Rijksinstituut voor Volksgezondheid
en Milieu
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FRONT OFFICE FOOD AND PRODUCT SAFETY

RISK ASSESSMENT OF VIBRIO CHOLERAE

Risk assessment requested by:	Netherlands Food and Consumer Product Safety Authority
Risk assessment performed by:	RIVM
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Subject

In a surveillance in which stationary fire extinguishing systems of 'BRZO' ('Besluit Risico's Zware Ongevallen') companies were tested for the growth of exotic species and specific pathogens, the sampling in December 2017 yielded *Vibrio cholerae* in three of the examined systems. *V. cholerae* was determined by applying matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometric analysis. It was questioned whether it concerned pathogenic strains or not. One isolated colony has been conserved and stored at TOPlab in Leiden.

Questions

1. Does it concern *Vibrio cholerae*?
2. Does it concern a pathogenic strain?
3. What is the associated risk? For example when using the fire extinguishing system concerned?

Conclusions

1) In the water samples of system B, *Vibrio cholerae* non-O1/O139 was detected. No *Vibrio cholera* was detected in the water samples of systems A and C.
2) *Vibrio cholerae* non-O1/O139 is a common pathogen in surface water that is able to cause ear and wound infections and gastrointestinal illness. *Vibrio cholerae* non-O1/O139 is NOT the causative agent of cholera.
3) There may be a risk of contracting wound infections when damaged skin is exposed to water that is contaminated with *Vibrio* species, particularly the presence of *Vibrio vulnificus*, which can cause severe wound infections and sepsis, requires precautionary measures.

Introduction

In a surveillance of stationary fire extinguishing systems of 'BRZO' companies, in which these systems were examined for the growth of exotic species and specific pathogens, the sampling of December 2017 yielded microorganisms that were suspected to be *Vibrio* species. The suspected *Vibrio* isolates were obtained from three different systems by

culturing on blood agar plates. Culturing of the same samples on a culture medium that is specific for growth of *Vibrio* species, Thiosulphate Citrate Bile Sucrose (TCBS) agar, did not yield any characteristic *Vibrio* colonies.
One isolate from each of the positive systems was identified by using MALDI-TOF (VITEK MS, Biomerieux). All three isolates appeared to be *Vibrio cholerae* (99.9% matches). TOPLab in Leiden performed the MALDI-TOF analyses. Upon acquisition of the MALDI-TOF results, one isolate was preserved in ethanol by TOPLab in Leiden, because this laboratory had no facilities to store or work with viable (potential) high risk pathogens. In January 2018, RIVM was asked to determine whether this isolate was a toxigenic *V. cholerae* strain, which can cause cholera. At the end of January 2018, the stored isolate was send to RIVM.

Examination of the isolate and additional water samples
Since the isolate was inactivated with ethanol, it was not possible to perform the standard culturing method to confirm previous results. It was only possible to extract DNA from the isolate in ethanol that was received by RIVM. PCRs for the *V. cholerae toxR* and *ctxA* genes were performed to confirm whether the isolate was *V. cholerae* (*toxR* gene) and contained the gene for toxin production (*ctx*A gene) or not. Both PCRs were negative, demonstrating that the isolate was not *V. cholerae*.
This may have been a true result, but may also have been due to the conservation of the isolate in ethanol. Storage in ethanol is not optimal when further identification is needed, and may affect the PCR. Since this is not a standard procedure, we have no data to verify whether this was actually the case or not. The DNA extraction worked well since PCR extraction controls gave the expected results.

Following the obtained results, it was decided to take new water samples from the three fire extinguishing systems concerned (A, B and C). The new samples were taken mid-April 2018 and analysed by RIVM within 24 hours after sample collection for the presence of *Vibrio* species. Sample volumes delivered were 500 ml (single for system A, in duplicate for system B and C). For all samples, the complete sample volume was analysed by using an MPN (most-probable number) method. Samples were enriched in ASPW (Alkali Saline Peptone Water), incubated at both 36 ± 2ºC and 41.5 ± 1 ºC for 18 ± 1 hours, and subsequently plated onto TSCB agar plates, which were incubated at 36 ± 2ºC for 24 ± 3 hours. Suspected *Vibrio* colonies were pure cultured on TSA (Tryptone Soy Agar) plates, incubated at 36 ± 2ºC for 24 hours. Subsequently, confirmation tests were done, including an oxidase test for all isolates, an API20E test system (for biochemical identification of bacteria) for oxidase positive isolates, PCR for isolates that were *V. parahaemolyticus*, *V. cholerae* or *V. vulnificus* based on the API20E test, an agglutination test for O1 and O139 antigens for confirmed *V. cholerae* isolates and finally MALDI-TOF for all isolates that were *Vibrio* species based on the API 20E test.

Results
Based on MPN enumeration, the estimated concentration of total *Vibrio* (i.e. concentrations were not obtained for individual species) in the analysed samples ranged between 0.03 and 4.6 per ml (Table 1).

Various *Vibrio* species were detected in the samples from all three locations (Table 1). These included the species commonly detected in Dutch surface water, such as *V. alginolyticus*, *V. parahaemolyticus*, *V. fluvialis*, *V. mimicus* and *V. cholerae*. Among the isolates were also *V. vulnificus* isolates, which have occasionally been detected in previous monitoring programs of Dutch surface waters (Schets et al., 2006; Schets et al., 2011a; Sterk et al., 2015).

V. cholerae was only detected in the samples from system B. These *V. cholerae* isolates were confirmed to be indeed *V. cholerae* by both PCR and MALDI-TOF. The isolates did, however, neither contain the *ctxA* gene, nor the O1 and O139 antigens. So, these isolates were confirmed to be *V. cholerae* non-O1/O139 and therefore non-toxigenic strains that do not cause cholera.

Table 1: Vibrio concentration in water from stationary fire extinguish systems and the detected Vibrio species

system	estimated concentration (MPN/ml)		detected species
	culture at 36 ± 2ºC	culture at 41.5 ± 1 ºC	
A	0.4	0.2	*V. alginolyticus*
B	2.4	4.6	*V. parahaemolyticus* *V. mimicus* *V. fluvialis* *V. vulnificus* *V. cholerae non-O1/O139*
C	0.07	0.03	*V. parahaemolyticus* *V. vulnificus*

Exposure and risk assessment

When the fire extinguishing systems are used, people using them may be exposed to water droplets or aerosols containing *Vibrio* species. All the *Vibrio* species that were detected in the water of these systems are considered to be human pathogens and are able to cause ear, wound and gastrointestinal infections (Oliver & Kaper, 1997).

The most relevant exposure route while using the fire extinguishing systems is skin contact with contaminated water. Swallowing water or submerging the head, which may result in gastrointestinal infections or ear infections, is not very likely. Contact of a damaged skin or an open wound (even if it is small) with water containing *Vibrio* species may lead to (serious) wound infections, as has also been reported for recreational water exposure in the Netherlands (Sterk et al., 2015).

In a previous study (Schets et al., 2011a), the concentrations of total *Vibrio* in recreational waters in The Netherlands ranged between 4 and 240,000 MPN/L. In the fire extinguishing systems examined, *Vibrio* concentrations ranged between 30 and 4600 MPN/L, which lies within the range detected in recreational waters, but with a lower maximum value. Cases of *Vibrio* infections related to recreational water exposure in The Netherlands are rare (Schets et al., 2011b; Sterk et al., 2015). Exposure to contaminated water droplets from the fire extinguishing systems may lead to wound infections, but based on previous results for recreational water exposure, and the observed *Vibrio* concentrations in the fire extinguishing systems, the risk does not seem very high.

However, the presence of *V. vulnificus* requires special attention. This bacterium is capable of causing serious, rapidly developing wound infections that may lead to septicemia and death (Oliver, 2013). It is therefore recommended to avoid contact with the contaminated water while having a damaged skin or wounds, or to seal wounds with a water tight cover.

References

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