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 relaxing 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 ± 2 °C for 24 ± 4 hours, respectively. Vibrio characteristic colonies on TCBS were subcultured onto Trypton Soy Agar (TSA; Oxoid, Badhoevedorp, the Netherlands) and incubated at 36±2 °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, disected, 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.
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 fish spas, but in all other spa types they were absent. The Vibrio spp. counts were all zero in foot and hand spas, and in four of five body spas. The Pseudomonas aeruginosa counts ranged from 0 to 109,000 MPN/100 mL, with a median value of 11 MPN/100 mL.

Table 1
Physical-chemical and microbiological parameters in the water of 24 fish spas in 16 companies, the Netherlands, October–November 2012

<table>
<thead>
<tr>
<th>Sample date (2012)</th>
<th>Company</th>
<th>Spa type and capacity</th>
<th>Water temperature (°C)</th>
<th>pH</th>
<th>Conductivity (µS/cm)</th>
<th>IE (n/100 mL)</th>
<th>Aeromonas spp. (n/100 mL)</th>
<th>Vibrio spp. (n/100 mL)</th>
<th>Pseudomonas aeruginosa (MPN/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 Oct A</td>
<td>Foot, 1 person</td>
<td>29.5</td>
<td>8.43</td>
<td>451</td>
<td>0</td>
<td>30,000</td>
<td>0</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>22 Oct A</td>
<td>Foot, 1 person</td>
<td>29.5</td>
<td>7.72</td>
<td>449</td>
<td>1</td>
<td>19,500</td>
<td>0</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td>22 Oct B</td>
<td>Foot, 2 persons</td>
<td>28.1</td>
<td>8.19</td>
<td>379</td>
<td>0</td>
<td>24,000</td>
<td>0</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>22 Oct C</td>
<td>Foot, 2 persons</td>
<td>29.0</td>
<td>8.14</td>
<td>552</td>
<td>1</td>
<td>82,000</td>
<td>411</td>
<td>178</td>
<td></td>
</tr>
<tr>
<td>30 Oct D</td>
<td>Foot, 4 persons</td>
<td>27.8</td>
<td>8.03</td>
<td>483</td>
<td>2</td>
<td>4,036</td>
<td>648</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>30 Oct E</td>
<td>Body, 1 person</td>
<td>31.2</td>
<td>8.49</td>
<td>294</td>
<td>0</td>
<td>32</td>
<td>5.4</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>30 Oct F</td>
<td>Body, 1 person</td>
<td>31.3</td>
<td>8.48</td>
<td>296</td>
<td>3</td>
<td>5,491</td>
<td>87</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>30 Oct F</td>
<td>Foot, 6 persons</td>
<td>31.3</td>
<td>8.59</td>
<td>251</td>
<td>0</td>
<td>4,145</td>
<td>270</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>30 Oct G</td>
<td>Foot, 2 persons</td>
<td>25.0</td>
<td>8.15</td>
<td>225</td>
<td>0</td>
<td>109,000</td>
<td>0</td>
<td>144</td>
<td></td>
</tr>
<tr>
<td>06 Nov H</td>
<td>Foot, 2 persons</td>
<td>26.8</td>
<td>8.49</td>
<td>348</td>
<td>0</td>
<td>5,864</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>06 Nov H</td>
<td>Hand, 2 persons</td>
<td>26.8</td>
<td>8.53</td>
<td>357</td>
<td>0</td>
<td>2,909</td>
<td>3</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>06 Nov I</td>
<td>Foot, 4 persons</td>
<td>28.6</td>
<td>8.09</td>
<td>483</td>
<td>0</td>
<td>32,700</td>
<td>4,290</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>06 Nov J</td>
<td>Foot, 4 persons</td>
<td>28.1</td>
<td>8.29</td>
<td>356</td>
<td>0</td>
<td>20,000</td>
<td>0</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>06 Nov K</td>
<td>Foot, 2 persons</td>
<td>27.8</td>
<td>8.32</td>
<td>513</td>
<td>18</td>
<td>30,500</td>
<td>0</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>06 Nov K</td>
<td>Hand, 2 persons</td>
<td>27.8</td>
<td>7.83</td>
<td>632</td>
<td>13</td>
<td>3,935</td>
<td>0</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>06 Nov L</td>
<td>Foot/hand, 1 person</td>
<td>33.0</td>
<td>7.36</td>
<td>1,083</td>
<td>0</td>
<td>18,500</td>
<td>6,900</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>06 Nov L</td>
<td>Body, 1 person</td>
<td>33.0</td>
<td>7.46</td>
<td>1,423</td>
<td>310</td>
<td>20,500</td>
<td>1,652</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>06 Nov L</td>
<td>Body, 2 persons</td>
<td>33.2</td>
<td>7.70</td>
<td>929</td>
<td>1</td>
<td>1,500</td>
<td>1,908</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>13 Nov M</td>
<td>Foot, 1 person</td>
<td>27.6</td>
<td>6.86</td>
<td>453</td>
<td>0</td>
<td>3,182</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>13 Nov M</td>
<td>Hand, 1 person</td>
<td>27.6</td>
<td>7.82</td>
<td>395</td>
<td>0</td>
<td>355</td>
<td>14</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>13 Nov N</td>
<td>Foot, 2 persons</td>
<td>28.6</td>
<td>6.98</td>
<td>416</td>
<td>0</td>
<td>16,500</td>
<td>850</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>13 Nov N</td>
<td>Body, 1 person</td>
<td>28.6</td>
<td>8.23</td>
<td>333</td>
<td>0</td>
<td>2,800</td>
<td>34</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>13 Nov O</td>
<td>Foot, 2 persons</td>
<td>27.6</td>
<td>7.74</td>
<td>218</td>
<td>0</td>
<td>44,000</td>
<td>11</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>14 Nov P</td>
<td>Foot, 1 person</td>
<td>27.8</td>
<td>8.11</td>
<td>470</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>88</td>
<td></td>
</tr>
</tbody>
</table>

IE: intestinal enterococci; MPN: most probable number.
were to Schets et al. [15] confirmed that the isolates toxigenic. 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-toxigenic. 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 (nFish spa = 21), M. conceptionense/M. senegalense (nFish spa = 16), M. abscessus (nFish spa = 15), and M. chelonae (nFish spa = 13). Less abundant were other M. chelonae complex isolates (nFish spa = 7), M. abscessus subsp. bolletii (nFish spa = 8), and M. phocaicum (nFish 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 surfaces water in the Netherlands during summer (0–4.2×10^6 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 >10^3 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 10^5 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.

**Acknowledgments**

The authors thank all owners and employees of the involved companies for their participation. John Klippe (province of South–Holland), Mariëlle Dirven (Public Health Service Rotterdam–Rijnmond), and Jerry van Druten (province of Overijssel) are acknowledged for their help in sampling. Miranda Kamst (RIVM) is acknowledged for assisting the mycobacteria analyses. The authors thank Olga Haenen (Central Veterinary Institute of Wageningen University), Joke van der Giessen, Corien Swaan, Thijss Veenstra (all from RIVM) for their contribution to this work. This work was funded by the Netherlands Food and Consumer Safety Authority (NVWA).

**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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