Water, viruses and human health

"Methods for virus monitoring in water samples"

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↑ Pressure

World Health Organization

"Water is essential to sustain life, and a satisfactory (adequate, safe and accessible) supply must be available to all."

World Health Organization, 2017

2017 → 71% of the global population (5.3 billion people) already used a safe-managed contamination-free water service

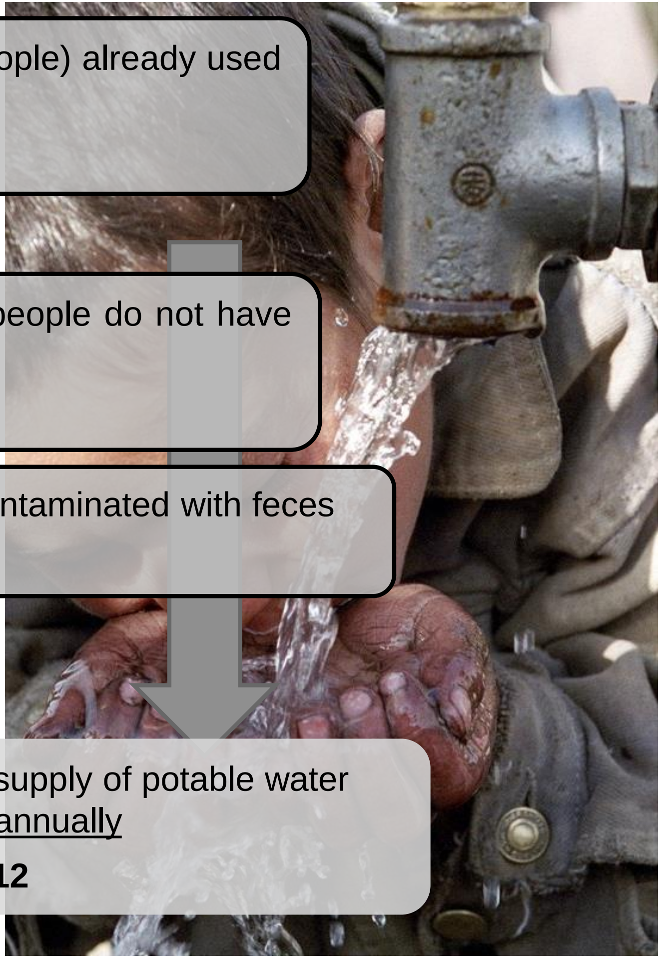
World Health Organization

According to the WHO, an estimated 785 million people do not have access to safe water

World Health Organization

At least 2 billion people have used a source of water contaminated with feces

World Health Organization

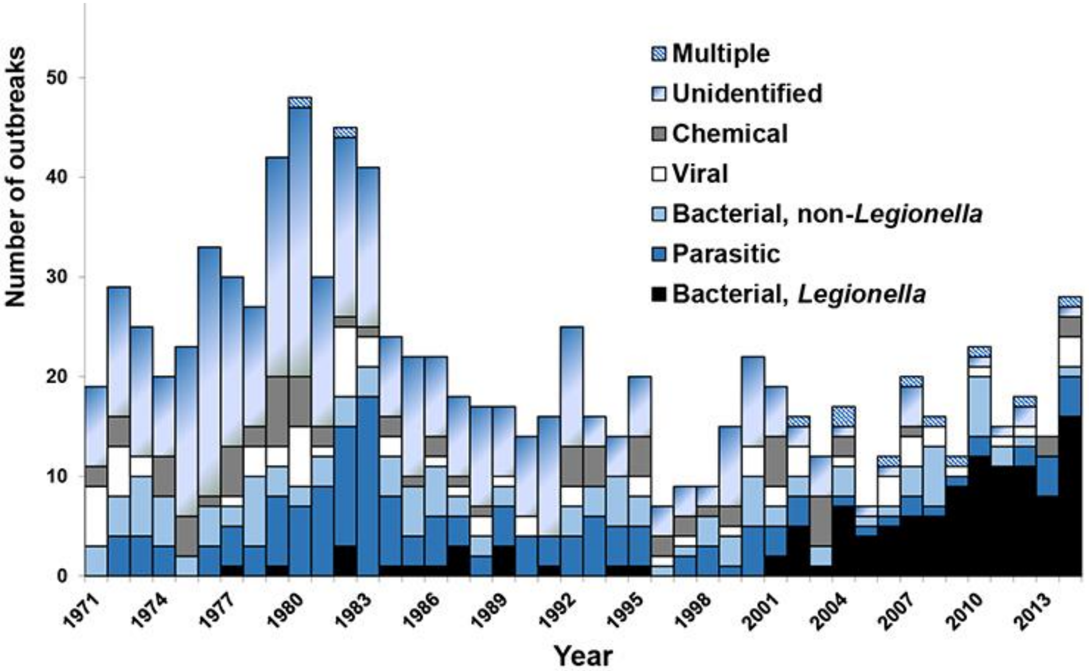


Global economic losses associated with improper supply of potable water and inadequate sanitation are around <u>$260 billion annually</u>

World Bank - Hutton, 2012

Waterborne outbreaks associated with drinking water

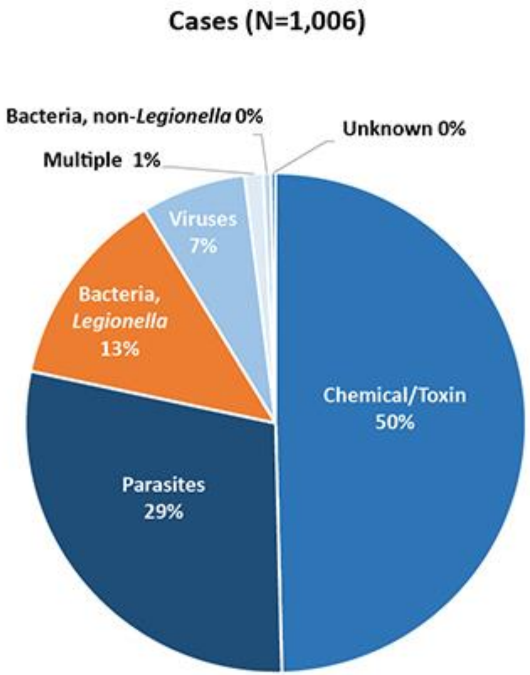
Etiology of drinking water–associated outbreaks, by year — USA, 1971–2014



Centers for Disease Control and Prevention - https://www.cdc.gov/healthywater/surveillance/drinking/2013-2014-figures.html

Waterborne outbreaks associated with drinking water

Etiology of Drinking Water Outbreak-related cases — USA, 2013–2014



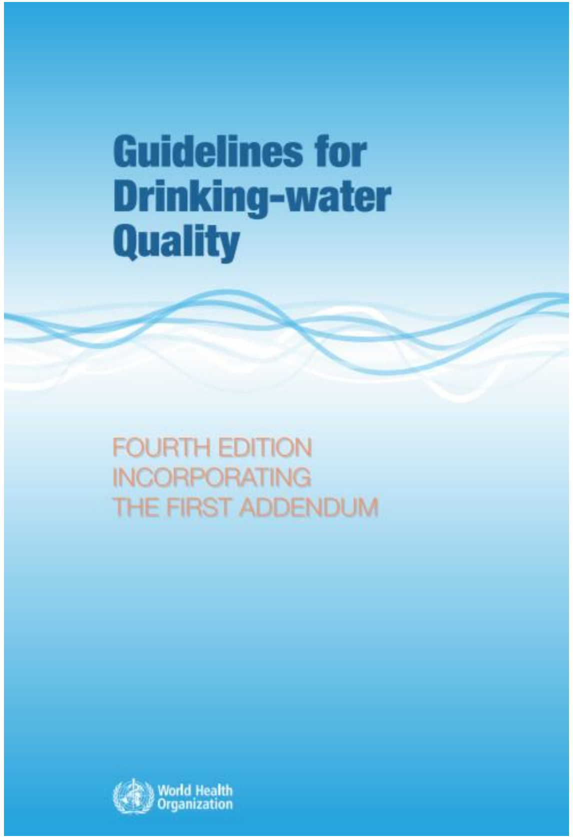
Centers for Disease Control and Prevention - https://www.cdc.gov/healthywater/surveillance/drinking/2013-2014-figures.html

Water in the world

- **6.1 “**By 2030, achieve universal and equitable access to safe and affordable drinking water for all.”
- **6.3** “By 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally.”

United Nations - https://sdgs.un.org/goals/

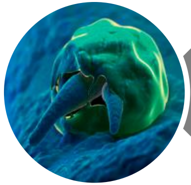
Water in the world



- **WHO, 2017** → guidelines on monitoring and quality control of drinking water
- This version of the document recommended the analysis of more pathogens, like enteric viruses

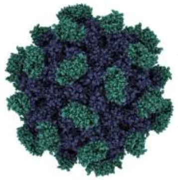
Water-related pathogens

Bacteria



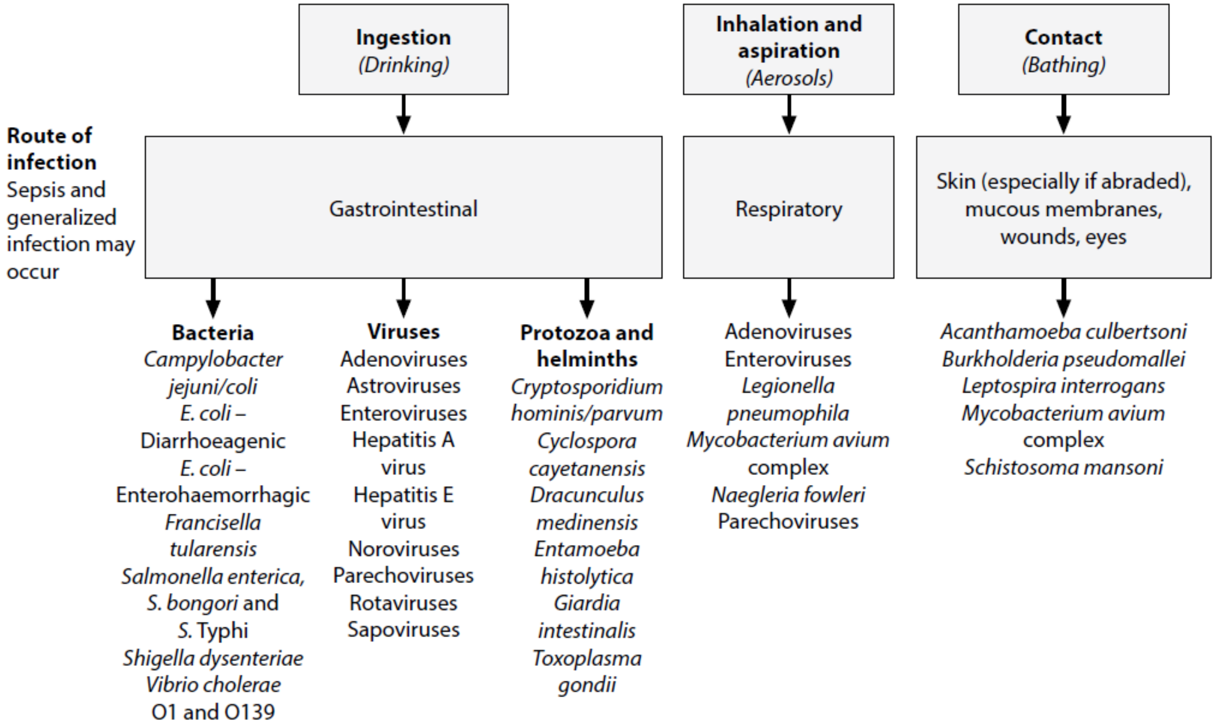
Protozoa

Helminths



Viruses

Transmission pathways for water related pathogens



WHO. Guidelines for Drinking-water Quality. 2017

Monitoring enteric viruses in different water matrices: implementation of methodologies and relevance of permanent surveys

Supervisor - Prof. Doutora Maria Filomena Caeiro (FCUL)

Co-supervisor – Prof. Doutora Maria de Fátima Serejo (FMUL)

Co-supervisor – Dra. Célia Serras Neto (EPAL)

Preface

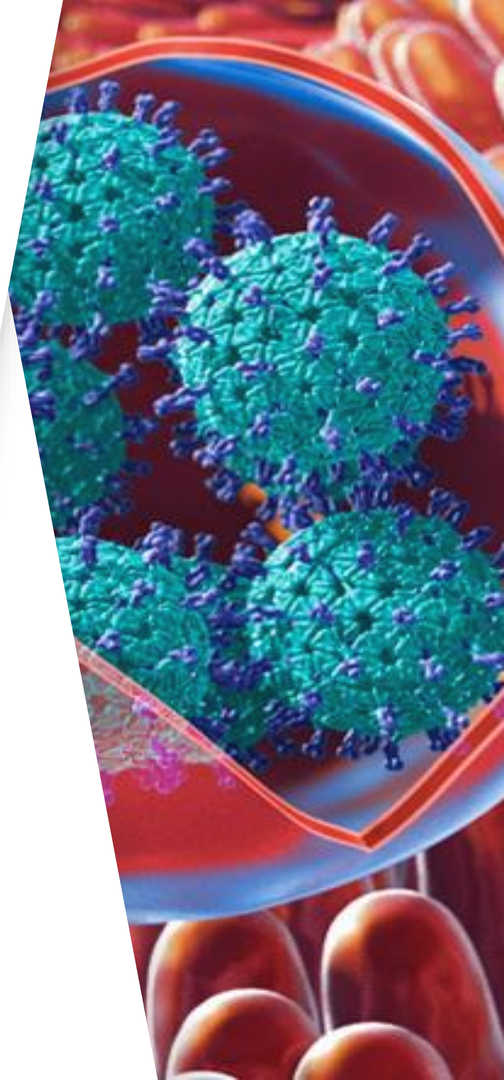
- Doctoral Program in Environmental Health (EnviHealth&Co)

- Hosting company: Empresa Portuguesa das Águas Livres (EPAL)

- Laboratories where the project was carried out:
  - Laboratório da EPAL – Equipa de Microbiologia (LAB/LMB)
  - Laboratório de Virologia (FCUL)

Enteric viruses

- One of the main causes of morbidity and mortality worldwide
- Several families: *Adenoviridae*, *Astroviridae*, *Caliciviridae*, *Hepeviridae*, *Picornaviridae* and *Reoviridae*
- Mainly transmitted by the fecal–oral route
- Very resistant to water treatments
- Robust capsids
- Capacity to remain infectious for a long time and in low doses
- Responsible for different illnesses/symptoms like fever, gastroenteritis, hepatitis, and respiratory diseases



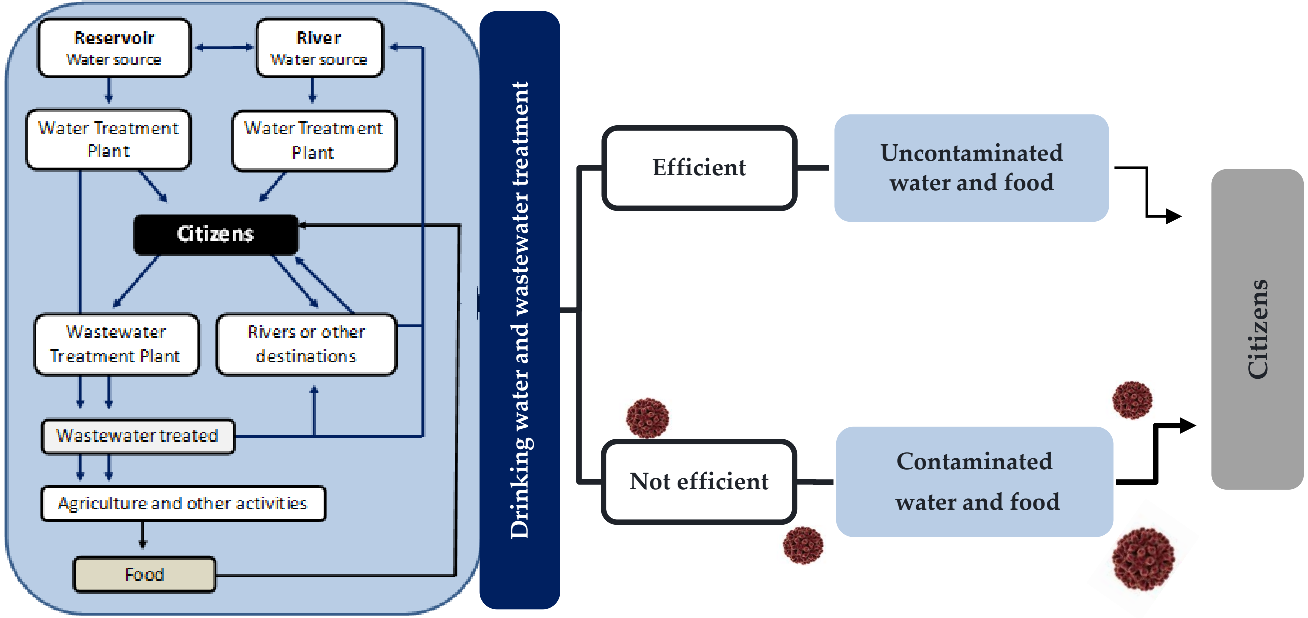
Sinclair et al. *Journal of Applied Microbiology* 2009 | Teixeira et al. *Microbiology Spectrum* 2020 | Salvador et al. *Water* 2020

Enteric viruses

- Sources of viral pollution:

Eifan, S. The Internet Journal of Microbiology . 2013| Gibson , K. Current Opinion in Virology . 2014

Water treatment



Salvador et al. *Microorganisms* 2020

Water treatment and monitoring are two key points to prevent outbreaks related to waterborne diseases.

Enteric viruses

Guidelines for Drinking Water Quality
(WHO, 2017)

→ **Enteric viruses are classified as having a moderate to high impact in human health**

Enterovirus

- RNA viruses of the *Picornaviridae* family

- One of the main causes of human infections worldwide

  -United States → cause more than 30 million infections and several thousand of hospitalizations per year

Instituto_Nacional de Saúde Doutor Ricardo Jorge

Observações_ Boletim Epidemiológico

artigos breves_ n. 5 _Doenças Infeciosas

_Vigilância laboratorial da infeção a Enterovirus entre 2010 e 2013

Paula Palminha, Carlos Ribeiro, Carla Roque, Elsa Vinagre

A study carried out by *INSA* in 625 fecal samples collected from 2010 to 2013 found 22.9% positive for *Enterovirus*

Palminha et al. INSA. Obs Bol Epidemiológico. 2015 | WHO. Guidelines for Drinking-water Quality. 2017

Enterovirus

- *Enterovirus* have been found in untreated natural water, drinking water, untreated and treated wastewater, and seawater in several countries

Untreated natural water

- **USA** (Wong et al. 2009; Lee et al. 2014)
- **Japan** (Haramoto et al. 2015)
- **Netherlands** (Schets et al. 2008)
- **Hawaii** (Updyke et al. 2015)
- **South Africa** (Lin & Sing 2015)
- **France** (Prevost et al. 2015)
- **Germany** (Leifels et al. 2016)
- **Uganda** (O´Brien et al. 2017)
- **Portugal** (Salvador et al. 2020)

Drinking water

- **Colombia** (Peláez-Carvajal et al. 2016)

Wastewater

- **USA** (Montazeri et al. 2015)
- **Bolivia** (Symonds et al. 2014)
- **Colombia** (Peláez-Carvajal et al. 2016)
- **Uganda** (O´Brien et al. 2017)
- **Portugal** (Salvador et al. 2020)

Hepatitis A virus (HAV)

- RNA virus of the *Picornaviridae* family
- It is the most common acute viral hepatitis in the world

  -1.4 million clinical cases reported annually globally

4.22. Hepatite A

DGS desde 1899 Direção-Geral da Saúde

Quadro 39 Número de casos notificados de Hepatite A, por classificação de caso e ano de notificação, Portugal, 2012-2015

Ano \ Classificação de caso	Confirmado	Provável	Possível	Total
2012	10	0	0	**10**
2013	15	2	0	**17**
2014	20	0	0	**20**
2015	29	0	0	**29**
Total	**74**	**2**	**0**	**76**

76 cases of hepatitis A have been reported between 2012 to 2015

554 cases of hepatitis A reported in 2017

WHO. Guidelines for Drinking-water Quality. 2017 | Direção-Geral de Saúde. Doenças de Declaração Obrigatória 2012-2015 Volume 1. Portugal. 2016

Hepatitis A virus (HAV)

- HAV have been found in untreated natural water, drinking water, untreated and treated wastewater, and seawater in several countries

Untreated natural water

- **USA** (Jiang & Chu, 2004)
- **Brazil** (Elmahdya et al., 2016)
- **Spain** (López-Gálvez et al., 2016)
- **Uganda** (O´Brien et al. 2017)
- **Republic of Korea** – groundwater (Shin et al. 2017)

Drinking water

- **Colombia** (Peláez-Carvajal et al. 2016)
- **Portugal** (Salvador et al. 2020)

Wastewater

- **Tunisia** (Béji-Hamza et al. 2014; Ouardani et al., 2015)
- **Sweden** (Hellmér et al. 2014)
- **Pakistan** (Ahmad et al. 2015)
- **South Africa** (Osuolale & Okoh 2015; Adefisoy et al. 2016)
- **Spain** (López-Gálvez et al., 2016)
- **Egypt** (Hamza et al. 2017)
- **Uganda** (O´Brien et al. 2017)
- **Portugal** (Salvador et al. 2020)

Hepatitis E virus (HEV)

- RNA virus of the *Hepeviridae* family
- In 2005 it was estimated that:
  - 20.1 million people were infected with HEV
  - 3.4 million symptomatic cases, 70 000 deaths and 3 000 stillborn infants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Observações_ Boletim Epidemiológico

artigos breves_ n. 10

_Diagnóstico da infeção por vírus da hepatite E no INSA, 2000-2012

Carla Manita Ferreira, João Almeida Santos, Teresa Lourenço, Camalavati Benoliel, Rita Matos, Helena Cortes Martins

A study carried out by INSA in 297 serum samples from 2000 to 2012 verified that 20.2% had antibodies to hepatitis E virus

Rein et al. Hepatology .2012 | Ferreira et al. INSA. Obs Bol Epidemiológico. 2013 | WHO. Guidelines for Drinking-water Quality. 2017

Hepatitis E virus (HEV)

- HEV have been found in untreated natural water, drinking water, untreated and treated wastewater in several countries

Untreated natural water	Drinking water	Wastewater
• **Colombia** (Baez et al., 2017) • **Portugal** (Salvador et al. 2020)	• **Portugal** (Salvador et al. 2020)	• **Italy** (La Rosa et al., 2010) • **Sweden** (Hellmér et al., 2014) • **Pakistan** (Ahmad et al., 2015) • **Colombia** (Baez et al., 2017) • **Portugal** (Salvador et al. 2020)

Norovirus

- RNA viruses of the *Caliciviridae* family
- It is one of the most common agents causing sporadic gastroenteritis

  -United States: cause 60% of acute gastroenteritis cases (21 million cases); 400 000 emergency department visits and 71 000 hospitalizations per year

ELSEVIER
Journal of Clinical Virology
journal homepage: www.elsevier.com/locate/jcv

Short communication

Surveillance of norovirus in Portugal and the emergence of the Sydney variant, 2011–2013

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[a] Laboratório Biologia Molecular, Departamento de Doenças Infeciosas, do Instituto Nacional de Saúde Dr Ricardo Jorge, Lisboa, Portugal
[b] Escola Superior Agrária, Instituto Politécnico de Viseu, Viseu, Portugal
[c] CIBIO/UP, Centro de Investigação em Biodiversidade e Recursos Genéticos/Universidade do Porto, Campus Agrário de Vairão, Vairão, Portugal
[d] Laboratório Nacional de Referência das Infeções Gastrintestinais, Departamento de Doenças Infeciosas, do Instituto Nacional de Saúde Dr. Ricardo Jorg Lisboa, Portugal
[e] Laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia da Universidade do Porto, Porto, Portugal

A study carried out by *INSA* in 580 stool samples from 13 Portuguese Hospitals between May 2011 and March 2012 found 11.6% were positive for *Norovirus*

Robilotti et al. Clinical Microbiollogy Reviews.2015| WHO. Guidelines for Drinking-water Quality. 2017

Norovirus

- *Norovirus* have been found in untreated natural water, drinking water, untreated and treated wastewater, and seawater in several countries

Untreated natural water	Drinking water	Wastewater
•**Finland** (Kukkula et al. 1999, Maunula et al. 2015) •**Norway** (Grøndahl-Rosado et al. 2014) •**Netherlands** (Lodder & Husman et al. 2005) •**Japan** (Haramoto et al. 2005) •**Portugal** (Salvador et al. 2020)	•**Finland** (Kukkula et al. 1999) •**Spain** - Bottled Water (Blanco et al. 2017) •**Portugal** (Salvador et al. 2020)	•**Norway** (Grøndahl-Rosado et al. 2014) •**France** (Silva et al. 2007) •**England** (Campos et al., 2016) •**Sweden** (Hellmér et al. 2014) •**Portugal** (Salvador et al. 2020)

Portuguese legislation

Drinking water
Decreto-Lei n.º 152/2017

Parte III — Parâmetros indicadores

Parâmetro	Valor paramétrico	Unidade	Observações
Alumínio	200	µg/l Al	
Amónio	0,50	mg/l NH_4	
Bactérias coliformes	0	N/100 ml	
Cálcio	-	mg/l Ca	Notas 1 e 2.
Carbono orgânico total (COT).	Sem alteração anormal	mg/l C	Notas 7 e 14.
Cheiro, a 25°C	3	Fator de diluição	
Cloretos	250	mg/l Cl	Nota 1.
Cloritos.	0,7	mg/l ClO_2	Nota 16
Cloratos	0,7	mg/l ClO_3	Nota 16
Clostridium perfringens (incluindo esporos).	0	N/100 ml	Nota 5.
Condutividade	2 500	µS/cm a 20°C	Nota 1.
Cor	20	[illegible]	
Desinfetante residual	-	mg[illegible]	N[illegible]
Dureza total	-	[illegible]$CaCO_3$	N[illegible]
Ferro	200	µg/l Fe	
Magnésio	-	mg/l Mg	Notas 1 e 3.
Manganês	50	µg/l Mn	
Microcistinas — LR total	1	µg/l	Nota 12.
Número de colónias a 22°C	Sem alteração anormal	N/ml a 22°C	Notas 13 e 14.
Número de colónias a 36°C	Sem alteração anormal	N/ml a 36°C	Notas 13 e 14.
Oxidabilidade	5,0	mg/l O_2	Nota 6.
pH	≥ 6,5 e ≤ 9,5	unidades de pH	Nota 1.
Sabor, a 25°C	3	Fator de diluição	
Sódio	200	mg/l Na	
Sulfatos	250	mg/l SO_4	Nota 1.
Turvação	4	UNT	Nota 8.
Dose indicativa (DI).	0,10	mSv	Nota 11.
Radão	500	Bq/l	Nota 9.
Trítio	100	Bq/l	Nota 10.

Enteric viruses

Wastewater for reuse
Decreto-Lei n.º 119/2019

ANEXO I

(a que se refere o artigo 16.º)

Normas de qualidade

A) REGA

Q[illegible] de água para reutilização para rega

Classe de q[illegible] ([1])	CBO$_5$ (mg/L O_2)	SST (mg/L)	Turvação (NTU)	*E. coli* (ufc/100 mL)	Ovos de parasitas intestinais (Nº/L) ([2])	Azoto amoniacal ([3]) (mg NH_4^+/L)	Azoto total ([3]) (mg N/L)	Fósforo total ([3]) (mg P/L)
A	≤10	≤10	≤5	≤10		10	15	5
B	≤25	≤35		≤100				
C	≤25	≤35		≤1000	≤1			
D	≤25	≤35		≤10000	≤1			
E ([4])	≤40	≤60		≤10000				

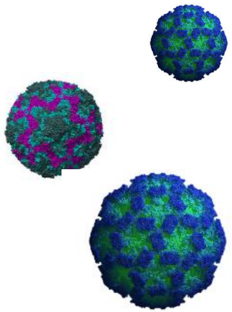
([1]) Descrição no Quadro 2.
([2]) Aplicável na rega de culturas agrícolas destinadas ao consumo animal.
([3]) Parâmetro facultativo. Poderá ser aplicável em alguns projetos de rega para minimização dos riscos de formação de biofilme e obstrução dos sistemas de rega.
([4]) Só aplicável a sistemas descentralizados ou descentralizados em simbiose.

Portuguese legislation

Fecal Indicator Bacteria

- In the current legislation, the microorganisms that indicate fecal contamination (coliform bacteria such as *Escherichia coli* and intestinal enterococci) have not been updated

- Although scientific knowledge has progressed, and it is important to include others such as enteric viruses



Main objectives

1. Implementation and validation of methodologies directed to water matrices, aiming the detection, quantification, and evaluation of potential risks of enteric viruses.

2. Monitoring the presence of *Enterovirus*, *Norovirus* (Genogroup I and Genogroup II), hepatitis A virus, and hepatitis E virus in four water matrices:

- natural water from two surface sources;
- drinking water sampled at the outlet of two Water Treatment Plants (WTPs), and in the water distribution network;
- untreated wastewater, sampled at the inlet of three Wastewater Treatment Plants (WWTPs);
- treated wastewater sampled at the outlet and the inlet of three WWTPs.

Specific objectives

1. To evaluate the potential infectivity of samples RT-qPCR positive for enteric viruses.
2. To evaluate the effectiveness of the water treatment systems in the elimination of enteric viruses.
3. To evaluate correlations between fecal indicator bacteria (FIB) and enteric viruses.
4. To evaluate correlations between several physical-chemical parameters of water and enteric viruses.
5. To evaluate the effectiveness of wastewater treatment systems in the eliminating of enteric viruses.

Materials and Methods

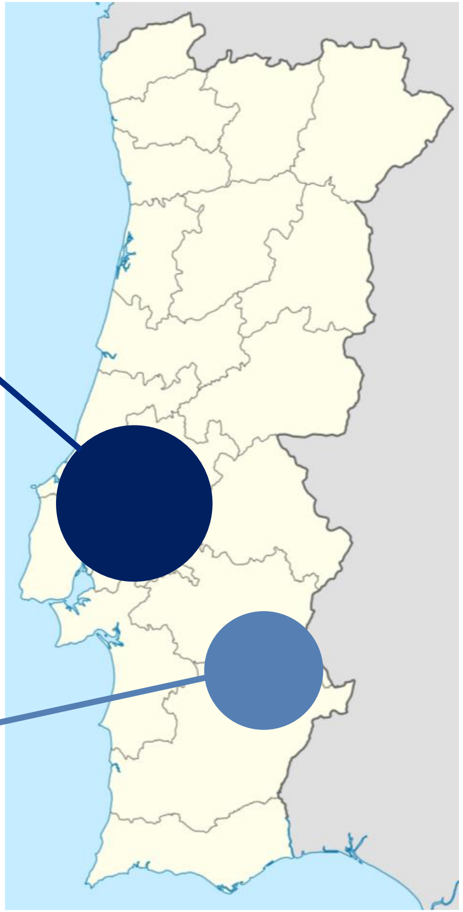
Sampling sites and sampling campaigns:

13 months sampling campaign (May 2018; Jan-Dec 2019)

- 2 surface water sampling points:
  - River (**R**)
  - Dam reservoir (**D**)
- 3 drinking water sampling points:
  - WTP_R (**ETA_R**)
  - WTP_D (**ETA_D**)
  - Water distribution network

5 months sampling campaign (Nov 2019-Mar 2020)

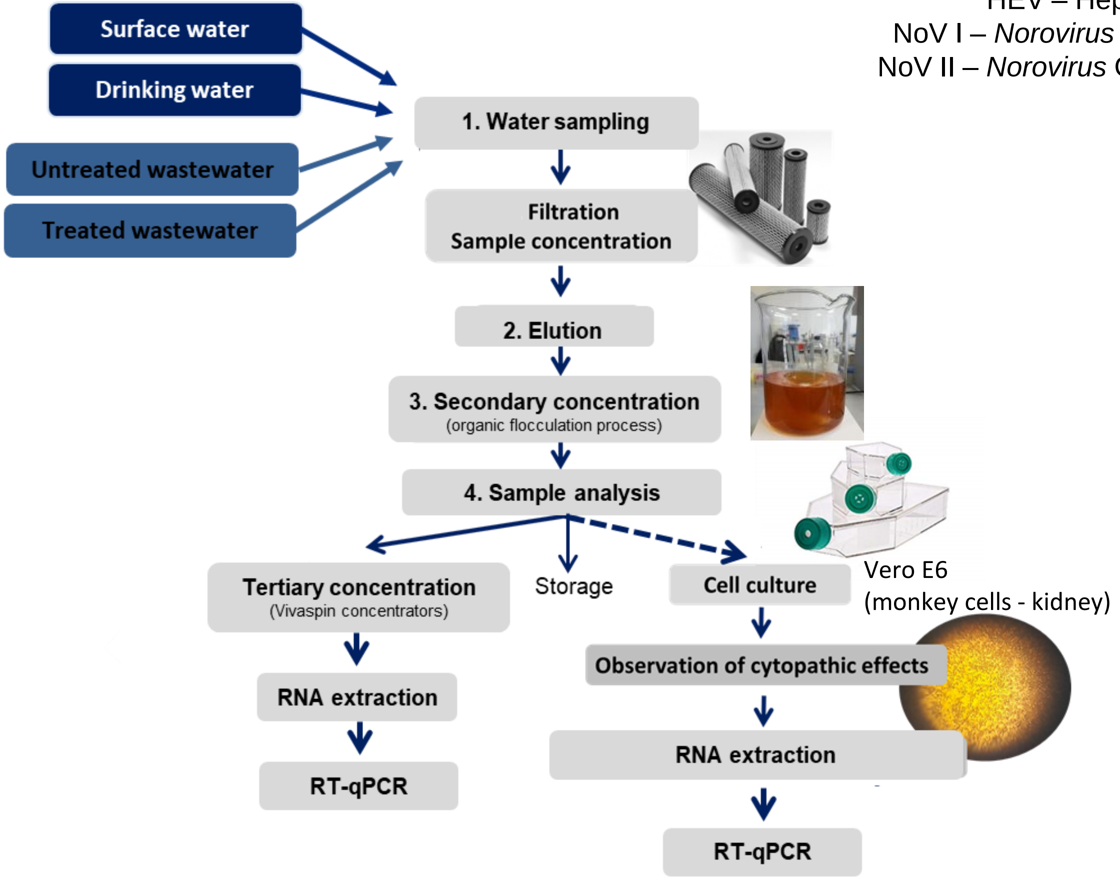
- 3 sampling points of untreated wastewater and 3 sampling points of treated wastewater:
  - WWTP_E (**ETAR_E**)
  - WWTP_R (**ETAR_R**)
  - WWTP_P (**ETAR_P**)



Materials and Methods

Adapted from
Method 1615
(EPA/600/R-10/181)

HAV – Hepatitis A virus
HEV – Hepatitis E virus
NoV I – *Norovirus* Genogroup I
NoV II – *Norovirus* Genogroup II



Materials and Methods
Natural Water/drinking water

Adapted from
Method 1615
(EPA/600/R-10/181)

NanoCeram® Virus Sampler

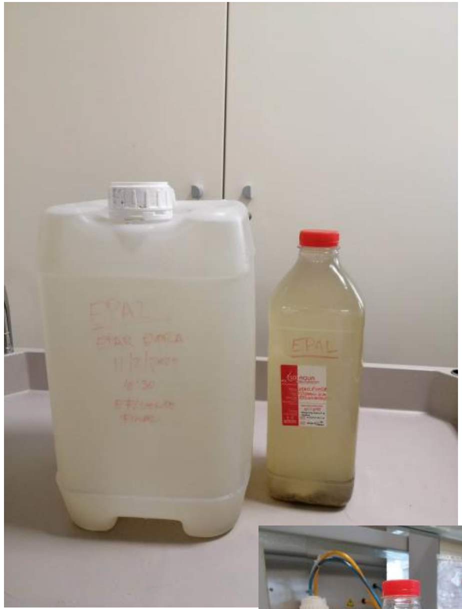
Adapted from
Method 1615
(EPA/600/R-10/181)

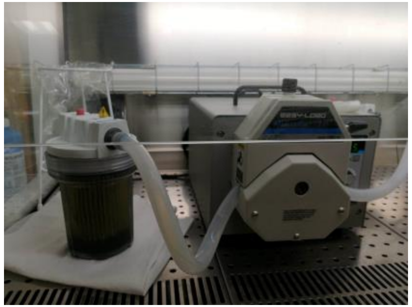
Materials and Methods
Natural Water/drinking water

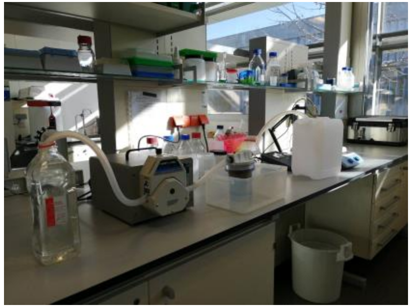
- **Surface water volume:** 130-2340 L
- **Drinking water volume:** 620-2000 L

Materials and Methods
Wastewater

Adapted from
Method 1615
(EPA/600/R-10/181)







- **Untreated wastewater volume:** 2 L
- **Treated wastewater volume:** 10 L

Results

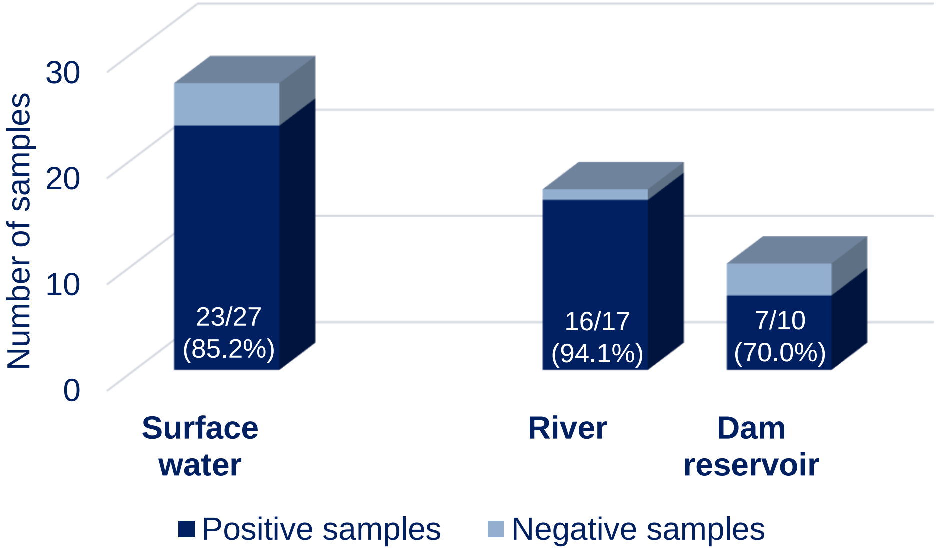
Surface and drinking water

Results

Detection and quantification of enteric viruses – surface water

2019 sampling campaign

- 27 collected samples
- Viral RNA detected:
  - HEV
  - NoV II
  - NoV I
  - *Enterovirus*
- HAV RNA - not detected
- NoV I RNA - only detected in River
- HEV RNA - the most frequently detected

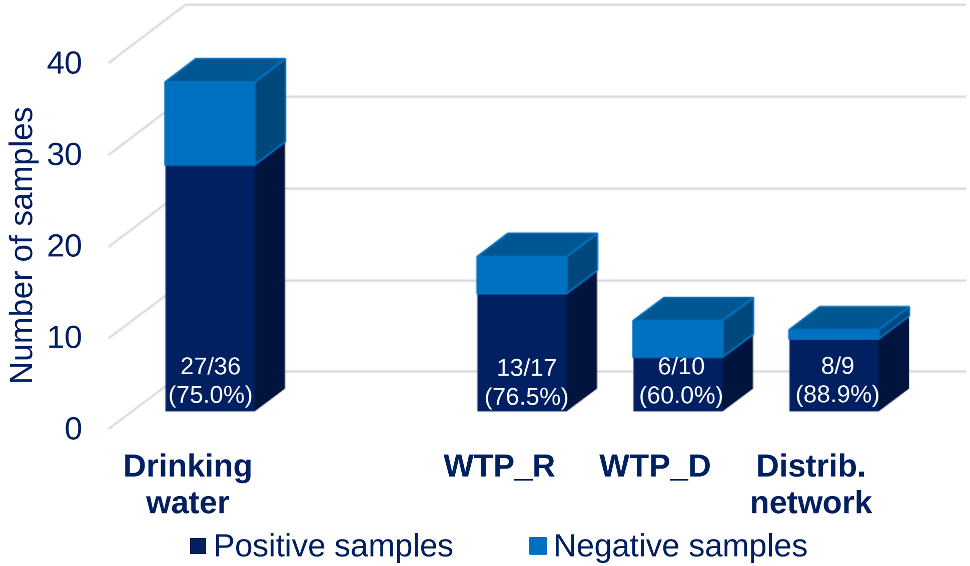


2019 sampling campaign

Results

Detection and quantification of enteric viruses – drinking water

- 36 collected samples
- Viral RNA detected:
  - HEV
  - NoV II
  - HAV



- *Enterovirus* and NoV I RNAs - not detected
- HAV RNA – only one sample (WTP_D)
- HEV RNA - the most frequently detected

Results

Detection and quantification of *Enterovirus*, NoV I, NoV II and HAV RNAs

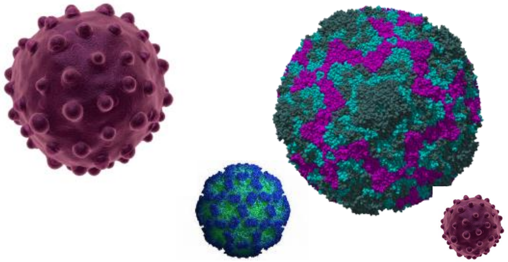
2019 sampling campaign

Table 2. Detection and quantification of enteric viruses in surface water ($n = 27$) and drinking water ($n = 36$) sampled in 2019.

Sampling	River				Dam Reservoir				WTP_R				WTP_D				Point in the Distribution Network			
	Entero	NoV I	NoV II	HAV	Entero	NoV I	NoV II	HAV	Entero	NoV I	NoV II	HAV	Entero	NoV I	NoV II	HAV	Entero	NoV I	NoV II	HAV
	gc/L				gc/L				gc/L				gc/L				gc/L			
January	ND	ND	ND	ND	0.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	12.5	ND
February (A)	ND	ND	0.4	ND	–	–	–	–	ND	ND	2.6	ND	–	–	–	–	–	–	–	–
February (B)	ND	3.6	19.6	ND	ND	ND	2.0	ND	ND	ND	9.7	ND	ND	ND	7.9	0.1	ND	ND	ND	ND
March (A)	4.4	0.2	ND	ND	–	–	–	–	ND	ND	ND	ND	–	–	–	–	–	–	–	–
March (B)	ND	5.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–	–	–	–
April	ND	10.6	2.4	ND	ND	ND	0.8	ND	ND	ND	ND	ND	ND	ND	1.4	ND	ND	ND	ND	ND
May	ND	ND	ND	ND	ND	ND	1.4	ND	ND	ND	1.5	ND	ND	ND	4.5	ND	ND	ND	4.6	ND
June	ND	ND	0.5	ND	ND	ND	78.6	ND	ND	ND	0.6	ND	ND	ND	0.9	ND	ND	ND	0.1	ND
July	ND	ND	ND	ND	–	–	–	–	ND	ND	0.2	ND	–	–	–	–	ND	ND	0.7	ND
August (A)	ND	ND	ND	ND	–	–	–	–	ND	ND	ND	ND	–	–	–	–	ND	ND	0.2	ND
August (B)	ND	ND	ND	ND	–	–	–	–	ND	ND	0.2	ND	–	–	–	–	–	–	–	–
September (A)	ND	0.4	ND	ND	–	–	–	–	ND	ND	0.1	ND	–	–	–	–	ND	ND	0.1	ND
September (B)	ND	2.0	0.8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.9	ND	–	–	–	–
October (A)	ND	137.0	ND	ND	–	–	–	–	ND	ND	0.2	ND	–	–	–	–	–	–	–	–
October (B)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.1	ND	ND	ND	ND	ND	ND	ND	0.1	ND
November	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–	–	–	–
December	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–	–	–	–

(A): sampling performed in the first half of the month; (B): sampling performed in the second half of the month; Entero: *Enterovirus*; HAV: hepatitis A virus; NoV I: *Norovirus* genogroup I; NoV II: *Norovirus* genogroup II; gc/L: genomic copies per liter of sampled water, based on the average value of two independent RT-qPCR results; ND: RNA not detected; -: absence of sampling.

Salvador et al. *Water* 2020



Results

Detection and quantification of HEV RNA

2019 sampling campaign

Table 2. Quantification of HEV RNA in concentrated samples from surface water sources and their associated water treatment plants, and evaluation of the treatment efficacy (reduction in RNA copies).

Date	HEV Concentration (gc/L)		Reduction (%) after Treatment	HEV Concentration (gc/L)		Reduction (%) after Treatment
	River	WTP_R		Dam Reservoir	WTP_D	
January	0	0	*	0	0	*
February, first half	355.5	320.8	9.8	-	-	-
February, second half	78.2	49.3	37.0	29.1	75.2	NR
March, first half	0	0	*	-	-	-
March, second half	4,029.1	0	100	0	0	*
April	7,383.1	2,379.3	67.8	109,687.5	5,617.1	94.9
May	1,936.5	428.0	77.9	2,412	0	100
June	1,394.9	126.0	91.0	0	58.7	NR
July	1,755.0	22.0	98.7	-	-	-
August, first half	206.5	24.2	88.3	-	-	-
August, second half	113.3	0	100	-	-	-
September, first half	23.3	1.9	91.9	-	-	-
September, second half	55.1	5.0	90.9	19.5	5.2	73.3
October, first half	36.3	4.5	87.6	-	-	-
October, second half	2.7	0	100	30.4	0.7	97.6
November	69.9	4.8	93.1	0.7	0	100
December	2.1	0	100	0	0	*

* Undetermined value or not calculated due to absence of detection; NR—no reduction with treatment; - no result, due to absence of sampling; gc/L: genomic copies per liter of sampled water, calculated with RT-qPCR results (average values of two independent reactions) and data from Table 1.

Salvador et al. *Microorganisms* 2020

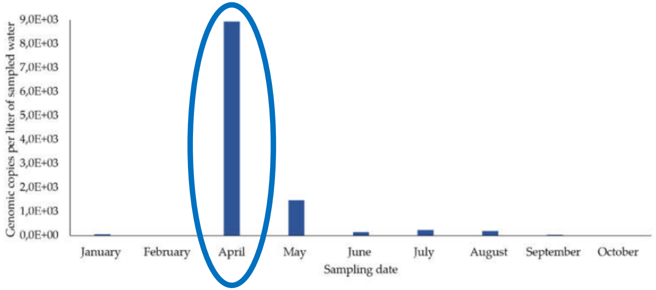
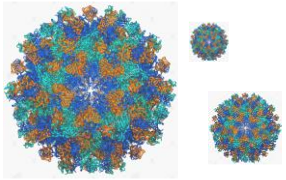


Figure 3. Variation in the concentration of HEV RNA detected in concentrated drinking water from the sampling point in the distribution network during 2019 ($n = 9$). RT-qPCR results (average values of two independent reactions), in gc/L, indicate estimated genomic copies per liter of sampled water (based on data from Table 1).

Salvador et al. *Microorganisms* 2020



HEV RNA was frequently detected

2019 sampling campaign

Results

Efficacy of Water Treatment Plants in the elimination of *Enterovirus*, NoV I and NoV II RNAs

Water Treatment Plant	Enteric virus	Reduction of viral RNA
WTP_R	*Enterovirus*	100%
	NoV I	100%
	NoV II	0–100%
WTP_D	*Enterovirus*	100%
	NoV I	Not detected
	NoV II	0–98.9%

More efficient in eliminating viral RNA (WTP_R)

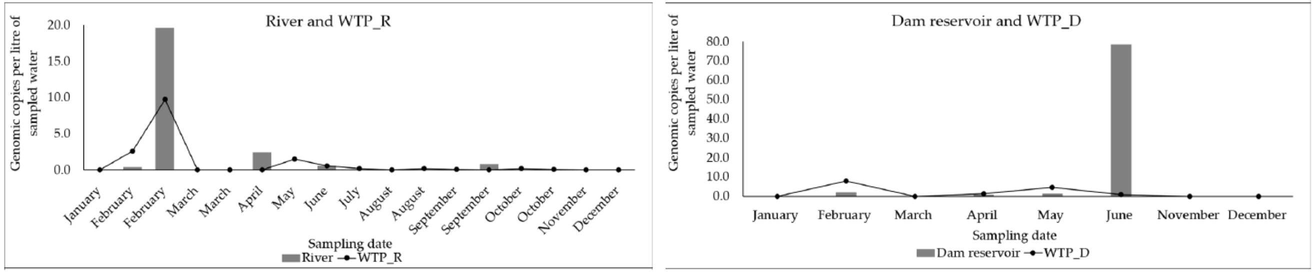


Figure 3. Variation in the NoV II RNA concentration throughout the 2019 sampling campaign, in River and WTP_R (n = 34) (first graph) and in Dam reservoir and WTP_D (n = 20) (second graph). Each concentration value, in gc/L, is the average of two independent RT-qPCR results.

Salvador et al. *Water* 2020

2019 sampling campaign

Results

Efficacy of Water Treatment Plants in HEV RNA elimination

- HEV found in surface water samples, were still detected in drinking water, although usually at lower concentrations

	Minimal reduction	Maximum reduction
WTP_R	9.8%	100%
WTP_D	0%	100%

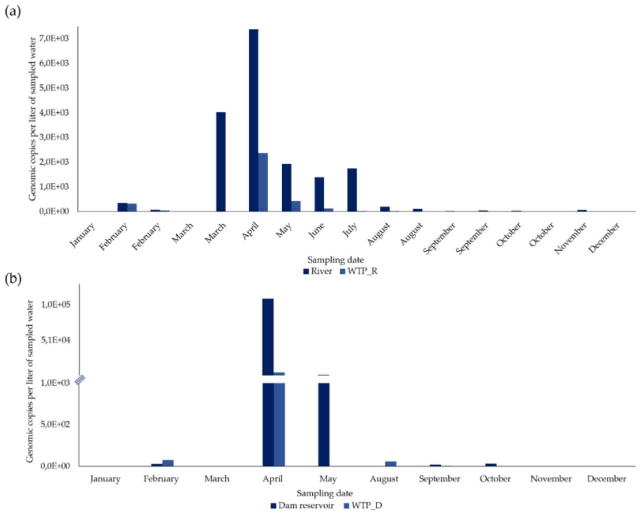


Figure 2. Variation in the concentration of hepatitis E virus (HEV) RNA detected in concentrated water sampled in four sampling sites during 2019. **(a)** River and WTP_R (n = 34). **(b)** Dam reservoir and WTP_D (n = 20). RT-qPCR results (average values from two independent reactions), in gc/L, indicate estimated genomic copies per liter of sampled water (based on data from Table 1).

Salvador et al. *Microorganisms* 2020

2019 sampling campaign

Results

Evaluation of sample's infectivity

- HEV infectivity was confirmed in samples from all matrixes (globally 25%)

Water matrice	Sampling point	Number of samples with infectious HEV
Surface water 3/13 (23.0%)	River	2/9 (22.%)
	Dam reservoir	1/4 (25%)
Drinking water 5/18 (27.7%)	WTP_R	3/9 (33.3%)
	WTP_D	1/4 (25%)
	Point in the distribution network	1/6 (16.6%)

Vero E6 cultures

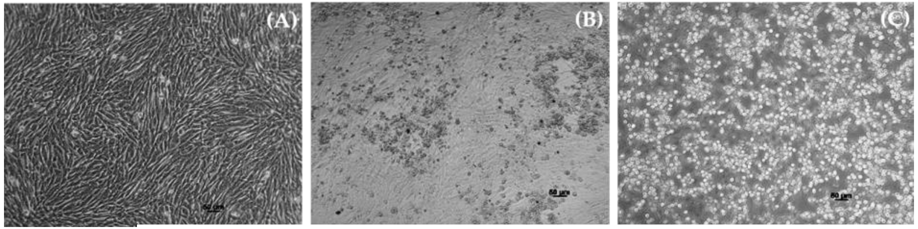


Figure 2. Photograph of Vero E6 cultures (a) mock infected (control) culture evidencing a monolayer of adherent cells; (b, c) mengovirus infected cultures evidencing cytopathic effect (CPE): (b) early CPE (infection foci), (c) complete CPE (rounded detached cells).

Salvador et al. *Journal of Water and Health*. Submitted.

Results

Detection and quantification of fecal indicator bacteria (FIB)

2019 sampling campaign

FIB detected in surface water:

- Coliform bacteria - 24/24 samples (100.0%)
- Fecal coliforms - 18/24 samples (75.0%)
- *E. coli* - 17/24 samples (70.8%)
- Intestinal enterococci - 16/24 samples (66.7%)

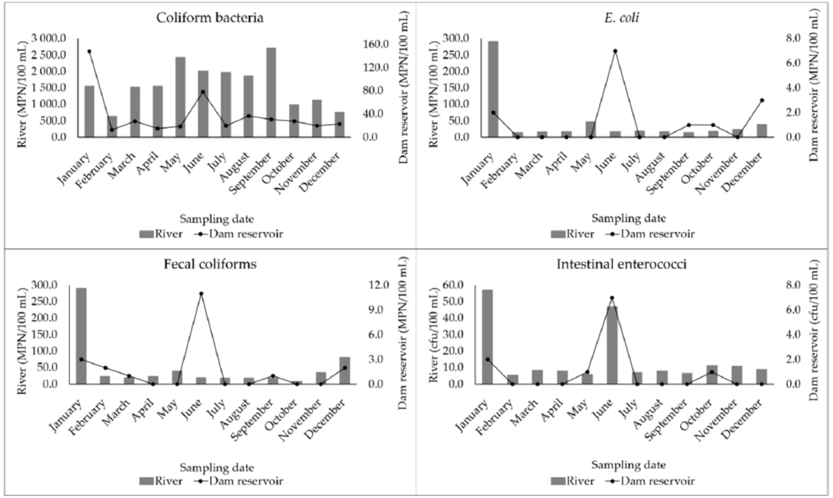


Figure 2. Microbiological characterization of the surface water collected during the 2019 sampling campaign (n = 96). The graphs represent the variation in concentration values from the four groups of FIB evaluated (coliform bacteria, *E. coli*, fecal coliforms, and intestinal enterococci) registered

Salvador et al. *Water* 2020

FIB→ not detected in drinking water

2019 sampling campaign

Results

Correlations between microbiological and physicochemical parameters (surface and drinking water)

Table 3. Spearman's correlation coefficients relating enteric viruses, microbiological, and physicochemical parameters of water quality.

	Entero	NoV I	NoV II	HAV	Coliform bacteria	Fecal coliforms	*E. coli*	Intestinal enterococci	Temp	pH	Total chlorine
Entero	1										
NoV I	0.2	1									
NoV II	−0.16	0.03	1								
HAV	−0.02	−0.05	0.2	1							
Coliform bacteria	0.14	−0.01	−0.08	−0.11	1						
Fecal coliforms	0.01	−0.1	−0.05	−0.09	0.92***	1					
E. coli	0.1	−0.16	−0.1	−0.09	0.92***	0.94***	1				
Intestinal enterococci	0.17	0.48**	−0.15	−0.09	−0.04	−0.17	−0.16	1			
Temp	−0.1	0.1	−0.12	−0.2	0.30*	0.16	0.22	0.19	1		
pH	−0.09	−0.11	0.17	0.14	−0.45**	−0.39*	−0.41**	−0.16	−0.34*	1	
Total chlorine	−0.19	−0.08	0.06	0.2	−0.85**	−0.76**	−0.73**	−0.16	−0.29	0.36*	1

Entero: *Enterovirus*; HAV: Hepatitis A virus; NoV I: *Norovirus* genogroup I; NoV II: *Norovirus* genogroup II; Temp: temperature;; *: weak correlation; **: moderate correlation; ***: strong correlation.

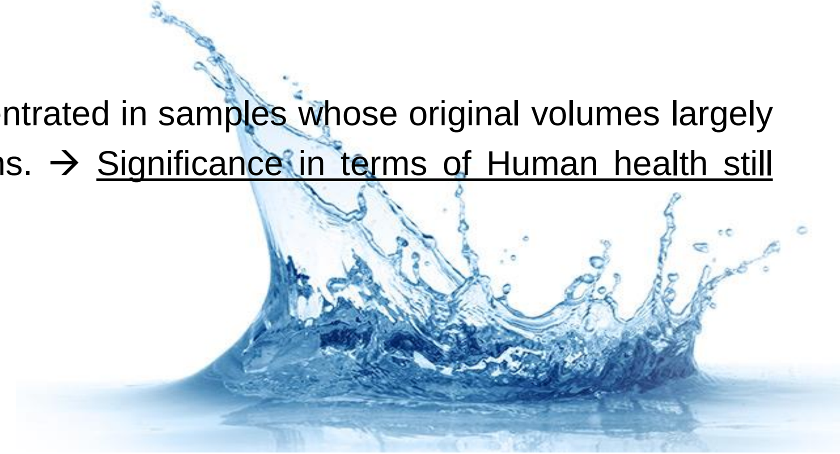
Salvador et al. *Water* 2020

Results

Wastewater

Final remarks

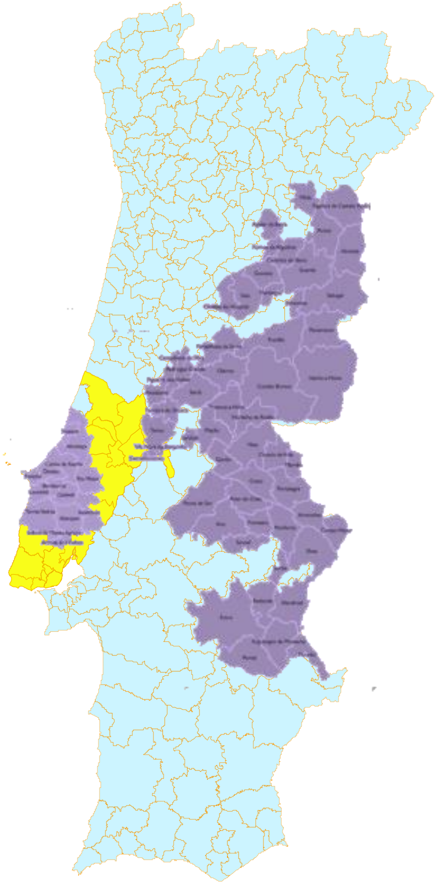
- In Portugal, viral RNAs and were detected in water matrices, as in other countries.
- Infectious HEV were found in the analyzed matrices.
- Variable effectiveness of WTPs and WWTPs in eliminating enteric viruses.
- Combination of molecular detection/quantification with assessment of infectivity in cell cultures.
- The detected viruses were highly concentrated in samples whose original volumes largely exceed those daily ingested by humans. → Significance in terms of Human health still deserve future assessments.



Laboratório de Lisboa – EPAL

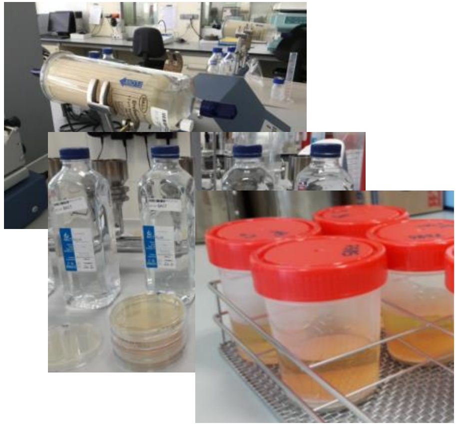
EPAL
Grupo Águas de Portugal

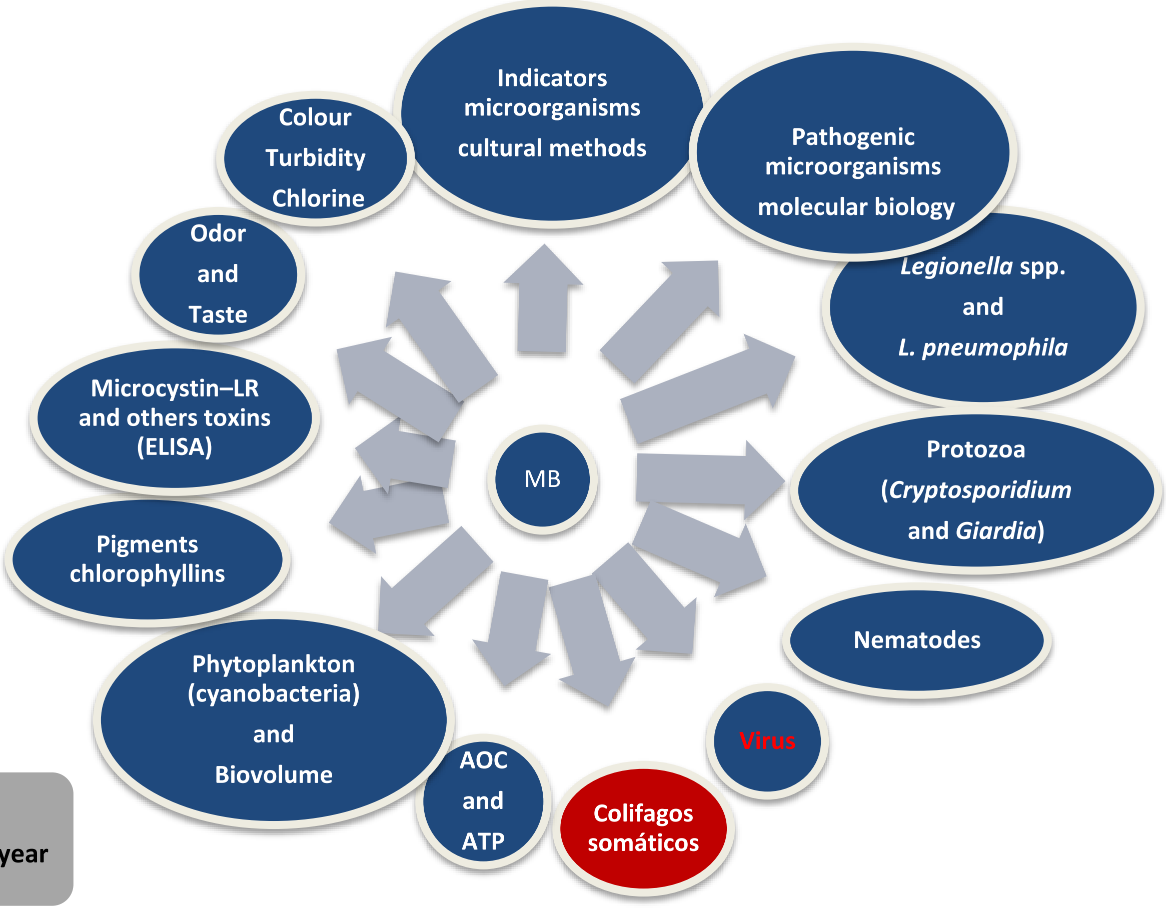
Sistema de Abastecimento da EPAL e AdVT



- ✓ **30% área de Portugal**
- ✓ Fornecimento em Alta a 87 Municípios + cidade de Lisboa em Baixa
- ✓ População Abastecida ≈ 3,5 M

Microbiology area – Laboratório de Lisboa





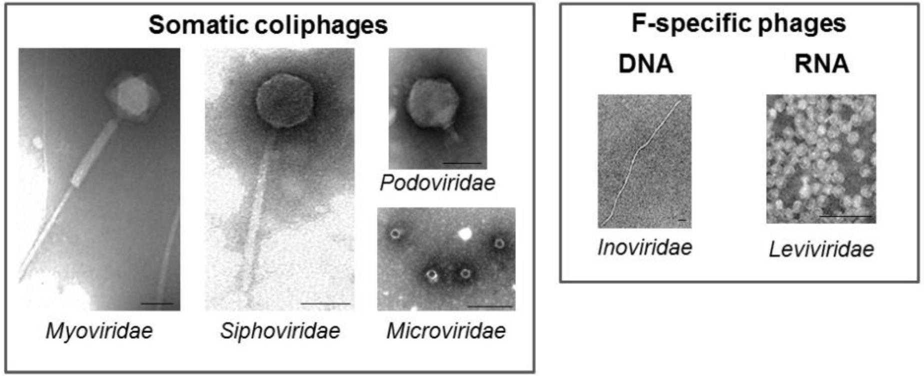
39 parameters

In different water, sediment and biofilm matrices

About 60 000 determinations per year

Source: Célia Neto (MB Supervisor)

- Bacteriófagos ou fagos são vírus que infetam bactérias
- Compostos pelo menos por um ácido nucleico e uma cápside proteica
- Só conseguem replicar dentro de bactérias hospedeiras suscetíveis
- Existem 2 grupos de bacteriófagos que infetam *E. coli*: colifagos somáticos e fagos F-específicos



Jofre et al. Coliphages as Model Organisms in the Characterization and Management of Water Resources. 2016 Water

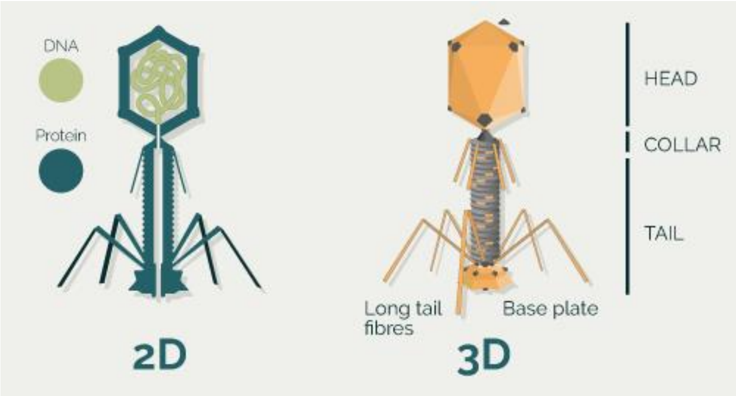
Colifagos somáticos

- Colifagos somáticos são um grupo heterogéneo de bacteriófagos que infetam *E. coli* através da parede celular

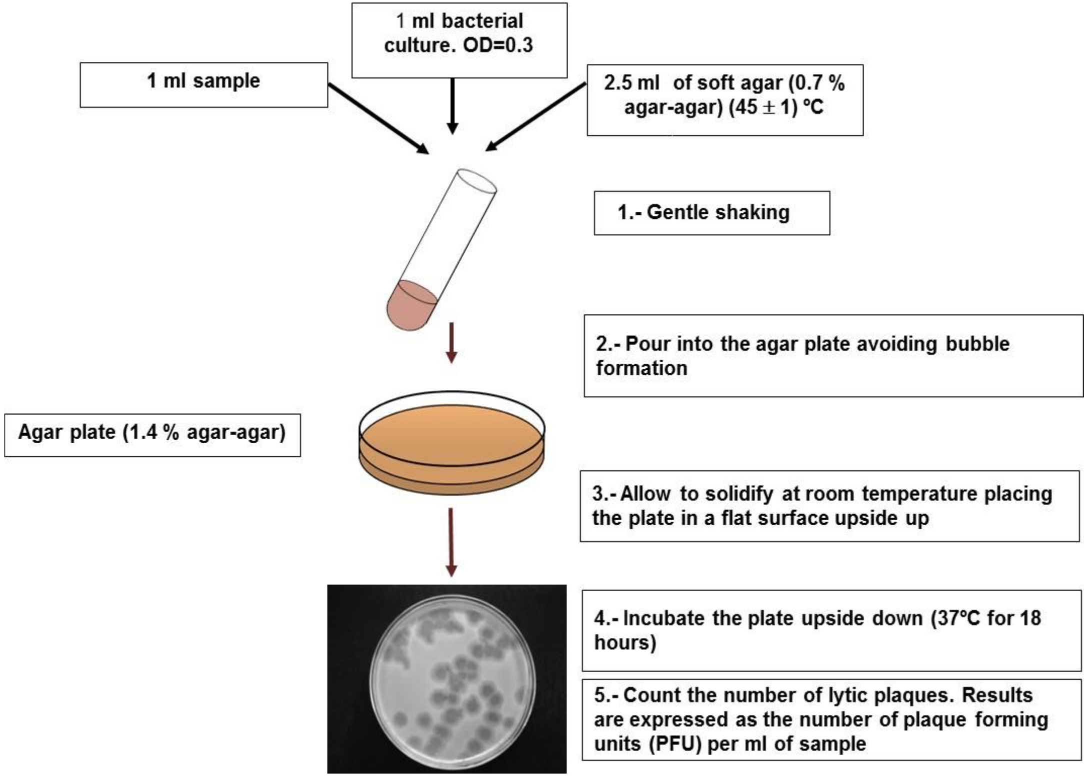
- Métodos de deteção fáceis, rápidos e económicos

- **Legislação Portuguesa → DL n.º 69/2023, de 21 de Agosto**

- Bons indicadores de contaminação viral?

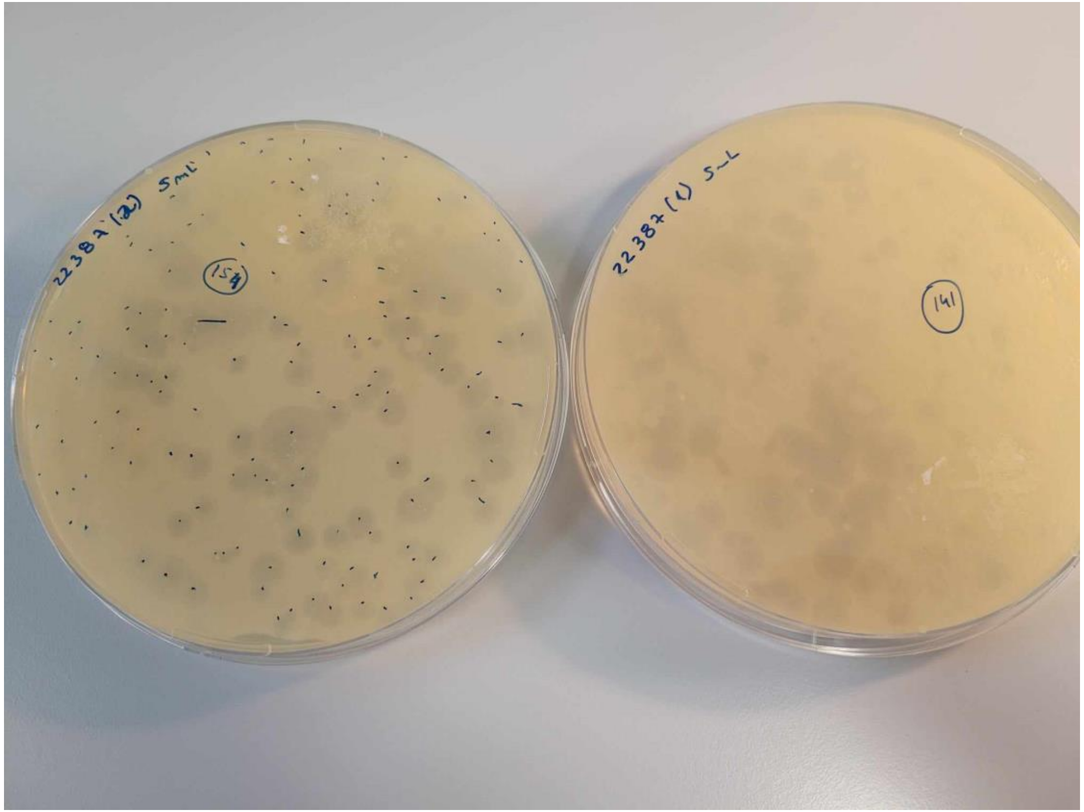


Colifágos somaticos



Jofre et al. Coliphages as Model Organisms in the Characterization and Management of Water Resources. 2016 Water

Colifagos somáticos



XIII JORNADAS DE ENGENHARIA
Grupo Águas de Portugal

ÁGUA, UM VALOR MOBILIZADOR

Avaliação da presença de RNA do vírus SARS-CoV-2 em águas naturais e para consumo do Sistema de Abastecimento da EPAL e AdVT

Daniel Salvador, M.Sc., Ph.D.
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Célia Neto, Rui Neves Carneiro

Direção de Laboratórios

EPAL
Grupo Águas de Portugal

pathogens MDPI

Article

One-Year Surveillance of SARS-CoV-2 Virus in Natural and Drinking Water

Daniel Salvador [1,2,*], Maria Filomena Caeiro [2], Célia Neto [1] and Rui Neves Carneiro [1]

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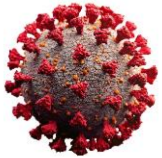
[2] Centro de Estudos do Ambiente e do Mar (CESAM), Departamento de Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa, Edifício C2-Piso 4, Campo Grande, 1749-016 Lisboa, Portugal
* Correspondence: daniel.salvador@adp.pt

Abstract: Although the SARS-CoV-2 virus has been detected in wastewater from several countries, monitoring its presence in other water matrices is still limited. This study aimed to evaluate the presence of this virus in natural and drinking water over one year of monitoring (2021). A survey of viral RNA was carried out by RT-qPCR in concentrated samples of surface water, groundwater, and drinking water from different regions of Portugal. SARS-CoV-2 RNA—quantified in genomic copies per liter (gc/L) of sampled water—was not detected in groundwater, but was detected and quantified in samples of surface water (two out of 43; 8035 and 23,757 gc/L) and in drinking water (one out of 43 samples; 7463 gc/L). The study also detected and quantified *Norovirus* RNA, intending to confirm the use of this enteric virus to assess variations in fecal matter throughout the sampling campaign. The samples positive for SARS-CoV-2 RNA also had the highest concentrations of *Norovirus* RNA—including the drinking water sample, which proved negative for fecal enteric bacteria (FIB). These results indicate that, to protect human health, it is advisable to continue monitoring these viruses, and noroviruses as fecal indicators (FI) as well—especially in low-flow water bodies that receive wastewater.

check for updates

Citation: Salvador, D.; Caeiro, M.F.; Neto, C.; Carneiro, R.N. One-Year

Keywords: human health; *Norovirus*; risk assessment; RT-qPCR; water monitoring; water safety; fecal indicator (FI)



Environmental Research 193 (2021) 110265

Contents lists available at ScienceDirect
Environmental Research
journal homepage: www.elsevier.com/locate/envres

SARS-CoV-2 coronavirus in water and wastewater: A critical review about presence and concern

Hai Nguyen Tran[a,b,*], Giang Truong Le[c], Dong Thanh Nguyen[d], Ruey-Shin Juang[e,f], Jörg Rinklebe[g,h], Amit Bhatnagar[i], Eder C. Lima[j], Hafiz M.N. Iqbal[k], Ajit K. Sarmah[l], Huan-Ping Chao[m,**]

Science of The Total Environment
Volume 737, 1 October 2020, 140405

First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan

Eiji Haramoto[a], Bikash Malla[a], Ocean Thakali[b], Masaaki Kitajima[c]

Comments (1)

Environmental Science & Technology Letters
pubs.acs.org/journal/estlcu
Letter

Persistence of SARS-CoV-2 in Water and Wastewater

Aaron Bivins,[#] Justin Greaves,[#] Robert Fischer,[#] Kwe Claude Yinda, Warish Ahmed, Masaaki Kitajima, Vincent J. Munster, and Kyle Bibby*

Cite This: Environ. Sci. Technol. Lett. 2020, 7, 937−942
Read Online

sk of SARS-CoV-2 infection from contaminated water systems

ie Shutler, Krzysztof Zaraska, Tom Holding, Monika Machnik, Kiranmai Uppuluri, Ian Ashton, Łukasz Migdał, vinder Dahiya

i: https://doi.org/10.1101/2020.06.17.20133504
w published in ACS ES&T Water doi: 10.1021/acsestwater.0c00246

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Available online at www.sciencedirect.com
Journal of Hospital Infection
journal homepage: www.elsevier.com/locate/jhin
Healthcare Infection Society

Short report

Preventing SARS-CoV-2 transmission in rehabilitation pools and therapeutic water environments

S. Romano-Bertrand[a,b,*], L-S. Aho Glele[c], B. Grandbastien[d], D. Lepelletier[e,f], on behalf of the French Society for Hospital Hygiene
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[b] Hospital Hygiene and Infection Control Team, University Hospital of Montpellier, Montpellier, France
[c] Department of Epidemiology and Infection Control, Dijon University Hospital, Dijon, France
[d] Department of Preventive Medicine, Infection Prevention and Control Team, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Lausanne, Switzerland
[e] MiHAR Lab, EE 1701 S, Nantes University, Nantes, France
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Research

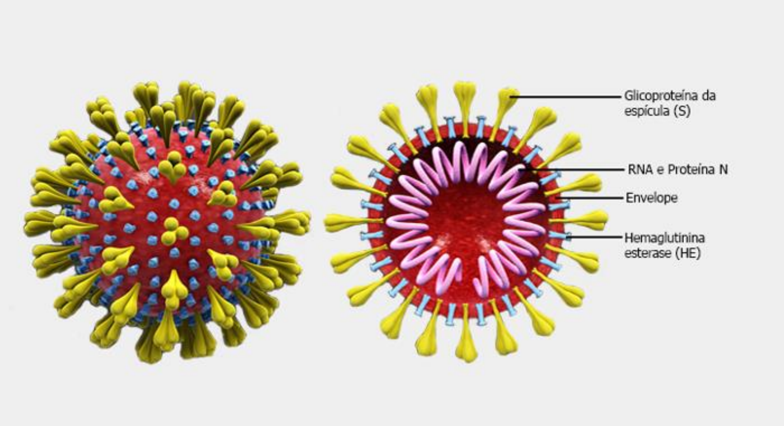
Evaluation of lockdown effect on SARS-CoV-2 dynamics through viral genome quantification in waste water, Greater Paris, France, 5 March to 23 April 2020

Check for updates

S Wurtzer[1], V Marechal[2,3], JM Mouchel[4], Y Maday[3,5], R Teyssou[6], E Richard[1], JL Almayrac[7], L Moulin[1]

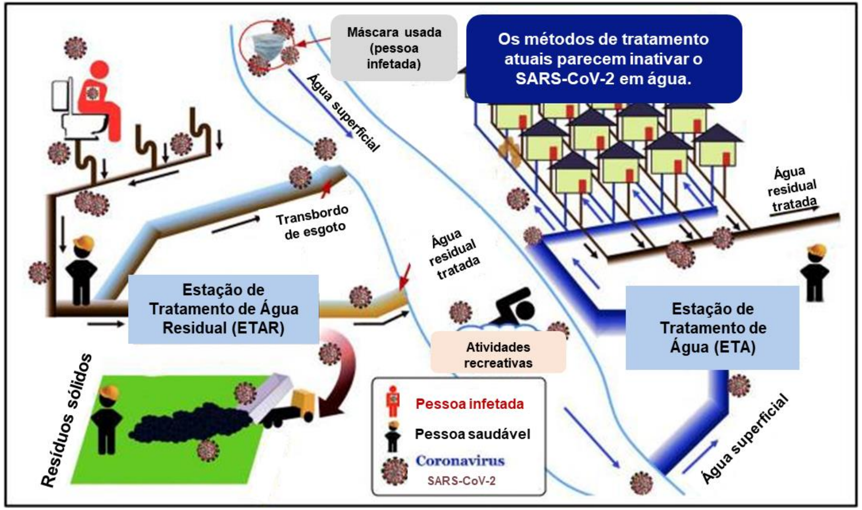
O vírus SARS-CoV-2

- Grupo de coronavírus - vírus de RNA
- Vírus com invólucro lipídico → ✚ mais sensível aos tratamentos da água
- Período de manutenção da capacidade de infetar humanos em ambientes aquáticos dependerá fortemente das propriedades da água
- Eliminado pelos sistemas respiratório superior e gastrointestinal
- Pode ocorrer a excreção destes vírus pelas fezes até quatro semanas



Fonte: Adaptado de https://www.scientificanimations.com/wiki-images/

Vias de transmissão



Possíveis vias de transmissão:

- Contato com fluídos contaminados
- Via aérea
- Via fecal-oral – água residual
- Via sanguínea
- Transmissão de mãe para filho
- Transmissão de animais para o ser humano
- Contaminação provocada pelas máscaras que são lançadas para o ambiente e que atingem a água

O RNA de SARS-CoV-2 nos últimos meses foi detetado em:

- Lamas de Estações de Tratamento de Águas Residuais
- Águas residuais municipais
- Águas residuais hospitalares
- Águas residuais de navios cruzeiro e de aviões comerciais de passageiros,
- Águas residuais tratadas (tratamento secundário)
- Água não potável
- Água superficial de rio

Resultados – Água Natural

XIII JORNADAS DE ENGENHARIA
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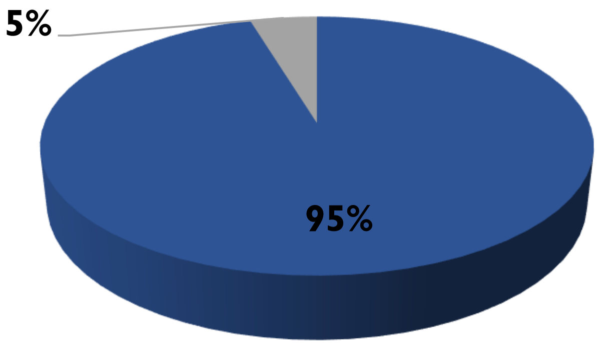
- Janeiro a dezembro de 2021 → 43 amostras de água natural, 35 provenientes de massas de água superficiais e 8 de furos
- RNA do SARS-CoV-2 detectado apenas em duas amostras de água superficial

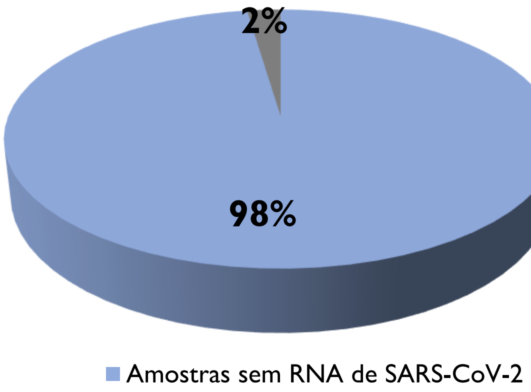


Matriz		Origem	Número de amostras colhidas	RNA de SARS-CoV-2	
				Número de amostras positivas	Concentração média (gc/L)
Água natural	Água superficial	Rio_T	10	0	-
		Albufeira_C	9	1	23,757
		Albufeira_M	5	0	-
		Albufeira_P	7	1	8,035
		Albufeira_S	4	0	-
	Água subterrânea	Furo_A	2	0	-
		Furo_L	2	0	-
		Furo_O	4	0	-

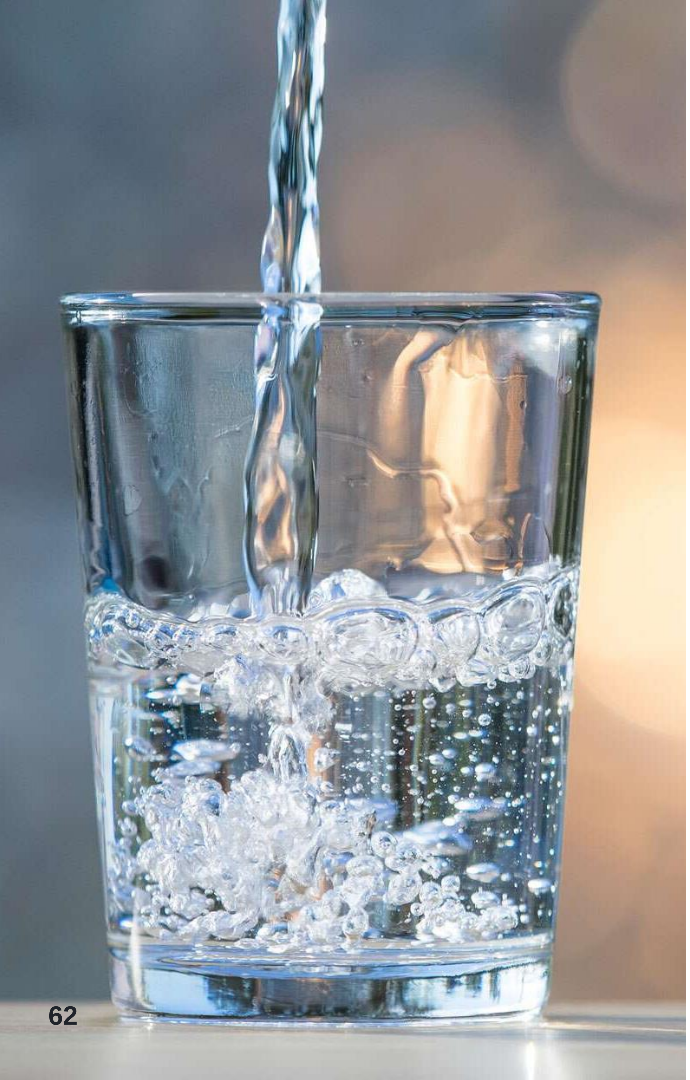
XIII JORNADAS DE ENGENHARIA
Grupo Águas de Portugal

Resultados – Água para consumo

- Janeiro a dezembro de 2021 → 43 amostras de água para consumo na saída das ETA ou nas estações de desinfeção de águas subterrâneas
- RNA do SARS-CoV-2 detectado apenas numa amostra



Matriz		Origem	Número de amostras colhidas	RNA de SARS-CoV-2	
				Número de amostras positivas	Concentração média (gc/L)
Água para consumo	Água para consumo proveniente de água superficial	ETA_T	10	0	-
		ETA_C	9	1	7,463
		ETA_M	5	0	-
		ETA_P	7	0	-
		ETA_S	4	0	-
	Água para consumo proveniente de água subterrânea	Furo_A_AC	2	0	-
		Furo_L_AC	2	0	-
		Furo_O_AC	4	0	-



Final remarks

- Supply of safe water is fundamental for maintaining human health
- Global climate change increases the risk of human exposure to waterborne pathogens, namely enteric viruses
- Pathogenic microorganisms transmitted by water have a high impact on human health
- The goal of cheap, fast, and reliable detection of many pathogens in natural water calls for innovative developments in analytical technologies and internationally compatible protocols for water quality assessment

Final remarks

- Enteric viruses are a current problem in developed and developing countries

  -This pathogens are resistant and can remain in the water for a long time

- Monitoring of microorganisms can be performed with different microbiology methods, but RT-qPCR is one of the most used

- With the advancement of knowledge, legislation needs to be updated with more parameters

- We need a different mindset to view the current and future water-related problems, and then collectively formulate and implement business unusual and out-of-the-box solutions

Thanks for the attention.