National cartography of water points for the presence of *Vibrio* spp. in Belgium

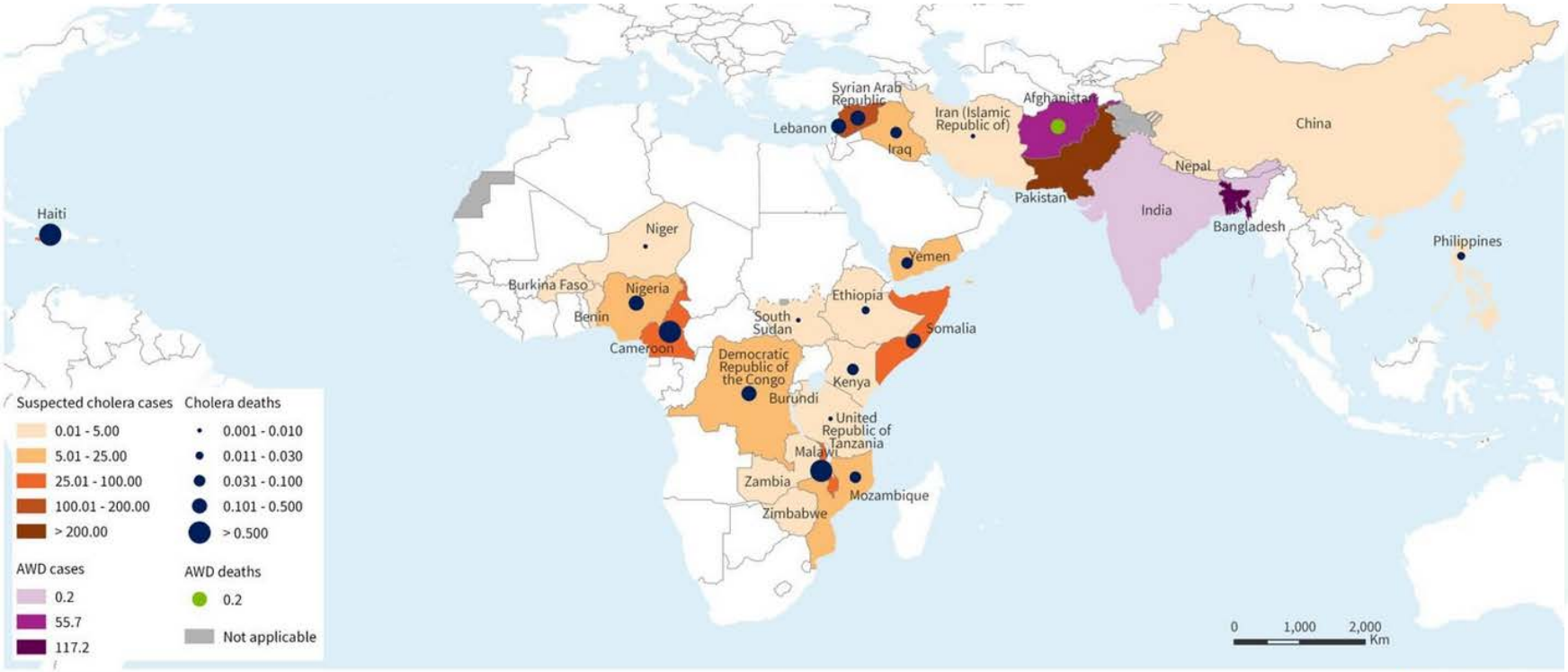
Rosalie Sacheli, Camile Philippe, Cécile Meex, Sami, Pierrette Melin, Marie-Pierre Hayette

CHU Liège-Clinical Microbiology-National Reference Center for *Vibrio cholerae* and *parahaemolyticus*

Sciensano Scientific Symposium on Infectious Diseases, Brussels, 11th May 2023

Introduction

Cholera incidence for 100 000/inhabitants notified by OMS between 1st January and 30 November 2022



The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of WHO concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Health Organization, United Nations Population Division (population prospect 2021)
Map Production: WHO Health Emergencies Programme
Map Date: 9 December 2022

World Health Organization

Introduction

The situation in Belgium 2012-2021 (NRC data - CHU Liege)

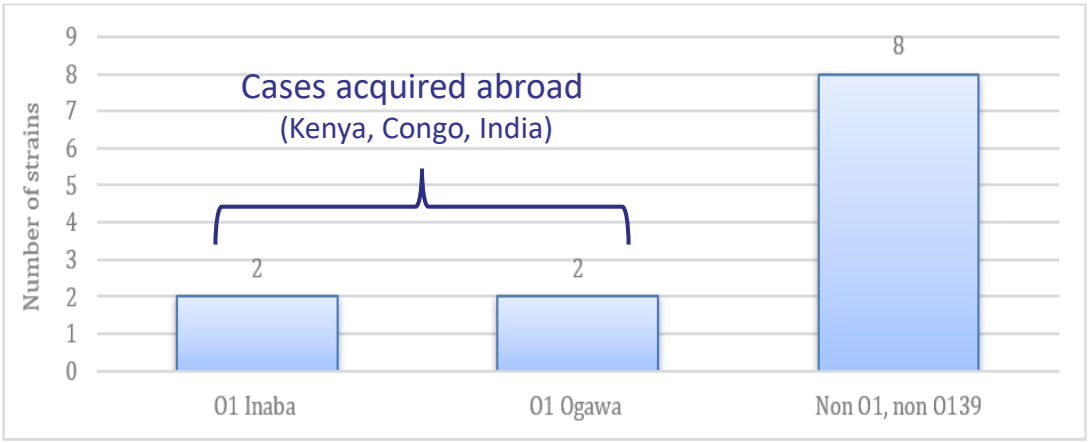


Figure 1: Distribution of the different serotypes identified among 12 *Vibrio cholerae* strains isolated from stools between 2012 and 2021 (Belgian NRC report 2020-2021)

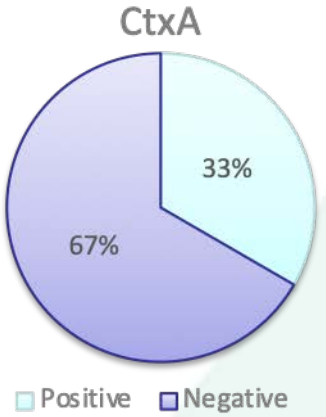
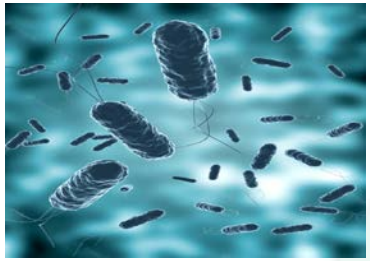


Figure 2: Graph showing the rate of presence of cholera toxin genes among the twelve *V. cholerae* strains isolated from stools. (Belgian NRC report 2020-2021)

Introduction

Vibrio spp. in water

- Non-toxigenic *Vibrio cholerae* and most *Vibrio spp.* are found in aquatic environment and are generally non-pathogenic
- **A few species** can **cause sporadically illnesses** such as wound infections, otitis, bacteremia and gastroenteritis.
- Recently, the number of reports of human infections, which can be life-threatening, involving non-O1, non-O139 *V. cholerae* and other *Vibrio* spp. **has increased** in Northern Europe as in Belgium and in France.
- Waters for recreational use such as lakes and sea water are **not yet monitored** for *Vibrio spp*.



Vibriosis in Belgium

ACTA CLINICA BELGICA
2018, VOL. 73, NO. 6, 463–465
https://doi.org/10.1080/17843286.2018.1483563

Taylor & Francis
Taylor & Francis Group

CASE REPORT

Check for updates

Atypical manifestation of *Vibrio cholerae*: fear the water!

Steven De Keukeleire[a], Paul Hoste[b], Mies Crivits[c], Naïma Hammami[d] and Anne Piette[a]

[a]Department of Laboratory Medicine, AZ Alma, Eeklo, Belgium; [b]Department of Gastro Enterology, AZ Alma, Eeklo, Belgium; [c]Department of Intensive Care, AZ Alma, Eeklo, Belgium; [d]Public Health and Surveillance Department, Zorg en Gezondheid Vlaanderen, Ghent, Belgium

ABSTRACT

Background: In recent years, there has been an universal increase in number of reports of infections involving non-O1, non-O139 *V. cholerae* (NOVC). NOVC infection is only rarely complicated by septicaemia or invasive extra-intestinal infection.

Case summary: We report the first documented case of NOVC bacteraemia in a Belgian immunocompetent male after ingestion of water when paddling in a creek.

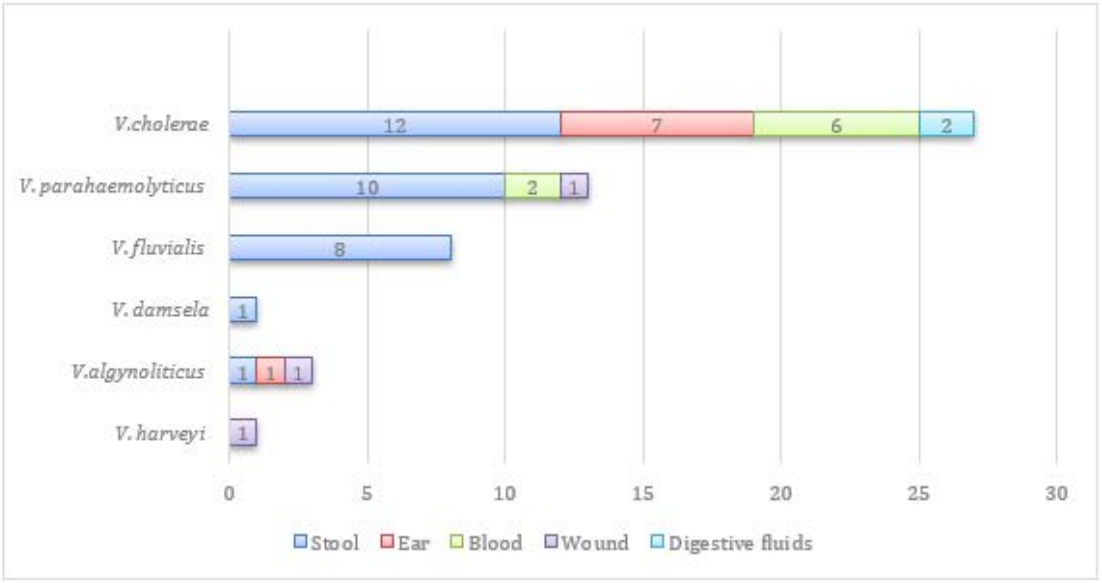
Conclusion: Clinicians should include *V. cholerae* in their differential diagnosis in patients presenting with a self-limiting gastroenteritis after ingestion of raw and undercooked seafood or bathing in potentially contaminated recreational waters during warm summers.

Abbreviations: NOVC: non-O1, non-O139 *V. cholerae*; MALDI- TOF: matrix-assisted desorption ionization time- -of-flight

KEYWORDS
Bacteraemia; gastroenteritis; non-O1, non-O139 V. cholerae; NOVC; Vibrio cholerae

Swallowed a considerable amount of water during a fall in brackish recreational water while 'sup-ping' ('stand-up paddling')

Water samples taken from the Creek on 14 June 2017 (14 days after exposure) yielded 5.10^4–10^5 colony forming units/100mL of non-O1/non-O139 *V. cholerae* **(NRC-CHU Liege)**



Belgian NRC report 2012-2021, R. Sacheli, P. Melin-CHU Liege

Belgian cartography of water points

The Belgian national reference center conducted a study, by doing a **cartography of Belgian water points** for screening the presence of ***Vibrio* spp**. in a few selected points to evaluate its possible impact on public health.

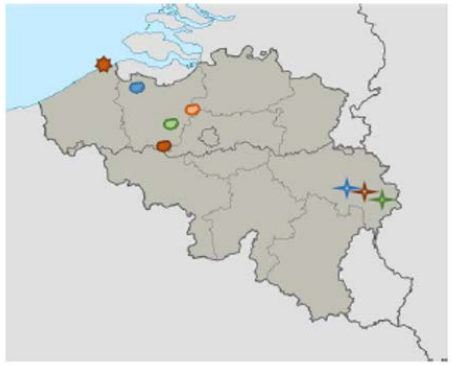
➔Master thesis in biomedical sciences by **Camille Philippe (Uliege)**

Material and Methods

Sampling

- According to recent clinical cases of vibriosis and to the distribution of recreational water locations, **8 areas were selected in Wallonia and Flanders including the North sea.**

Boerekreek 9982
Donkvijver 9700
Blaarmeersen 9000
Donk lake 9290
Knokke Heist 8300
Butgenbach lake 4750
Robertville lake 4950
Warfaaz lake 4900

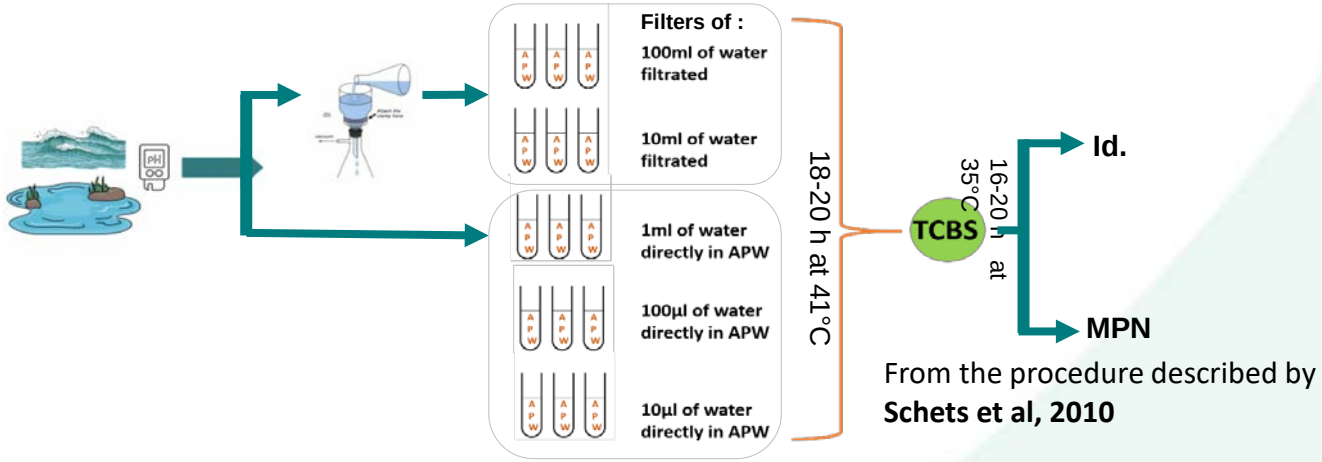


- **Sampling** of water was done at each site once per month **between May and September 2021.**
  - Use of a telescopic device for collection of 1 litre sample
  - Water poured in sterile bottle, transported on ice and kept at 4°C until analysis within 24 hours.
  - **Temperature and pH of water measured and recorded** at time of each sampling.

Material and Methods

Culture

- **Most Probable Number (MPN) culture method**: serial dilutions in alcaline peptone water (APW)
  - Upon reception in the laboratory, for each sample of water, in triplicate:
    - 10 mL and 100 mL were filtered (0.45 µm) and filters were inoculated in 50 mL of APW.
    - 10 µl, 100 µl and 1 mL were inoculated in 9 mL of APW
    - All inoculated APW were incubated 18-20h at 41°C and further sub-cultured (10 µL) on thiosulfate citrate bile saccharose agar medium (TCBS), then incubated 16-20h at 35°C.



From the procedure described by **Schets et al, 2010**

- **Identification and estimated quantification of *Vibrio spp***
  - **Identification** of growing colonies on TCBS by **MALDI-TOF mass spectrometry + agglutination and PCR**
  - The positive TCBS plates originating from the serial dilutions in APW, allowed the estimation of the concentration of *Vibrio spp* in the different samples according to **MPN** interpretation.

Results

- Results of culture on TCBS and Maldi-Tof MS identification
  - **No *Vibrio* sp. found in Walloon lakes** (Butgenbach, Robertville, Warfaaz) and in one lake in Flanders (Donk)

	Inland water			Coastal water
	Blaarmeersen	**Donkvijver**	**Boerekreek**	**Knokke-Heist**
May	/	*V. cholerae*	*V. cholerae*	/
June	/	/	*V. cholerae*	*V. cholerae*
	/	/	/	*V. alginolyticus*
July	*V. cholerae*	/	*V. cholerae*	*V. cholerae*
	/	/	/	*V. alginolyticus*
	/	/	/	*V. parahaemolyticus*
August	/	*V. cholerae*	*V. cholerae*	*V. cholerae*
	/	/	/	*V. alginolyticus*
	/	/	/	*V. parahaemolyticus*
September	/	/	*V. cholerae*	*V. cholerae*
	/	/	/	*V. alginolyticus*

- All *Vibrio cholerae* found were **Non O1, Non O139, CtxA –**
- All *Vibrio parahaemolyticus* were **Tdh-/Trh-**

Results

- Estimation of the concentration of *Vibrio cholerae* (non-01, non-0139) and *Vibrio* spp. (by Most Probable Number method, MPN) in four water points in Flanders

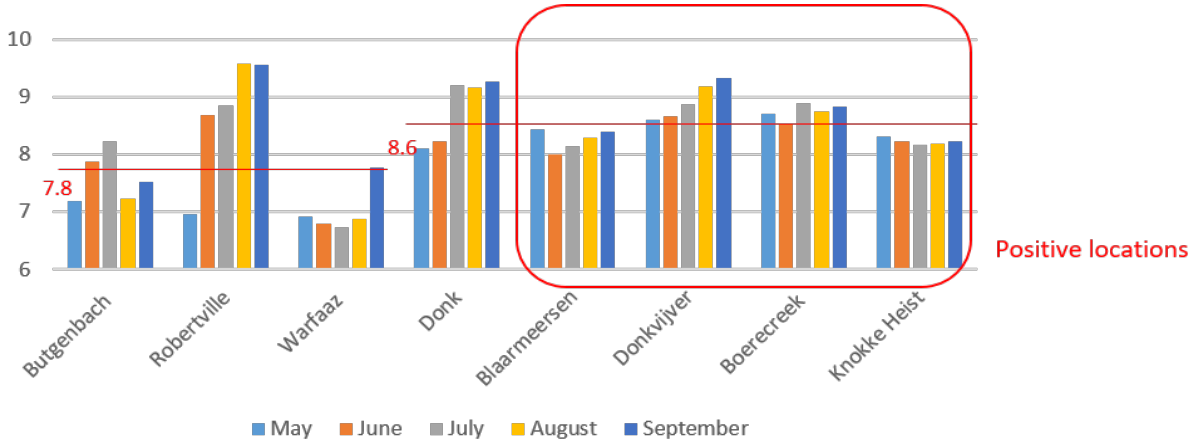
	Blaarmeersen (CFU/100mL)	Donkvijver (CFU/100mL)	Boerekreek (CFU/100mL)	Sea (Knokke) (CFU/100mL)
May	/	**240** (42-1000)	7,5 (1,7-20)	/
June	7,5 (1,7-20)	/	**240** (42-1000)	**110** (18-410)
July	/	/	110 (18-410)	**210** (45-420)
August	/	**460** (90-2000)	**2400** (420-10000)	**>11000** (4200-400000)
September	/	/	**460** (90-2000)	**240** (42-1000)

Excellent quality	Inland water (CFU/100ml)	Coastal water (CFU/100ml)
E.coli	<500	<250
Intestinal enterococci	<200	<100

Directive 2006/7/EC of the European parliament concerning the management of bathing water quality

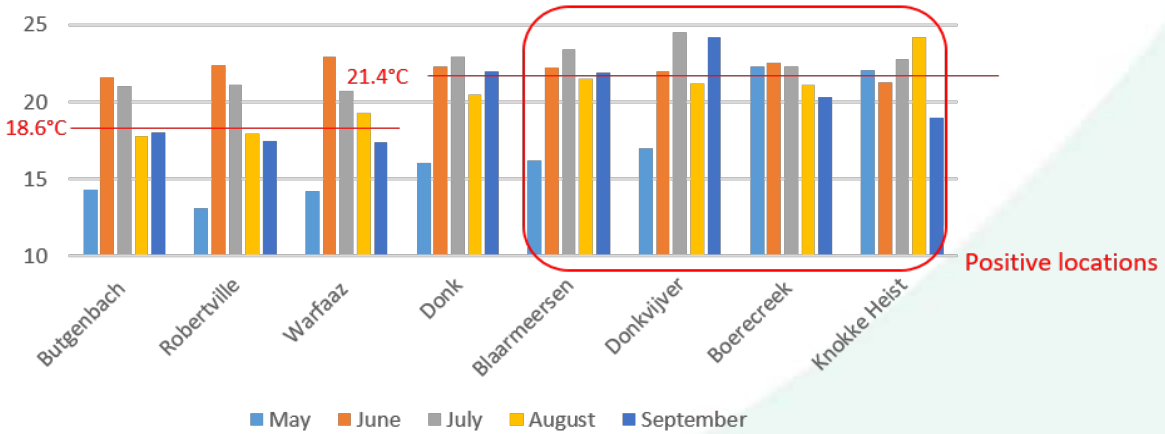
Results

pH



Post hoc test of Scheffé showed a **statistically significant** difference of the pH in Flanders than in Wallonia **(p=0.0009).**

T°



Post hoc test of Scheffé showed **statistically significant** difference between the temperature of water in Flanders and in Wallonia **(p=0.0019)**

Conclusion

- Our study demonstrate the **presence of *Vibrio* cholerae (non-O1, non 0-139) and *Vibrio spp***. at concentrations able to cause human infections in different water points in the North of Belgium.
- **Mean temperatures and pH were higher in Flemish selected locations** than in Walloon selected lakes. They can be favorable factors for the growth of *Vibrio spp*. Other factors such as salinity should be also included in future surveillance.
- This study **supports the recommendation to include *Vibrio* spp. in water quality controls in** order to define if water recreational activities are harmless for humans in Belgium.

CHU de Liège

RÉGION WALLONNE

LIÈGE université

Center for Interdisciplinary Research on Medicines

Thank you for your attention

FONDS LEON FREDERICQ

CENTER FOR INTERDISCIPLINARY RESEARCH ON MEDICINES

fnrs

LA LIBERTÉ DE CHERCHER

CIRM ULiège