removed, but the plants rapidly grow to their original size. Mallards (*Anas platyrhynchos*), great crested grebes (*Podiceps cristatus*) and cormorants (*Phalacrocorax carbo*) colonize the lake. The IJzeren Man is used for both swimming and other recreational activities such as sailing, windsurfing, rowing and cycling by pedal boat. The IJzeren Man has a history of swimmers' itch.

Outbreak report the Wed

On July 22nd, 2004, the public health services of the Kennemerland region reported that 11 of 170 children attending a children's holiday camp developed a skin condition with macular eruptions and mild to severe itching after swimming in the Wed on July 16th. The local authorities released a press communication to inform the public of possible swimmers' itch. Despite information in the local news papers, numerous bathers continued visiting the lake and many other cases of presumptive *cercarial dermatitis*

were subsequently reported to the public health services. In July 2005 the public health services of the Kennemerland region received renewed reports of swimmers' itch among school children after swimming in the Wed.

Outbreak report the IJzeren Man

On August 11th, 2004, the municipal health services of the city of Eindhoven reported skin complaints among 30 people who swam in the IJzeren Man on August 8th and 9th. All 30 people visited general practitioners who diagnosed *cercarial dermatitis*. The manager of the IJzeren Man informed the visitors of possible swimmers' itch and advised showering after swimming. A period of bad weather during which visits to the IJzeren Man declined to a very low level was followed by a weekend of fair weather on 4–5 September. On September 7th new cases of *cercarial dermatitis* were reported and the IJzeren Man was subsequently closed for the remainder of the bathing season.

Epidemiological investigation

Following the reported skin complaints associated with the Wed, a standardized questionnaire was designed for participants of the holiday camp. The questionnaire was set up to obtain information about age, sex, the duration of water exposure for each day from July 26th up until July 30th 2004, the part of the lake where persons swam, nature of symptoms (tick boxes for diarrhoea, vomiting, itch, reddening of the skin, skin eruptions, fever, headache or 'other complaints'), onset and duration of symptoms. Participants were also asked to provide information on the size of skin eruptions, location of the eruptions on the body, previous complaints of itching skin eruptions and swimming in other open waters during the 2004 bathing season. However, the forms could not be distributed to participants of the holiday camp from which the first cases were reported because the camp management refused collaboration. Nevertheless, the management of two other simultaneously held holiday camps, from which cases of presumptive *cercarial dermatitis* were also reported, agreed to collaborate. On July 29th questionnaires were distributed to 200 participants.

Questions for visitors of the IJzeren Man were identical, but comprised the period August 5th up until August 9th 2004. Due to logistical problems, the forms could not be distributed to all people visiting the IJzeren Man, but only ten cases who consulted a general practitioner could be asked to complete the form.

Environmental investigation

Both the Wed and the IJzeren Man are official European bathing sites at which water quality was tested at a two-weekly interval. The water was examined for the presence of total and faecal coliforms by using membrane filtration methods according to Dutch standards (Anonymous 1982a, b); pH was measured and the aesthetic parameters transparency, colour, odour, presence of scum, oil and litter were assessed.

Approximately 500 snails were collected from the Wed on both July 23rd and 30th, 2004. On August 12th, 2004, 25 snails were collected from the IJzeren Man, whereas 60 snails were collected from this lake on September 7th. The snails were transported to the laboratory in a container with water from the lake from which they were collected. Shell height was measured and the snails were identified using an identification key (Werkgroep Zwemmersjeuk 2004; <http://www.infamil.nl/contents/pages/132715/zwemmersjeukprotocolnov2004.pdf>). The snails were transferred to a beaker with fresh tap water which was placed under a desk lamp for approximately 3 h to stimulate release of cercariae (Sluiter *et al.* 1980). The tap water was subsequently examined for the presence of ocellate furcocercariae by microscopy (magnification 50–100 ×).

For isolation of Desoxyribo Nucleic Acid (DNA), a volume of 30–50 ml of the tap water in which the snails were stimulated to shed cercariae was filtered through a 0.4 µm pore size polycarbonate membrane filter (Isopore; Millipore Corporate, Billerica, USA). DNA was extracted by using a DNeasy kit (Qiagen Benelux BV, Venlo, the Netherlands) according to the manufacturers' instructions. Polymerase Chain Reaction (PCR) detection of the *Trichobilharzia* tandem repeated DNA sequence ToSau3A was performed as described by Hertel *et al.* (2002). The isolated PCR products were sequenced directly. PCR detection of

the ITS1 and ITS2 regions was performed as described by Dvorák *et al.* (2002).

On July 18th, 2005 water samples (20 L) were taken from the Wed at a beach with onshore wind and a beach with offshore wind. Small snails (< 4 mm shell height) were abundant in the bathing area, but were not collected; larger snails were collected. Water and snails were transported to the laboratory for analyses. Shedding of cercaria from snails was stimulated as described previously. Water samples were concentrated by filtration through Envirochek filtration capsules (Pall Gelman Laboratory, Ann Arbor MI, USA) at a flow rate of 2 L/min following the manufacturers' instructions and ISO 15553 (Anonymous 2006b). Filtered samples were eluted from the filter with approximately 130 ml elution buffer (Anonymous 2006b) by agitation in a wrist-action laboratory shaker for 5 min at 600 rpm. After decanting the eluate into a 250 ml conical centrifuge tube, the elution procedure was repeated. After centrifugation (10 min, 1080 × G, without centrifuge break) of the total eluate the supernatant was aspirated. DNA was extracted from the pellet and PCR was performed as described earlier.

On July 24th, 2006, public health services Zuidoost Brabant and district water board Aa en Maas reported tens of cases of swimmers' itch among people who visited the recreational lake Prinsenmeer during the previous three weeks. Four water samples were taken from this lake on July 27th. Sample concentration, DNA extraction and PCR of the ToSau3A repeat were performed as described above.

RESULTS AND DISCUSSION

Epidemiological investigation

Fifty-one (25.5%) of the forms which were distributed to visitors of the Wed were returned. The respondents were (parents of) children from three months to 13 years of age, 25 girls and 26 boys (Figure 1). Twenty-seven (53%) of the respondents (16 girls, 11 boys) reported adverse health effects after swimming in the Wed. Ninety-six percent of the children with health complaints reported skin conditions which comprised skin eruptions as a single symptom or in combination with itching or red macula (Table 1). The macular eruptions generally appeared on arms, legs and trunk. Twenty of the 27 (74%) children with symptoms had

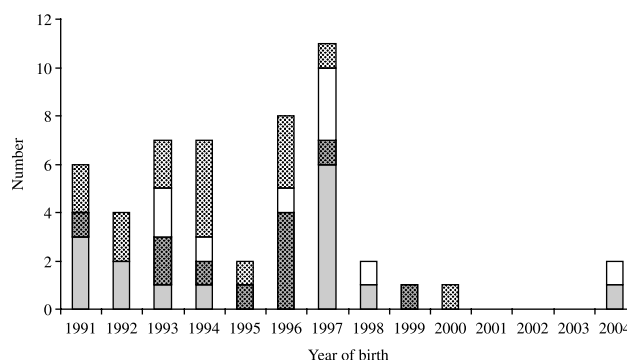


Figure 1 | Distribution of age, sex and presence or absence of health complaints among children who visited the Wed in the summer of 2004. ■ boys with complaints; □ boys without complaints; ▨ girls with complaints; □ girls without complaints.

no history of similar symptoms after swimming in open water in 2004 or previous years, two (7.4%) did not know and five (18.5%) reported to have had similar symptoms after swimming in dune lakes, including the Wed, in 2003.

On average, children with skin complaints had contact with the water on 3.6 days of the 5-day study period, whereas children without complaints had water contact on 3.2 days of these 5 days (Table 2). In general, children with symptoms spent more time in the water on the days of exposure than children without complaints. Both groups almost equally used the deep and the shallow part of the lake for their recreational activities (Table 2).

Seven of the ten forms which were sent to cases from the IJzeren Man were returned. Six of seven persons

Table 1 | Frequency of observed health complaints among 27 children who visited the Wed during the study period in the summer of 2004

Health Complaint	Frequency	
	n	%
Diarrhoea	1	3.7
Vomiting	0	0
Itch*	20	74.1
Reddening of skin*	11	40.7
Skin eruptions*	25	92.6
Fever	1	3.7
Headache	3	11.1
Other	3	11.1

*In total 26 children reported at least one of these.

Table 2 | Bathing behaviour of children with and without health complaints who visited the Wed during the study period in the summer of 2004

	Children with complaints		Children without complaints	
	Number	(%)	Number	(%)
Bathing area visited				
Deep and shallow	19	(70.3)	18	(75.0)
Deep	4	(14.8)	2	(8.3)
Shallow	4	(14.8)	4	(16.7)
Number of days with water contact				
Total whole group	98		76	
Average	3.6		3.2	
Frequency of reported hours of water contact per day				
> 1	49	(50)	28	(37)
0.5 – 1	7	(7)	16	(21)
< 0.5	3	(3)	3	(4)
Don't know	39	(40)	29	(38)

reported itching, whether or not accompanied by macular eruptions, after swimming in both the deep and shallow part of the IJzeren Man on 3–6 August 2004. They all stayed in the water for more than two hours each day they swam and never had similar symptoms after swimming in open water before. The seventh person only visited the shallow part of the lake and reported fever and headache.

Despite the relatively low response, the results suggest that longer and more frequent exposure to the Wed resulted in an increase in reported symptoms of swimmers' itch. These results confirm previous epidemiological studies which identified exposure as one of the risk factors for *cercarial dermatitis* (Chamot *et al.* 1998; Verbrugge *et al.* 2004). The high attack rate (53%) observed in our study is probably biased since (parents of) children with complaints probably were more prone to return questionnaires than (parents of) children without complaints, resulting in a possible overestimation of the attack rate. Similar (55%) or higher (81–82%) attack rates are however known from other outbreak reports (Bourée & Caumes 2004).

Environmental investigation

During the 2004 bathing season, water quality in the IJzeren Man and the Wed complied with European bathing water

legislation (Table 3). Although ca. 1000 fresh water snails (*Lymnaea stagnalis*) from the Wed were examined in 2004, *Trichobilharzia* was neither detected microscopically nor by PCR. The collected snails had an approximate shell height of 4 mm. About 0.5% of the snails ($n = 3$) had shells of approximately 40 mm high, which is the average size of adult snails. The small, presumably young snails may not yet have been infected or may have had pre-patent infections which could not be detected in the laboratory. Both in field and laboratory studies with lymnaeid *Stagnicola elrodi* snails Graham (2003) demonstrated a positive relation between snail size and infection intensity, with larger snails displaying higher infection intensities and shedding larger numbers of cercariae. At the time of sampling in 2004 only a few larger snails could be retrieved from the bathing area at the Wed. Large specimens inhabiting the part of the lake which was difficult to reach for snail sampling may however have shed large numbers of cercariae, which were transported to the beach by onshore wind and water flow. It has been demonstrated that single infected snails can release huge numbers of cercariae (Sluiter *et al.* 1980), whereas in areas known for the occurrence of *cercarial dermatitis*, the prevalence of *Trichobilharzia* in *L. stagnalis* and *Radix sp.* is generally low (Kolárová *et al.* 1997; Niewiadomska *et al.* 1997; Loy & Haas 2001). In a pond

Table 3 | Microbiological, physical and esthetic parameters determined in water from the recreational lakes the IJzeren Man and the Wed during the 2004 bathing season and the standard values for these parameters as required by European Bathing Water Directive 76/160/EEG

Parameter	The IJzeren Man	The Wed	EU Standard
Total coliforms (cfu / 100 ml)	44*	43 [†]	10.000
Faecal coliforms (cfu / 100 ml)	83*	40 [†]	2.000
Transparency (m)	1.7*	0.9 [†]	1–2
pH [‡]	8.1–10.0	8.5–9.5	6–9
Water temperature (°C)	16–22	14–23	
Colour, odour, scum, oil, litter [§]	no abnormalities	no abnormalities	no abnormalities

*average of 7 data points.

[†]average of 6 data points.[‡]observed range.[§]identical observation on all sampling dates.

system in Southern Germany *T. ocellata* showed a constant prevalence of 0.17% in *L. stagnalis* during a 20 year study period (Loy & Haas 2001).

Two *Lymnaea stagnalis* snails with shells of approximately 35 mm high were collected from the Wed in 2005. One snail appeared dead, the other shed large numbers of *Trichobilharzia* cercariae as was confirmed by microscopy. PCR detection of the ToSau3A repeat confirmed the presence of *Trichobilharzia* in the snail and in the water sampled from the lake at the beach with onshore wind; the sample taken at the beach with offshore wind was negative. Sequence analysis of the obtained PCR product showed 95% similarity with *T. ocellata* DNA sequences in the GenBank database (GenBank accession no. AF442689). PCR detection of the ITS site confirmed the presence of *Trichobilharzia* DNA in the snail; sequence analysis of the PCR product showed 98% similarity with *T. szidati* DNA (GenBank accession no. AY713973). PCR products obtained after performing ITS PCR on water samples were purified for sequence analysis, which revealed *Echinostoma* DNA sequences but no *Trichobilharzia* sequences. Water quality was in compliance with European bathing water legislation (data not shown).

Although the symptoms suggested that the cases reported from the Wed in 2004 had *cercarial dermatitis*, the diagnosis was not supported by the detection of the parasite *Trichobilharzia*. The detection of *Trichobilharzia* in a water sample and a snail from the Wed in 2005 however confirmed the cases of presumptive *cercarial dermatitis*

reported in 2005 and supports the hypothesis that the children participating in the holiday camps in the summer of 2004 indeed had swimmers' itch.

Snails (*Radix* spp.) collected from the IJzeren Man in August 2004 did not shed ocellate furcocercariae; data on snail size are not available. The *Radix* spp. collected in September 2004 had shell heights ranging from 5 to 15 mm, with the majority of shells being 10 mm high. In the tap water in which release of cercariae from *Radix* spp. was stimulated, large numbers of ocellate furcocercariae were detected by microscopy. The presence of *Trichobilharzia* was confirmed by PCR detection of the ToSau3A repeat. ToSau3A positive bands were purified for sequence analysis. Comparison of the detected sequences with other sequences in the GenBank database showed 95% similarity with the ToSau3A region of *T. ocellata* (GenBank accession no. AF442689) and 94% with the ToSau3A region of *T. regenti* (GenBank accession no. AF442688). Sequence analysis of purified ITS PCR products encountered problems with *Echinostoma* DNA as described earlier.

In two of four water samples from Prinsenmeer taken in 2006, PCR detection of the ToSau3A repeat confirmed the presence of *Trichobilharzia*. Sequence analysis of the PCR products showed 95–97% similarity with *T. ocellata* DNA sequences in the GenBank database (GenBank accession no. AF442689). No snails were found in this lake and an epidemiological study was not performed. Water quality was in compliance with European bathing water legislation (data not shown).

The detection of *Trichobilharzia* in fresh water snails and water samples from recreational lakes which met European bathing water standards confirms that legislation based on faecal indicator parameters does not protect bathers from conditions caused by micro-organisms of non-faecal origin. Although the revised European Bathing Water Directive (Anonymous 2006a) does require appropriate monitoring and adequate management measures when proliferation of cyanobacteria, which are also of non-faecal origin, is expected and health risks are presumed, it does not address *cercarial dermatitis*.

The presence of *Trichobilharzia* in snails from the IJzeren Man in 2004, water and snails from the Wed in 2005 and water from Prinsenmeer in 2006 was demonstrated by microscopy and confirmed by PCR. Dvorák *et al.* (2002) showed that sequences of the internal transcribed spacers ITS1 and ITS2 can be used for species identification. We were however unable to obtain *Trichobilharzia* ITS sequences from the IJzeren Man and the water sample from the Wed due to the presence of *Echinostoma* DNA. The primers which were used in the ITS PCR (Dvorák *et al.* 2002) amplified a generic DNA fragment which resulted in equally sized PCR products for both *Trichobilharzia* and *Echinostoma*. For further characterization of the *Trichobilharzia* DNA optimization of the PCR protocol is required and will be performed. Species identification by sequence analysis was further hampered by the complicated phylogeny within the genus *Trichobilharzia*. Nomenclature is still subject of debate (Horák *et al.* 2002) and at present DNA sequence data are only available for four *Trichobilharzia* species: *T. ocellata*, *T. szidati*, *T. regenti* and *T. franki* (Rudolfová *et al.* 2005). Sequence analysis of the DNA of various *Trichobilharzia* isolates demonstrated that recent European descriptions of *T. ocellata* (including those from the Netherlands by Sluiter (1983)) were identical with *T. szidati*. We observed that ToSau3A sequences obtained from *L. stagnalis* from the Wed identified *T. ocellata*. ITS sequences obtained from the same specimen identified *T. szidati*, suggesting that these *Trichobilharzia* species may be identical.

We demonstrated that direct detection of *Trichobilharzia* in water samples by concentration followed by PCR is possible. The method however requires further evaluation since PCR can detect DNA from both live as well as

deteriorated organisms. Background signals of *Trichobilharzia* DNA in lakes which do and do not have a history of *cercarial dermatitis* need to be established. When sampling water for direct detection of *Trichobilharzia*, it is important to take into account the wind direction, and sample the upper water layer. Cercariae released from snails display a positive phototactic and a geonegative orientation and tend to be present in the upper layers of the water body (Horák *et al.* 2002). Cercariae are capable of active movement but wind and water flow transport them over longer distances (Horák *et al.* 2002). As we demonstrated, samples containing cercariae are most likely taken at beaches with onshore breeze. If this method appears broadly applicable, it saves the time-consuming and sometimes hazardous collection of snails and the tedious microscopic examination which does not always produce unambiguous results (Rudolfová *et al.* 2005). The method is a valuable tool for confirmation of presumptive outbreaks when snails cannot be found in the suspected water and public health protecting measures are necessary at short notice.

CONCLUSIONS

The detection of *Trichobilharzia* in fresh water snails and water samples from recreational lakes which met European bathing water standards confirmed that legislation based on faecal indicator parameters does not protect bathers from conditions caused by micro-organisms of non-faecal origin.

Despite a relatively low response, epidemiological data suggested that longer and more frequent exposure to the water resulted in an increase in reported symptoms of *cercarial dermatitis* and confirmed the importance of exposure as a risk factor.

Optimization of the current ITS PCR protocols is needed to enable *Trichobilharzia* species identification when DNA of other parasites such as *Echinostoma* is present.

A novel method for direct detection of *Trichobilharzia*, which includes concentration of a water sample by filtration and detection of the parasite by PCR proved a valuable and simple tool for confirmation of presumptive outbreaks, particularly when snails could not be found in

the suspected water and public health protecting measures were necessary.

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