

The microbiological quality of water in fish spas with *Garra rufa* fish, the Netherlands, October to November 2012

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In fish spas, clients may submerge their hands, feet or whole body in basins with *Garra rufa* fish, for dead skin removal. Skin infections may result from using these spas, transmitted from fish to clients, through either fish or water, or from client to client. The microbiological water quality was determined in 24 fish spas in 16 companies in the Netherlands through analysis of a single water sample per fish spa. Water samples were tested for the presence of *Aeromonas* spp., *Vibrio* spp., *Pseudomonas aeruginosa*, nontuberculous mycobacteria, and faecal indicator bacteria by using standard culture methods. The majority of the examined fish spas contained *Aeromonas* spp. (n=24), *P. aeruginosa* (n=18), *Vibrio* spp. (n=16) including *V. cholerae* non-O1/O139 and *V. vulnificus*, and several rapid growing *Mycobacterium* spp. (n=23) including *M. fortuitum*, *M. conceptionense*, *M. abscessus* and *M. chelonae*. Faecal contamination of the fish spa water was low. Based on the detected concentrations of *Aeromonas* spp., *Vibrio* spp., and *P. aeruginosa*, the detected *Mycobacterium* spp., and the health implications of these bacteria, the health risk from using fish spas is considered limited for healthy people with an intact skin and no underlying disease.

Introduction

Originating from Turkey, fish spas are increasingly popular and available throughout the world. In fish spas, clients submerge their feet, hands, or their whole body, in basins with *Garra rufa* fish that nibble dead and thickened skin from the submerged body parts during a 15 to 30 minutes treatment. *Garra rufa* are small toothless fish that belong to the carp family (*Cyprinidae*). The use of fish spas is mostly considered a cosmetic and relaxing treatment, but it is also offered to relieve skin disorders such as eczema and psoriasis [1-3]. Studies performed in Turkey and Austria suggested a beneficial effect of fish spa therapy, or

ichthyotherapy, on psoriasis. However, several factors could have contributed to this outcome, including limited follow-up of patients, selenium in the therapy water, and simultaneous ultraviolet light A (UVA) treatment [2,3].

Since the same water and fish are used for several simultaneous and subsequent clients, and the fish are potential carriers of pathogens, the most important health risk from fish spas is the possible transmission of infections [1]. The water temperature in fish spas is kept at 25–30 °C thus providing opportunities for many bacteria to thrive. Pathogens may be transmitted from fish to man, from water to man, and from man to man with water and/or fish. An appropriate water quality is important to reduce the risk of infection for clients. Conventional water treatment and disinfection are, however, not possible, because these processes would kill the fish [1]. Commonly, the water is filtered, but most applied filters do not remove micro-organisms and those that do, have little effect on micro-organisms in biofilms or on the fish skin [1]. Continuous partly refreshment of the water results in dilution of the microbial contamination and may have a beneficial effect on water quality when no fresh contamination is introduced. However, in daily practice, fish spas are continuously re-contaminated while in use [1].

The handling of fish, and keeping fish in crowded aquaria, may lead to chronic stress, reduced fish health, and poor fish immunity [4]. Apparently healthy fish may carry (human) pathogens without having visible symptoms. When such fish are exposed to unfavourable conditions, outbreaks of infectious diseases may occur among the fish, resulting in an increased number of waterborne bacteria in the spas, with an accompanying increased zoonotic risk of transmission to humans [5]. Recently, *Aeromonas sobria* was implicated as the cause of massive die-off in *Garra rufa* at

a breeding farm in Slovakia [6], and a batch of ill *Garra rufa* in England appeared to be contaminated with *Streptococcus agalactiae* [7].

Examination of several batches of *Garra rufa* that entered the United Kingdom (UK) from Indonesia and several other Asiatic countries, demonstrated the presence of, among others, *Aeromonas* spp., *Vibrio* spp., *Mycobacterium senegalense* and *Streptococcus agalactiae*. All of these bacteria are capable of causing disease in humans [7].

Concerns about possible transmission of infections from fish spas to humans have led to the publication of guidance [1] and risk assessment documents [8] in the UK and France respectively, and a negative advice against starting new companies or continuation of existing companies offering fish spa treatment in Belgium [9]. Fish spas have been banned in some provinces in Germany due to animal welfare considerations, and the German veterinary authority has drafted a requirements document for new fish spas [10]. Animal welfare and hygiene considerations have resulted in a ban of fish spas in several states in the United States and some provinces in Canada [1].

In this study, the water in a random selection of fish spas throughout the Netherlands was tested for the presence of *Aeromonas* spp., *Vibrio* spp., *Pseudomonas aeruginosa*, nontuberculous mycobacteria (NTM) and the faecal indicator bacteria *Escherichia coli* and intestinal enterococci, to assess the microbiological water quality. Possible implications for public health are discussed.

Materials and methods

Recruitment of participants and sampling of fish spas

A random selection of 25 companies that offered fish spa treatment was asked by letter to participate in the study in September 2012.

At each participating company, one fish spa was sampled, however, when several types of fish spas were present (hand, foot, body), one spa of each type was sampled. Whenever there was visible dirt on the fish spa walls, Enviro Swabs (3M) were used to take swab samples from these walls. Water samples of 1.5 L were taken according to ISO 19458:2006. All samples were transported to the laboratory in insulating containers with melting ice and analysed within 24 hours from sampling. Samples were taken in October and November 2012, during opening hours, almost always unannounced, and at different times during the sampling days.

Sample analyses

All water samples were examined for the number of *Escherichia coli* (in 10, and 40 mL volumes), intestinal enterococci (in 10, and 40 mL volumes), *Aeromonas*

spp. (in 0.1, and 1.0 mL volumes), and *Vibrio* spp. (in 0.1, 1, 10, and 40 mL volumes) by membrane filtration of appropriate volumes of the samples according to ISO 9308–1:2000 (Rapid Test), ISO 7899–2:2000, Havelaar et al. [11], and incubation of the membrane filters on Thiosulphate Citrate Bile salts Sucrose agar (TCBS; Tritium Microbiologie, Eindhoven, the Netherlands) at $36\pm 2^\circ\text{C}$ for 24 ± 4 hour, respectively. *Vibrio* characteristic colonies on TCBS were subcultured onto Tryptone Soy Agar (TSA; Oxoid, Badhoevedorp, the Netherlands) and incubated at $36\pm 2^\circ\text{C}$ for 20–24 hours.

From each sample, a maximum of five presumptive *Aeromonas* spp., and ten presumptive *Vibrio* spp. (five blue and five green colonies) were confirmed by using Matrix Assisted Laser Desorption Ionization Time of Flight mass spectrometry (MALDI–TOF) [12]. Occasionally, e.g. when large amounts of background flora were present, presumptive *E. coli* and enterococci were confirmed by MALDI–TOF.

P. aeruginosa was enumerated in 100 mL samples by using Pseudalert (IDEXX Laboratories Inc., Westbrook, ME, US) according to the manufacturer's instructions. A most probable number (MPN) table (provided by the manufacturer) was used to determine the *P. aeruginosa* concentration in the samples. Occasionally, the content of a fluorescent well was subjected to confirmation by MALDI–TOF.

For analysis of NTM, 1 L water samples were decontaminated by 30 minutes incubation with 0.005% cetylpyridinium chloride (CPC) at room temperature. Two 0.5 L fractions of the decontaminated samples were filtered through 0.45 μm pore size black membrane filters (Millipore, Amsterdam, the Netherlands). Subsequently, the two membrane filters were washed with 300 mL sterile distilled water, dissected, and placed onto the moderately selective sides (7H10) and the antimicrobial-supplemented highly selective sides (7H11) of two Middlebrook 7H10/7H11 agar bi-plates (Becton Dickinson, Erembodegem, Belgium). The plates were sealed in air permeable bags; one plate was incubated at 30°C and the other at 36°C for up to three weeks, with weekly inspection for growth of colonies with characteristic morphology. Presumptive mycobacterial colonies were picked, to a total of 10 per sample, and subcultured onto Middlebrook 7H10 agar plates supplemented with Delvocid (Tritium Microbiologie, Eindhoven, the Netherlands) at 30°C and 36°C , and identified by sequencing of the *rpoB* gene [13,14].

Water temperature of all samples was determined on site, whereas pH, turbidity, and conductivity were determined in the laboratory, using standard laboratory equipment.

TABLE 1

Physical–chemical and microbiological parameters in the water of 24 fish spas in 16 companies, the Netherlands, October–November 2012

Sample date (2012)	Company	Spa type and capacity	Water temperature (°C)	pH	Conductivity (µS/cm)	IE (n/100 mL)	<i>Aeromonas</i> spp. (n/100 mL)	<i>Vibrio</i> spp. (n/100 mL)	<i>Pseudomonas aeruginosa</i> (MPN/100 mL)
22 Oct	A	Foot, 1 person	29.5	8.43	451	0	30,000	0	124
22 Oct	A	Foot, 1 person	29.5	7.72	449	1	19,500	0	137
22 Oct	B	Foot, 2 persons	28.1	8.19	379	0	24,000	0	42
22 Oct	C	Foot, 2 persons	29.0	8.14	552	1	82,000	411	178
30 Oct	D	Foot, 4 persons	27.8	8.03	483	2	4,036	648	59
30 Oct	E	Body, 1 person	31.2	8.49	294	0	32	5.4	<1
30 Oct	F	Body, 1 person	31.3	8.48	296	3	5,491	87	6.4
30 Oct	F	Foot, 6 persons	31.3	8.59	251	0	4,145	270	150
30 Oct	G	Foot, 2 persons	25.0	8.15	225	0	109,000	0	144
06 Nov	H	Foot, 2 persons	26.8	8.49	348	0	5,864	2	1
06 Nov	H	Hand, 2 persons	26.8	8.53	357	0	2,909	3	<1
06 Nov	I	Foot, 4 persons	28.6	8.09	483	0	32,700	4,290	<1
06 Nov	J	Foot, 4 persons	28.1	8.29	356	0	20,000	0	24
06 Nov	K	Foot, 2 persons	27.8	8.32	513	18	30,500	0	>200
06 Nov	K	Hand, 2 persons	27.8	7.83	632	13	3,955	0	<1
06 Nov	L	Foot/hand, 1 person	33.0	7.36	1,083	0	18,500	6,900	>200
06 Nov	L	Body, 1 person	33.0	7.46	1,423	310	20,500	1,652	>200
06 Nov	L	Body, 2 persons	33.2	7.70	929	1	1,500	1,908	>200
13 Nov	M	Foot, 1 person	27.6	6.86	453	0	3,182	11	<1
13 Nov	M	Hand, 1 person	27.6	7.82	395	0	355	14	<1
13 Nov	N	Foot, 2 persons	28.6	6.98	416	0	16,500	850	1
13 Nov	N	Body, 1 person	28.6	8.23	333	0	2,800	34	14
13 Nov	O	Foot, 2 persons	27.6	7.74	218	0	44,000	11	2
14 Nov	P	Foot, 1 person	27.8	8.11	470	0	100	0	88
Minimum values			25.0	6.86	218	0	32	0	<1
Maximum values			33.2	8.59	1,423	310	109,000	6,900	>200
Median values			28.4	8.12	432	0	11,182	11	33

IE: intestinal enterococci; MPN: most probable number.

Results

Participants and samples

Fifteen companies positively responded to the request to participate in the study, a sixteenth company was included through intervention of the local public health service. The sixteen participating companies included wellness centres (n=4), beauty salons (n=1), and companies that solely offered fish spa treatment (n=11). A total of 24 samples were collected: 15 samples from foot spas, five samples from body spas, three samples from hand spas, and one sample from a combined hand–foot spa. None of the fish spas had visible dirt on the spa walls, and therefore swab sampling of the spa walls was not done.

Physical-chemical water quality parameters

The water temperature in the spas ranged from 25.0 to 33.2 °C, with a median of 28.4 °C (Table 1). In body spas, the water temperature was generally higher than

in foot and hand spas. In four of five body spas, the water temperature was above 31 °C. The pH value of the fish spa water ranged from 6.9 to 8.6, with a median value of 8.1. In all fish spas, water turbidity values of 0.00 Formazine Turbidity Units (FTU) were measured, indicating that the turbidity was very low and below the detection limit of the equipment used. The conductivity ranged from 218 to 1,423 µS/cm, with a median of 432 µS/cm. The highest conductivity values were exclusively found in the three spas of one company (Table 1).

Microbiological water quality

In most fish spas, faecal contamination based on the faecal indicator parameter intestinal enterococci was limited (Table 1). The *E. coli* analyses were seriously hampered by abundant growth of *Plesiomonas shigelloides*, which may have masked *E. coli* colonies. We therefore consider the *E. coli* data unreliable. *Aeromonas* spp. were present in all samples from all

TABLE 2

Water treatment and management in 16 fish spa companies, the Netherlands, 2012

Parameter/management	Number of companies	
	Yes	No
Water filter present	16	0
Water filter per fish spa	13	3
One water filter for several fish spas	3	13
UVC treatment of the water only	5	11
Ozone treatment of the water only	4	12
UVC and ozone treatment of the water	6	10
Neither UVC nor ozone treatment of the water	1	15
Monitoring of water temperature	15	1
Microbiological water testing	5	11
Keeping a log of water quality	11	5
Complete water replacement ^a	6	9
Partial water replacement ^b	9	6
No water replacement, only replenishment	1	15

UVC: ultraviolet light C.

^a Once to twice a week.

^b Daily to once to twice a week, for 10 to 80%.

fish spas, although in varying numbers, ranging from 32 to 1.10^5 colony forming units (CFU) per 100 mL (Table 1). The median value of 1.10^4 CFU per 100 mL indicates that most numbers were on the high end of the range.

P. aeruginosa was detected in 18 of the 24 examined fish spas, while in six of 24 spas the most probable number was below the Pseudalert lowest detection limit of 1 per 100 mL. In four of the 18 positive spas, the most probable number was above the Pseudalert highest detection limit of 200 per 100 mL (Table 1). MALDI-TOF confirmed the presence of *P. aeruginosa* in positive wells, in those samples ($n=4$) where it was applied.

Vibrio spp. were present in 16 of 24 fish spas; in positive samples, the *Vibrio* numbers ranged from two to 6,900 CFU per 100 mL, but most concentrations were low (Table 1). Species identification with MALDI-TOF demonstrated the presence of *V. cholerae* in all positive fish spas. PCR of the *toxR* and *ctxA* genes according to Schets et al. [15] confirmed that the isolates were *V. cholerae* and demonstrated that they were non-toxicogenic. *V. vulnificus* was found in one full body spa

only. PCR of the *vvhA* gene according to Canigral et al. [16] confirmed the MALDI-TOF identification.

Incubated samples could only be read for the presence of rapid growing mycobacteria (RGM). Reading the plates for the presence of slow growing mycobacteria was not possible due to the growth of large amounts of disturbing background flora. RGM were present in 23 fish spas; the incubated sample from the 24th fish spa could not be read due to overgrowth by fungi. *Mycobacterium* species that were frequently found included *M. fortuitum* ($n_{\text{fish spa}}=21$), *M. conceptionense*/*M. senegalense* ($n_{\text{fish spa}}=16$), *M. abscessus* ($n_{\text{fish spa}}=15$), and *M. chelonae* ($n_{\text{fish spa}}=13$). Less abundant were other *M. chelonae* complex isolates ($n_{\text{fish spa}}=7$), *M. abscessus* subsp. *bolletii* ($n_{\text{fish spa}}=8$), and *M. phocaicum* ($n_{\text{fish spa}}=6$). *M. alvei*, *M. peregrinum*, *M. porcinum*, *M. wolinski*, and three novel, yet unknown environmental mycobacteria, were only incidentally isolated.

Management

The investigated companies had fish spas from various suppliers, whereas some of them constructed their own spas. Most companies purchased the fish from the same supplier.

All companies filtered the fish spa water, using biological filters with zeolite; the majority had one filter per fish spa installed. Additionally, most companies treated the water with ultraviolet light C (UVC), ozone or both. Almost all companies checked the water temperature on a regular basis, and many of them used a test kit designed for aquarium and pond owners to test a basic set of chemical parameters, including pH value, hardness, ammonia, nitrite, nitrate, phosphate, iron, and copper. Keeping a log was not a standard procedure. Five of the 16 companies had the microbiological water quality checked by a laboratory. Refreshment policies varied from total replacement of the water once to twice a week, to partial replacement of the water with a daily to weekly frequency (Table 2).

Discussion

All bacteria the fish spa water was tested for, were detected, although not in all spas and in varying concentrations. Whether or not *Aeromonas* spp. *Vibrio* spp., *P. aeruginosa* or RGM were found, could not be related to a specific type of spa (foot, hand, or body), a specific water treatment process (filtration with UVC, ozone or both), or a specific regime of water refreshment (total or partly, with varying frequency). Physical-chemical parameters did also not relate to presence or absence of bacteria or high or low bacterial counts. It is plausible that the small number of fish spas contributed to the inability to determine a possible correlation with microbiological water quality. Also, the presence of bacteria may depend on occasional contamination of the water by clients and the subsequent expansion of the contamination for prolonged time periods. Most owners could, however, not provide clear figures on spa use in terms of number of users per day; so a

relation between intensity of fish spa use and microbiological water quality could not be established.

When comparing the *Vibrio* spp. concentration in fish spas (0 – 6,900 CFU/100 ml, median 11 CFU/100 ml) with the numbers detected in various surface water in the Netherlands during summer (0 – 4.2.10⁵ MPN/100 ml, median 37 MPN/100 ml [15], the numbers in fish spas were mostly lower, or sometimes in the same order of magnitude, even though the water temperature in fish spas was ca 5 to 10°C higher than the surface water temperature during summer [15]. Current *Vibrio* spp. concentrations in surface water led to a limited number of reported cases of illness in the Netherlands, which were mostly cases of ear complaints as a result of *V. alginolyticus* infections [15]. This suggests that the *Vibrio* spp. numbers in fish spas do not pose a major health risk. However, the presence of *V. cholerae* non-O1/O139 and *V. vulnificus*, which are a well-known cause of wound infections in humans [15,17], may pose a risk for people with a damaged skin. Moreover, the high water temperatures in fish spas can allow *Vibrio* spp. to proliferate to numbers higher than those observed in the sampled fish spas, resulting in an increased risk.

In chlorinated pools, *P. aeruginosa* should be absent in 100 ml samples of the pool water [18]; a requirement that was not met in 75% of the fish spas. There are no requirements for *P. aeruginosa* in recreational surface waters. Comparing the *P. aeruginosa* concentrations in fish spas (range 1 – 178 MPN/100 ml, median 33 MPN/100 ml) with those previously found in Dutch surface waters, generally shows lower concentrations in the latter (0 – 9 CFU/100 ml) [19], also in recreational water related to outbreaks of otitis externa (0.4 CFU/100 ml) [20]. In the swimming pool environment, *P. aeruginosa* commonly causes folliculitis through infection of disrupted follicles, and although dose response relationships for dermal exposure are unclear, the suggested levels of concern for healthy individuals are > 10⁵ per 100 ml [21]. Concentrations in that range were not found in the examined fish spas, suggesting that the health risk related to *P. aeruginosa* in fish spas is limited for people with an intact skin. *P. aeruginosa* may however pose a greater risk for people with a damaged skin, and in spas where numbers largely exceed 10⁵ per 100 ml.

Aeromonas spp. are ubiquitous in the aquatic environment, in a broad range of concentrations [22], and have also been detected in all examined fish spas. Concentrations in fish spas were in the order of magnitude as those typical for rivers receiving sewage discharge [22]. A health risk from the isolated *Aeromonas* spp. cannot be fully ruled out, but it may be limited for healthy individuals with an intact skin and no underlying disease or reduced immunity, since human skin and soft tissue infections with *Aeromonas* spp. are commonly associated with wounds after water related trauma, or occur in persons with underlying disease [23].

RGM, that have been detected in all examined fish spas, are known environmental opportunistic pathogens that are increasingly recognised as causative agents of human and fish disease, both in sporadic cases and outbreaks [24,25]. Their transmission to humans from an environmental source, with subsequent clinical disease, is however rarely proven, except for cases in hospital settings [24]. Most of the species isolated from fish spas have previously been found as the cause of illness in immune competent persons after exposure to various water sources, including whirlpool footbaths in a nail salon (*M. fortuitum*) [26], a fish tank after contact with broken glass (*M. senegalense*) [27], and therapy pool water (*M. phocaicum*) [28]. In the Netherlands, RGM have been isolated from tap and shower water without a direct link to illness [14]. *M. chelonae*, *M. abscessus*, and *M. fortuitum* are frequently found in clinical samples whereas the other species recovered from fish spas are less frequently observed [25]. The three yet unclassified environmental *Mycobacterium* species have not previously been isolated from human samples in the Netherlands and have not been found in the BLAST database.

The low level of faecal contamination confirmed the expected low input of intestinal enterococci with human faeces through hands and feet, as well as the limited contribution of the fish [29]. Dilution and regular cleaning policies probably enhance these low levels. A higher level of faecal contamination could be expected in full body spas, but was not observed. *Pl. shigelloides*, which appeared to be present in most fish spas and hampered the *E. coli* detection, is naturally present in warm surface water and causes human gastroenteritis [30]. *Pl. shigelloides* is known to disturb *E. coli* enumeration in samples from warm surface waters while using the Rapid Test in ISO 9308-1:2000 [30]. Although the presence of *Pl. shigelloides* in fish spas was not foreseen, it appeared that the bacterium is often part of the microbial flora in aquariums [31].

This study provided insight in the microbiological condition of fish spa water, albeit the number of data are too limited to suggest ranges for acceptable microbiological contamination. Fish spas do, however, require their own set of microbiological parameters and guide values. Swimming pool and recreational water guidelines are not particularly appropriate, since they focus on an environment with a residual effect of disinfectants, or apply to surface waters with different contamination sources.

The detected concentrations of *Aeromonas* spp., *Vibrio* spp., and *P. aeruginosa* in the water of the examined fish spas, in combination with the health implications of these bacteria at such levels, and the detected *Mycobacterium* spp. and their health implications, suggest a low health risk from fish spas. The data from this study support the opinion of Health Protection Agency (now Public Health England) and the French

Agency for Food, Environmental and Occupational Health and Safety (ANSES) that the health risk from using fish spas is limited for healthy people with an intact skin and no underlying disease. Persons with underlying disease affecting the immune system, such as diabetes, and skin conditions, such as psoriasis and eczema, and persons with a damaged skin, may be at a greater risk. Transmission of infection through ingestion of intestinal pathogens in contaminated water is of limited importance in hand and foot spas, but it may play a role in body spas. Moreover, head immersions in body spas, may lead to ear infections with *P. aeruginosa* or *Vibrio* spp. In all spa types, pathogens can be transmitted through hand–mouth contact. This transmission route is likely to be more relevant when the water is heavily contaminated [32].

In addition to establishing microbiological guidelines for fish spas, drafting a code of practice on hygiene in fish spas and risk communication to clients is recommended.

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Conflict of interest

None declared.

Authors' contributions

FMS and HHvdB designed the study, HHvdB and RdZ carried out microbiological analyses, FMS performed data analysis and drafted the manuscript, FMS, DvS and AMdRH interpreted data, and all authors reviewed and revised the first and final drafts of this manuscript.

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