



International Symposium
Improving Access to Safe Water
Perspectives from Africa and the Americas
São Carlos, 13-17th September 2010

Aquatic Virology

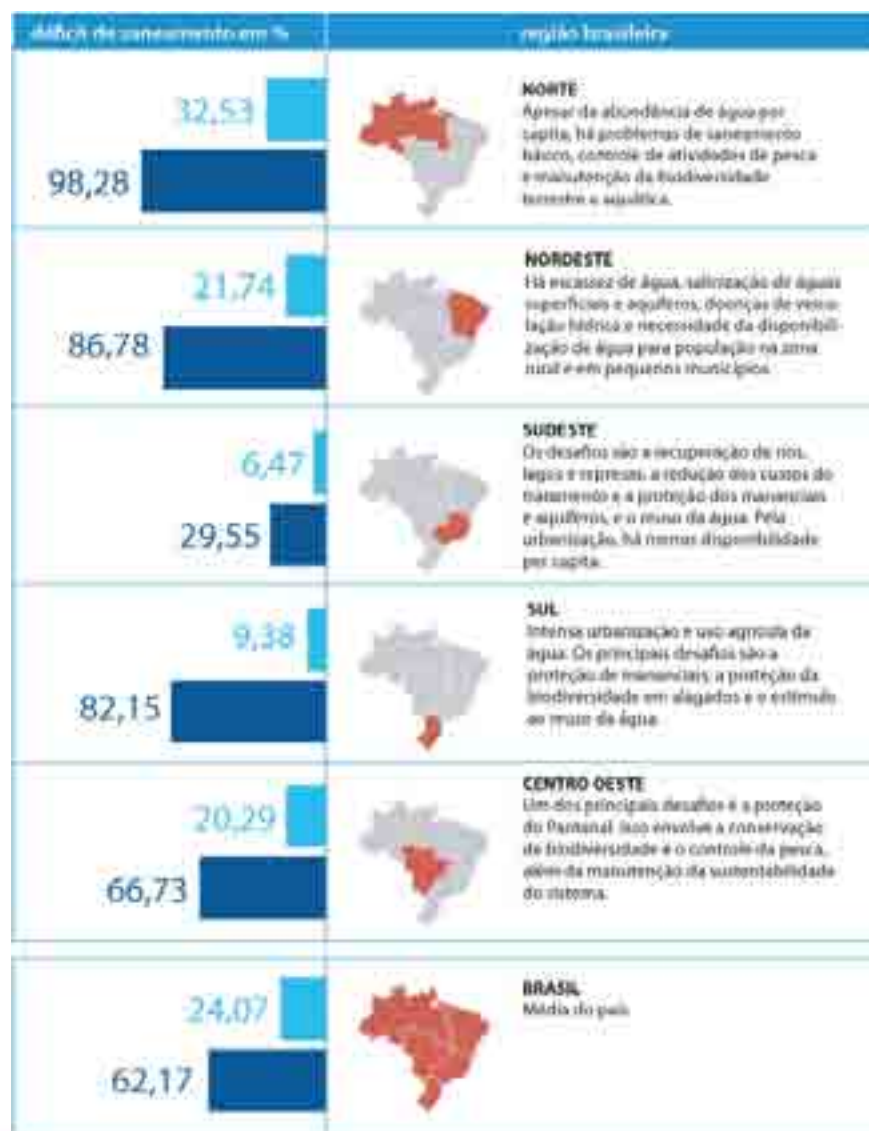
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Laboratório de Microbiologia Molecular

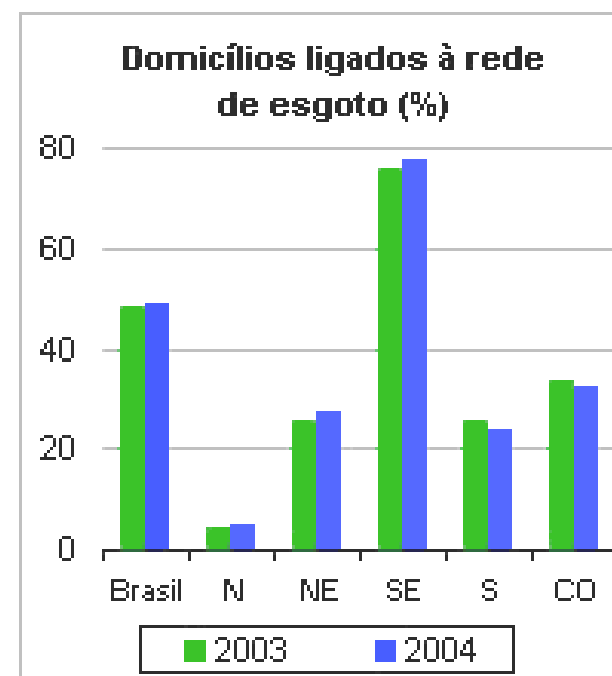
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 déficit de água
 déficit de esgoto

Fonte: CUNHA, Nelson. SPAN, Jairo. O Meio de Água. São Paulo: Publifolha, 2005, p. 76-79. 06



Fonte: Programa das Nações Unidas para o Desenvolvimento – Pnud

Aquatic Virology

- Environmental virology
- Enteric Viruses
 - Monitoring, treatment, health risks
- Virioplankton
 - Influence on aquatic ecosystems



Viriplankton

Viriplankton may be defined as the most abundant part of the microscopic communities in aquatic ecosystems, including viruses infecting bacterio- and phytoplankton. Those viruses are present in seawater and freshwater

More than 10^8 viral particles may exist in 1L of marine water

More than 10^{29} viral particles constitute the Earth's viriplankton community

In general, viral lysis affects the carbon and nutrient flow through different compartments of the food web

In marine pelagic systems, between 6% and 26% of the photosynthetically fixed carbon is channelled or 'shunted' to the dissolved organic matter (DOM) pool by viral lysis of cells at all trophic levels

Viriplankton is a strong force driving microbiological diversity on water ecosystems



Minireview

Are viruses driving microbial diversification and diversity?

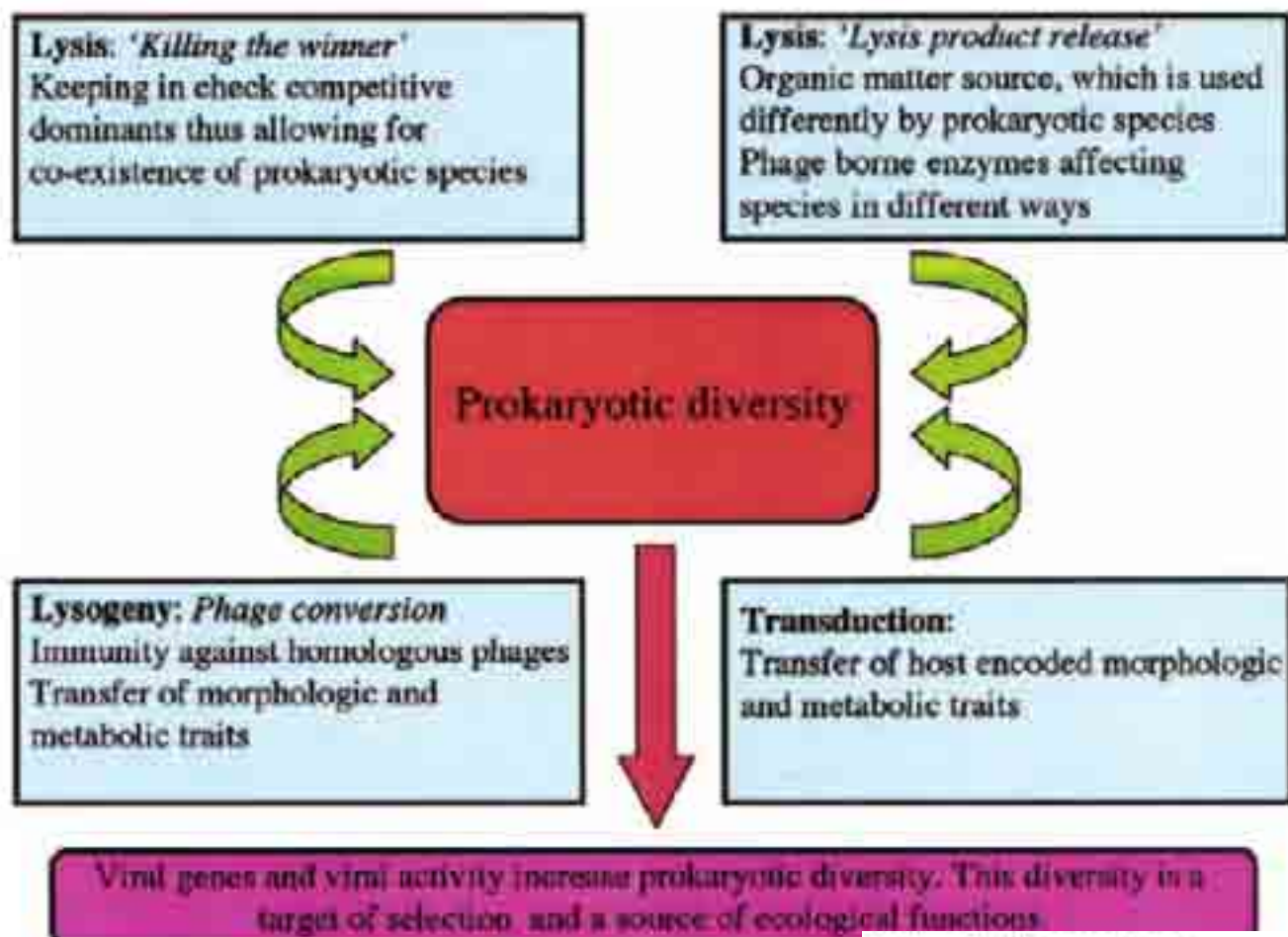


Fig. 1. Summary of potential effects of viruses on prokaryotic diversity.

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Water Quality monitoring

Physical and chemical parameters

Microbiological analysis

Markers:

Classic X Advanced



Detecção de vírus entéricos como medida de qualidade em água potável: situação atual e limitações

Detection of enteric viruses as a measure of drinking water quality: current situation and limitations

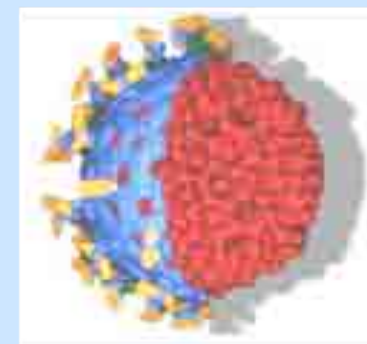
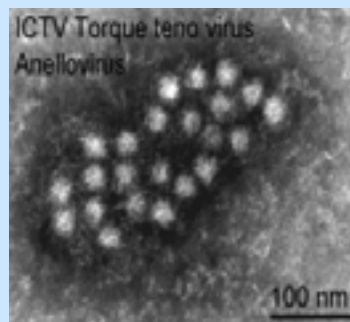
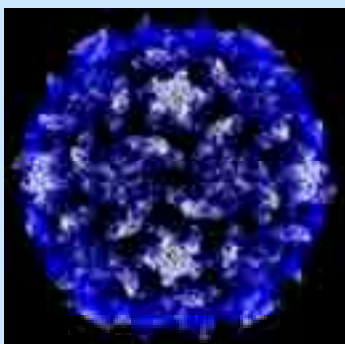
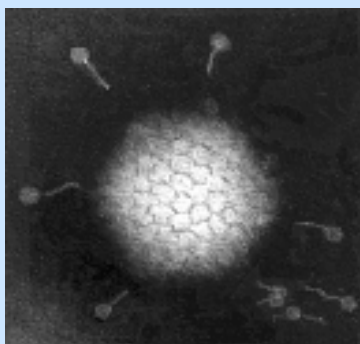
Fernando Rosado Spilki¹

Tabela 1. Vírus entéricos com possível transmissão por água e respectivas doenças causadas em humanos.

Vírus	Doença que causa
Poliovírus	Meningite (inflamação das meninges), paralisia
Echovírus	Meningite, diarreia, doenças respiratórias
Coxsackievírus A	Meningite, doenças respiratórias
Coxsackievírus B	Miocardite (inflamação do músculo cardíaco), anomalias cardíacas no feto, doenças respiratórias, meningite
Enterovírus 68 a 71	Meningite, encefalite, conjuntivite, doenças respiratórias
Enterovírus 72	Hepatite (inflamação do fígado)
Vírus das Hepatites A e E	Hepatite
Norovírus	Diarreia, vômito
Calicivírus	Gastroenterite
Astrovírus	Gastroenterite
Reovírus	Não determinada, possivelmente doença respiratória
Rotavírus	Diarreia em crianças
Adenovírus	Conjuntivites, gastroenterites, doenças respiratórias
Coronavírus	Doenças respiratórias
Parvovírus	Doenças respiratórias
Torovírus	Gastroenterites
Torquetanovírus	Não determinadas, possivelmente hepatite

Diarrhea remains the second cause of morbidity (disease) in humans (WHO, 2010)

> maybe the first for developing countries!



Adenovírus

*DNA de fita dupla

*Causador de diarréias, pneumonias e conjuntivites

Enterovírus

*RNA fita simples

*Causador de diarréias e conjuntivites

Torque teno vírus

*DNA circular fita simples

*Provável causador de hepatites

Norovírus

*RNA fita simples

*Causa surtos de diarréia e vômitos

Rotavírus

*RNA dupla fita

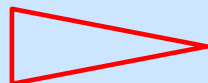
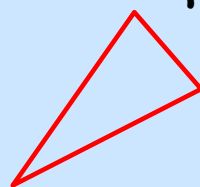
*Principal responsável por gastroenterites em crianças

Vírus não-envelopados e icosaédricos



Transmissão fecal-oral

Trato gastrointestinal



Contaminação do solo, alimentos e água

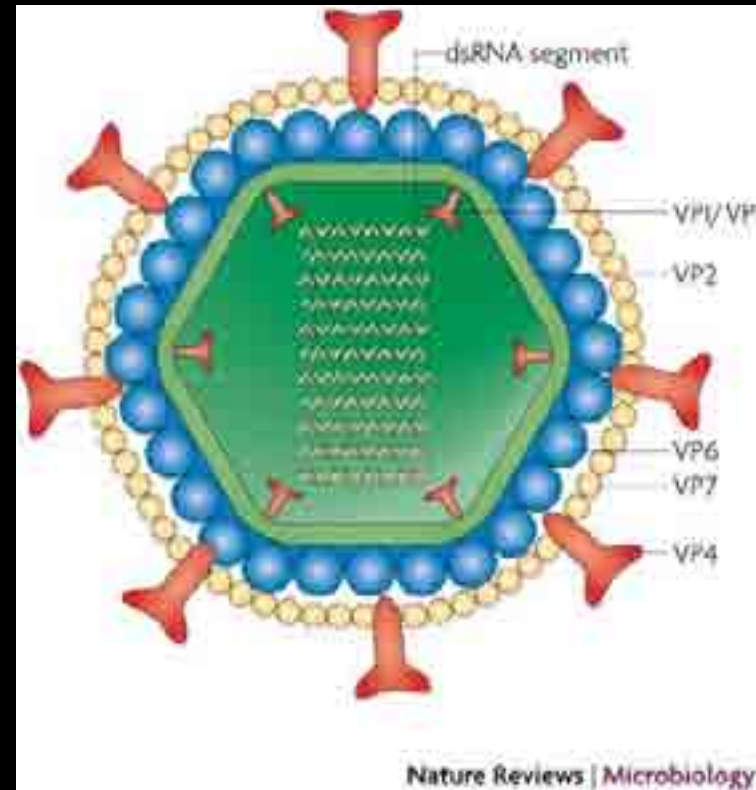


Rotavirus

Waterborne > fecal-oral route transmission

Diarrhea, vomiting, wasting, death

Children under 5-years of age, developing countries



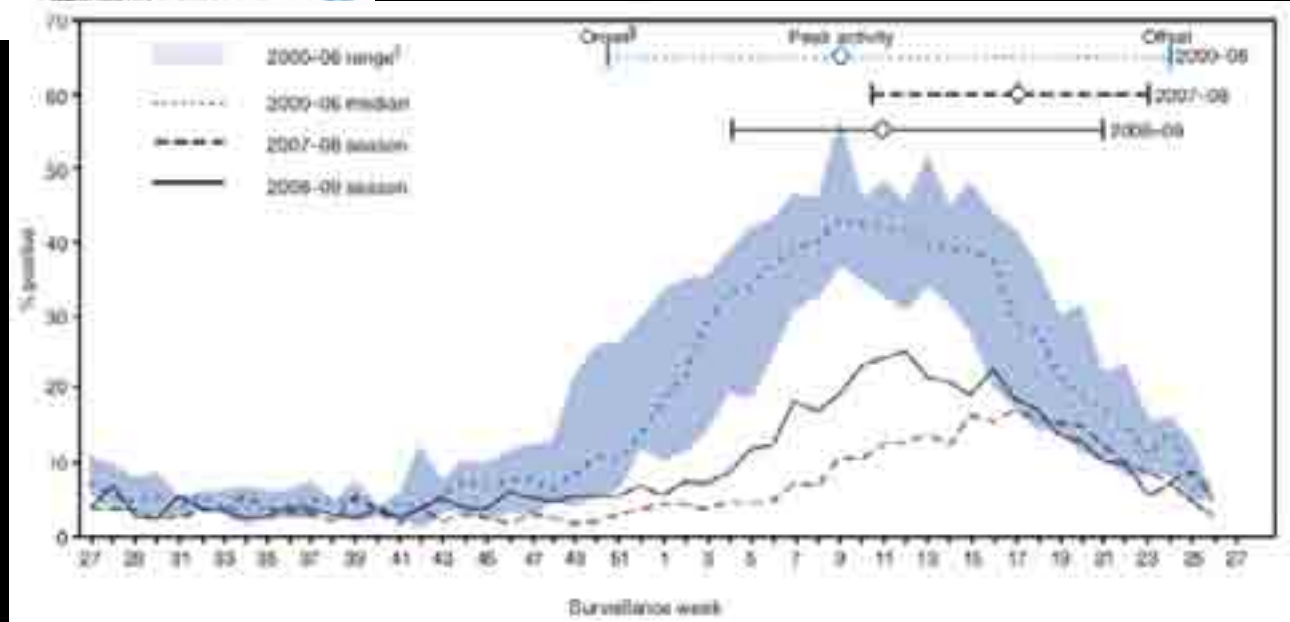
97 500 global child respiratory deaths, 2004
as of March 2007



Worldwide child deaths, 2004
100-500
10-100
More than 100

Source: Department of International Economics & Strategy, WHO

The information contained on this page is intended to provide a general overview of the situation in the world and is not intended to be used for any specific purpose. It is not intended to be used for any specific purpose. It is not intended to be used for any specific purpose.



History of Environmental Virology

- 1940s- Melnick samples East River
- 1950s- Hepatitis epidemic in New Delhi
- If viruses can infect cell cultures, they can infect animals & humans
- G. Berg: "Any amount of virus in drinking or recreational water that is detectable in appropriate cell cultures constitutes a hazard to those drinking such water."

Enteric Viruses

- Viruses that “grow” in the gut and can spread to other organs
- Spread via fecal-oral route
- Over 100 types isolated from human feces >>> na verdade 140
- Can be in concentrations as high as 10^4 - 10^6 pfu/g feces >> ***em muitos casos mais que isso!!!!***
- Some can survive secondary treatment, i.e. chlorination

Sanitation



Portaria MS

Nº 518 de 25/03/04

Estabelece os procedimentos e responsabilidades relativos ao controle e vigilância da qualidade da água para consumo humano e seu padrão de potabilidade , e dá outras providências.




Tabela 8 - Número mínimo de amostras mensais para o controle da qualidade da água de sistema de abastecimento, para fins de análises microbiológicas, em função da população abastecida.

PARÂMETRO	SISTEMA DE DISTRIBUIÇÃO (REDE E RESERVATÓRIOS)			
	População abastecida			
	< 5.000 hab	5.000 a 20.000 hab	20.000 a 250.000 hab	> 250.000 hab
COLIFORMES TOTAIS	10	1 para cada 500 hab.	30 +(1 para cada 2.000 hab.)	105 + (1 para cada 5.000hab.) máximo de 1.000

NOTA: Na saída de cada unidade de tratamento devem ser coletadas, no mínimo, duas amostras semanais, recomendando-se a coleta de, pelo menos 4 amostras semanais.

How about the viruses?

Not only on the Brazilian case of Brazilian water regulations, but for the vast majority of developing countries the “common behaviour” is a little scary and dangerous...



Drinking water should be free of fecal contamination due to health issues

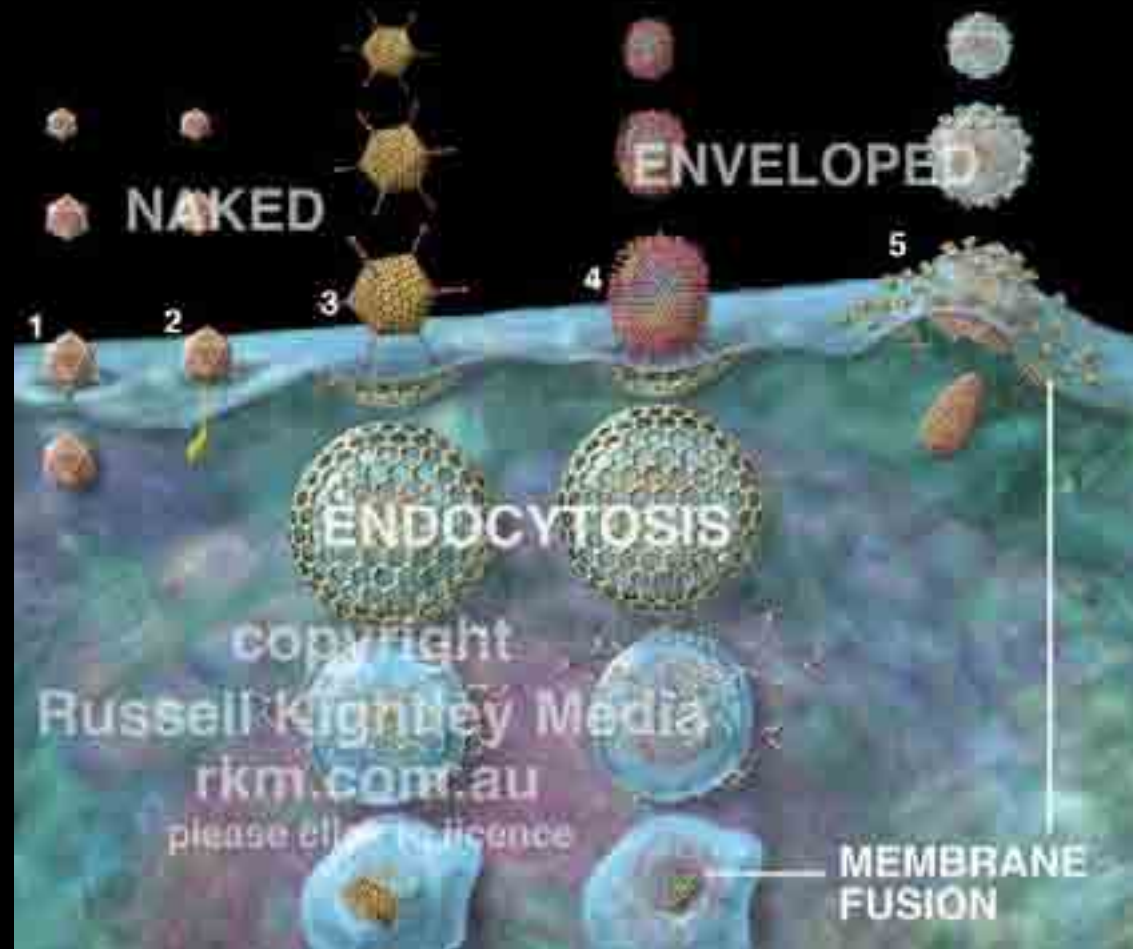
Fecal contamination is mainly determined by the detection and quantification of fecal coliforms

The absence of fecal coliforms does not ensure the safety of water since enteric viruses are more resistant than bacteria to both environmental conditions and conventional water treatment



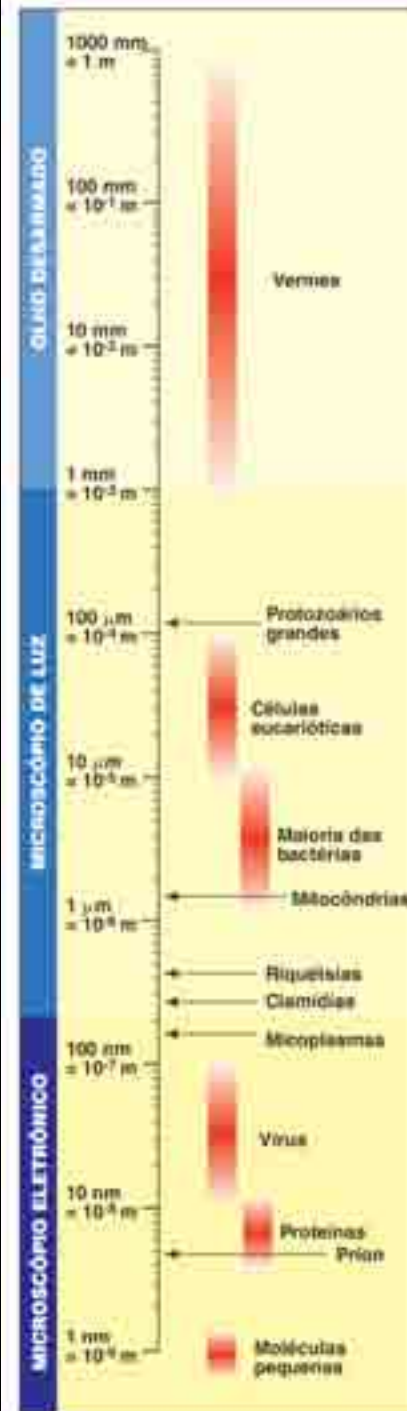
Non-enveloped (naked) viruses

Viral capsids are highly resistant to extremes of pH, temperature, disinfectants, etc



Filtration

Ineficaz:
20 - 200nm!



Disinfectants

Adenoviruses, enteroviruses, and rotaviruses are highly resistant to chlorine and other disinfectants commonly used for water treatment



Persistence under environmental conditions

E. coli ~3 weeks > regrowth outside the host in tropical/subtropical areas!

Enterovirus 120 days

Adenovirus ~ unknown

Microbial source tracking (MST)

Which are the hosts involved?

E. coli = very difficult to discover the host, since it is non-specific

Enteric viruses = mainly host specific > both genomic and antigenic tools may allow to discover the host's species



MST may be a very useful tool, specially for risk analysis and decision making



Conceptual challenges

Infectious (complete) X Non-infectious (partly destroyed viral particles)

Viral isolation X molecular detection

More training X more money

Since we commonly found at least one of these viruses in water samples:

Which will be the “maximum accepted dosis”?

Risk analysis

How to control the contamination?

UV (at least for Adenoviruses...)

New chemicals, new physical methods for destruction and removal



How to start: collecting points

Risk analysis

Geoprocessing

Contamination plumes



Volume adopted for sampling

On the past = huge! (10-1000L)

Today = the less, the better > concentration of molecular detection inhibitors and cell culture toxic substances



Journal of Virological Methods 359 (2009) 104–109

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Comparison of methods for concentrating human adenoviruses, polyomavirus JC and noroviruses in source waters and drinking water using quantitative PCR

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Concentration of samples

Viruses are commonly highly diluted in water, thus requiring previous concentration of samples to undergo detection

Filtration using electric charged membranes

Dialysis

Ultracentrifugation

Flocculants (alum, PEG, etc)

Imunoaffinity

Shellfish and fish



Concentração das amostras

Amostras de água (500ml)



Homogeneizar com 0,3g de MgCl_2 e ajustar pH (5,0)



Concentrar em Membrana negativa HA 0,45 μm



Rinsar com 87,5 ml de 0,5 mM H_2SO_4 pH 3.0



Filtrar com 2,5 ml de 1 mM NaOH pH 10,5



Neutralizar o filtrado com 12,5 μl de 50 mM H_2SO_4 e 100X tampão TE



Figura 2. Processo de concentração das amostras com auxílio da bomba a vácuo.
Foto: Joseane (bolsista-Feevale)





Figura 2. Vista aérea da estação de tratamento de esgoto ETE São João – Navegantes, identificando os locais das coletas das amostras, 1: afluente (esgoto bruto) e 2: efluente (esgoto tratado). 3: tanques de aeração. (<http://www2.portoalegre.rs.gov.br/dmae/default.php>).

Viruses in sewage

Pontos de coleta		Períodos de coletas							
		Mar	Abr	Mai	Jun	Jul	Ago	Set	Out
AFLU	TTV	-	-	-	-	-	-	-	-
	Enterovírus	-	-	-	-	-	-	-	-
	C. totais	4,2x10 ⁷	4,4x10 ⁷	1,3x10 ⁷	2,0x10 ⁷	1,6x10 ⁷	1,7x10 ⁷	9,9x10 ⁶	1,4x10 ⁷
	E.coli	1,7x10 ⁷	8,7x10 ⁶	3,1x10 ⁶	6,5x10 ⁶	1,6x10 ⁷	3,1x10 ⁶	1,6x10 ⁶	2,4x10 ⁶
	pH	7,1	7,1	7,2	7,3	7,1	7,0	6,8	6,8
EFLU	TTV	-	-	-	-	-	-	+	-
	Enterovírus	+	-	+	-	-	-	-	-
	C. totais	6,1x10 ⁵	4,4x10 ⁴	1,1x10 ⁵	5,2x10 ⁵	1,2x10 ⁵	1,2x10 ⁵	3,6x10 ⁴	5,1x10 ⁴
	E.coli	1,2x10 ⁵	1,1x10 ⁴	1,8x10 ⁴	5,8 x10 ⁴	6,1x10 ⁴	2,5x10 ⁴	2,5x10 ⁴	2,9x10 ³
	pH	6,8	6,7	6,6	6,6	6,7	6,7	6,7	6,7

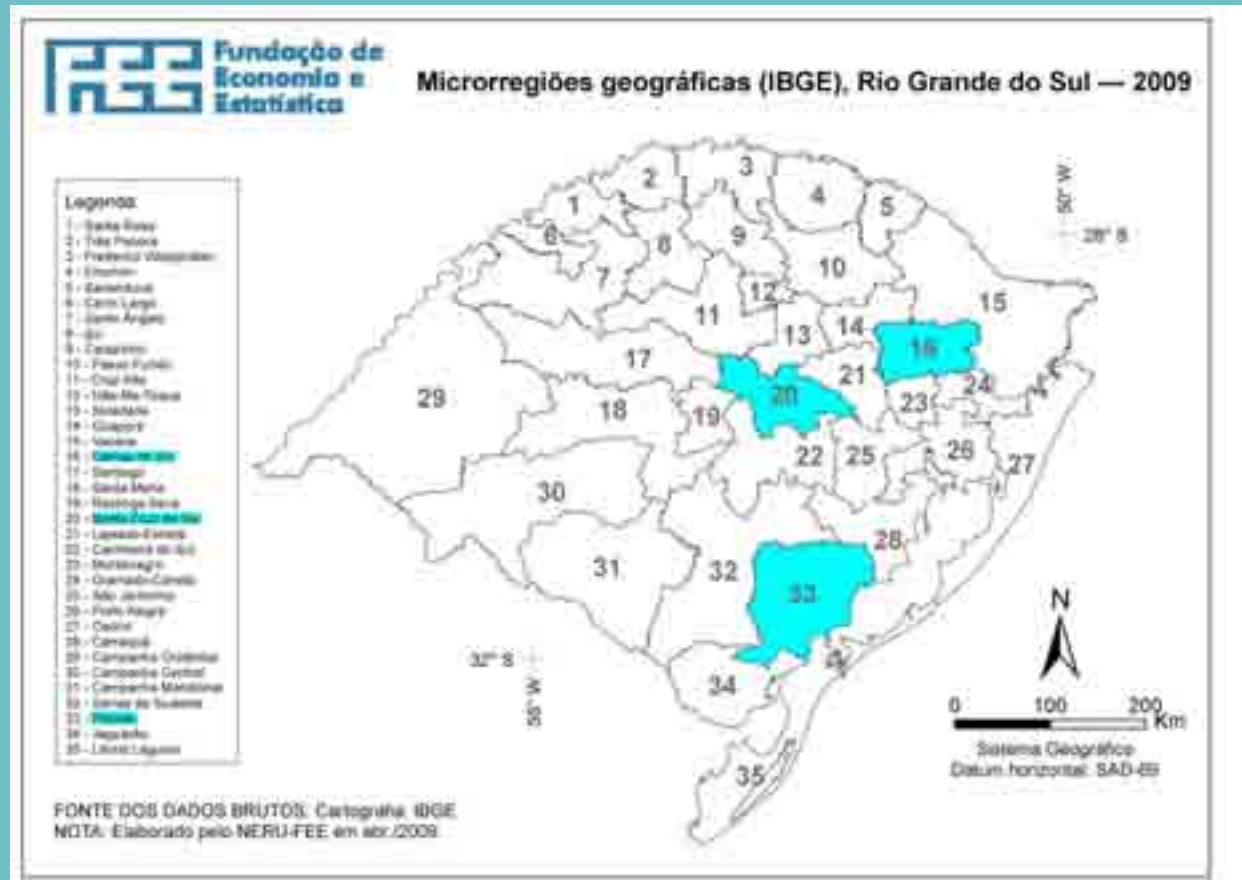
Resultados das análises da presença de TTV, Enterovírus, Coliformes totais (NMP/100mL), *Escherichia coli* (NMP/100mL) e pH dos pontos Afluente (afllu) e Efluente (eflu), da ETE São João – Navegantes Porto Alegre.



Table 2. Results for detection of EV and TTV genome in water samples collected from the Arroio Dilúvio, Porto Alegre, Brazil.

Harvesting point	EV			TTV		
	January	April	July	January	April	July
1	-	+	-	+	+	-
2	-	+	-	+	-	-
3	-	+	-	+	+	-
4	-	+	+	+	+	+
5	-	+	-	+	+	-

Drinking water collected on schools (tap/ drinking water)



.T= 34 amostras;
- Jul/set/2009;
- 19 Caxias;
- 8 Pelotas;
- 7 Sta. Cruz do Sul;
- ICBS, UFRGS.

Figura 4. Mapa do Rio Grande do Sul e suas microrregiões. Em destaque as três microrregiões utilizadas no estudo, número 16: Caxias do Sul; 20: Santa Cruz do Sul e 33: Pelotas. (Mapa modificado, disponível em: http://mapas.fee.tche.br/wp-content/uploads/2009/08/microrregioes_rs_2009.png).

TTV and/ or Enterovirus in tap water

	Número total de amostras	Positivas para TTV	Positivas para Enterovírus
Amostras de água de torneira de escolas municipais e estaduais do RS	34	4/34	10/34

SINOS River



Coleta em 4 pontos, desde a nascente até o delta



Peixes Piava (*Leporinus obtusidens*)



Gene da
proteína vp6



PCR

Rotavírus

Rotavírus	
Controle	+
Ponto 1	-
Ponto 2	-
Ponto 3	-
Ponto 4	+

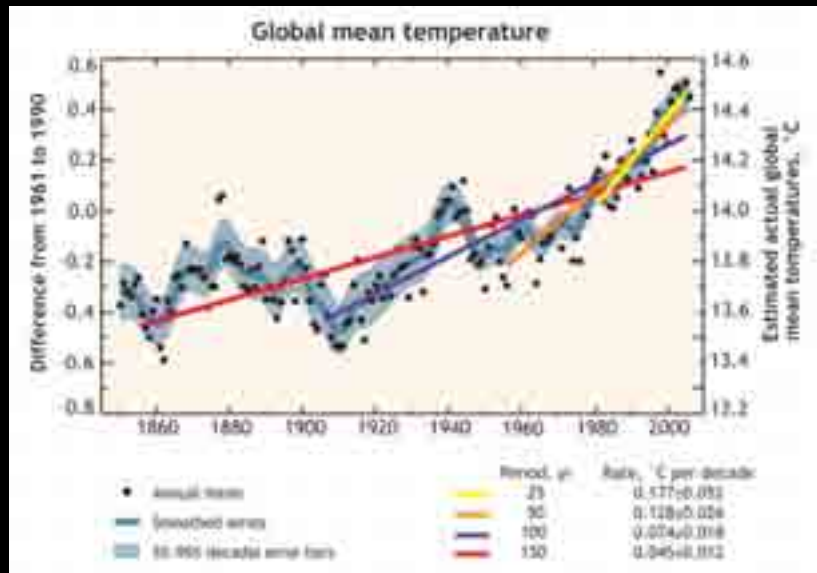
>>> Uma coleta,
direto da amostra do
Rio

Tabela 5 - Resultados de detecção viral nos hepatopâncreas de peixes referentes aos pontos de coleta. Comparação entre presença de rotavírus e detecção de Coliformes Fecais (*Escherichia coli*).

Detecção viral por <u>Rotavírus</u>	Peixe <u>1</u>	Peixe <u>2</u>	Peixe <u>3</u>	Peixe <u>4</u>	Peixe <u>5</u>	Peixe <u>6</u>	Peixe <u>7</u>	Peixe <u>8</u>	<u>E.coli</u>
Ponto <u>1</u>	+	-	-	-	-	-	-	-	29,4
Ponto <u>2</u>	-	-	-	-	-	-	-	-	10,1 x 10 ²
Ponto <u>3</u>	+	+	-	-	-	-	-	-	47,3 x 10 ²
Ponto <u>4</u>	+	-	-	-	-	-	-	-	53,8 x 10 ²



Climatic changes and human health

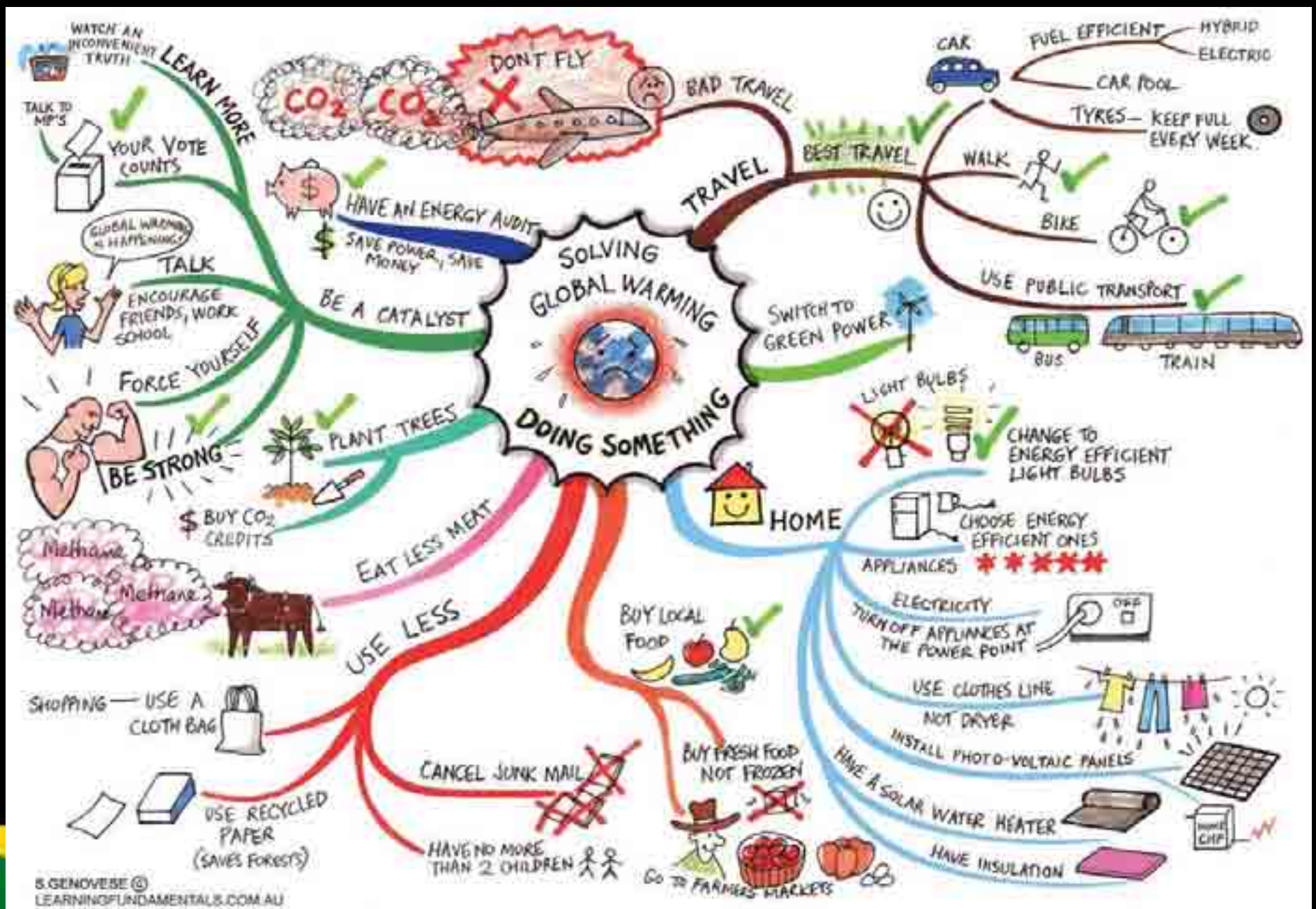


✓ Direct effects

- ✓ Heat consequences: circulatory diseases, respiratory disorders, skin diseases, etc

✓ Indirect effects

- ✓ Increase of chemical risks
- ✓ Toxins (algal blooms, pesticides)
- ✓ Diseases transmitted by arthropods
- ✓ **Waterborne diseases!**



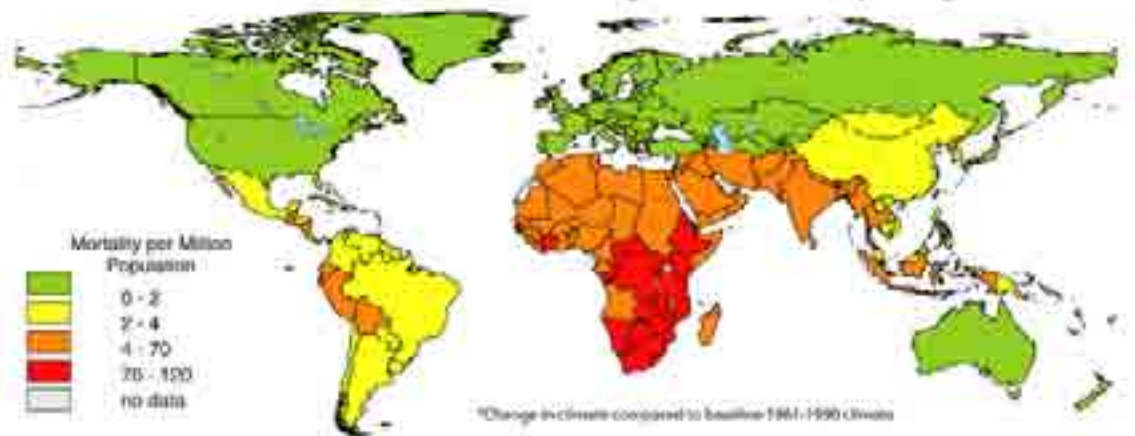
Total CO₂ Greenhouse Gas Emissions in the Year 2000, by Country



Data source:
 Marland, G., TA. Boden, and R. J. Andres. 2002. Global, Regional, and National Fossil Fuel CO₂ Emissions in Tera. A Compendium of Data on Global Change. Carbon Dioxide Information Analysis Center Oak Ridge National Laboratory, U.S. Department of Energy Oak Ridge, Tenn., U.S.A.

Map produced by the Green for Sustainability and the Global Environment (GAGE)

Estimated Deaths Attributed to Climate Change in the Year 2000, by Subregion*



Data source:
 M. Michael, B. Campbell-Lendrum, D. Kovats, et al. Global Climate Change in Cooperative Quantification of Health Risks (Global and Regional Burden of Disease due to Selected Major Risk Factors, M. Ezzini, Lopez, A.L., Rodgers, A., Murray, C.L. Geneva, World Health Organization, 2004

Map produced by the Green for Sustainability and the Global Environment (GAGE)

Mudanças climáticas globais e recursos hídricos com enfoque para as bacias hidrográficas

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Pesquisador, Embrapa Meio Ambiente, Jaguariúna - SP

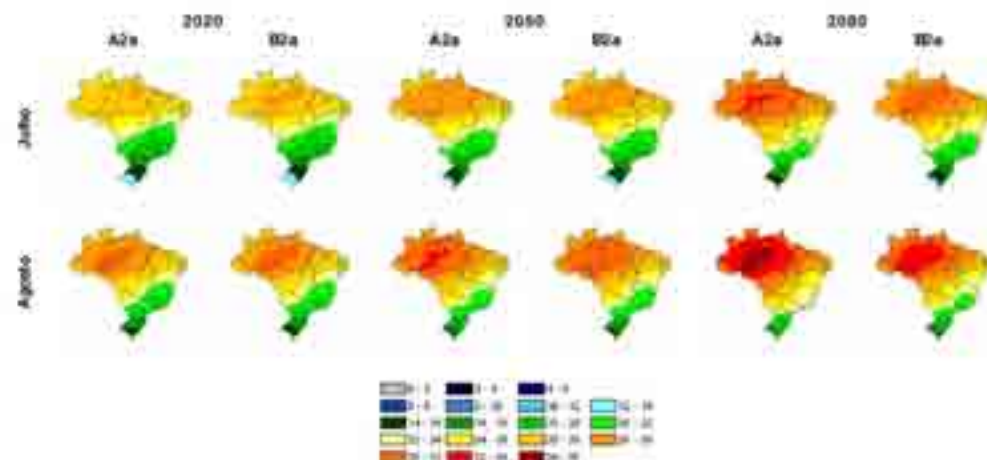


Figura 2. Temperatura média (°C) de Julho e Agosto para os anos de 2020, 2050 e 2080, dos cenários A2 e B2, representada pela média de 6 modelos climáticos globais do Terceiro Relatório de Avaliação (TAR) do IPCC.

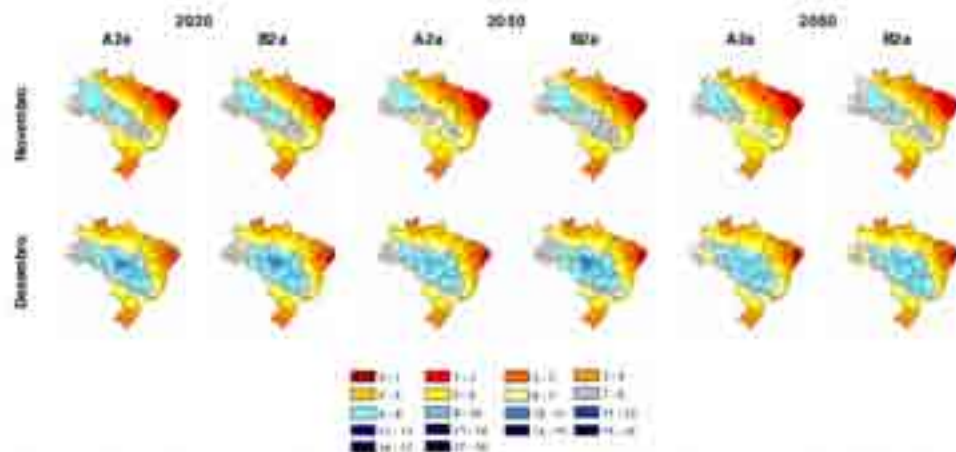
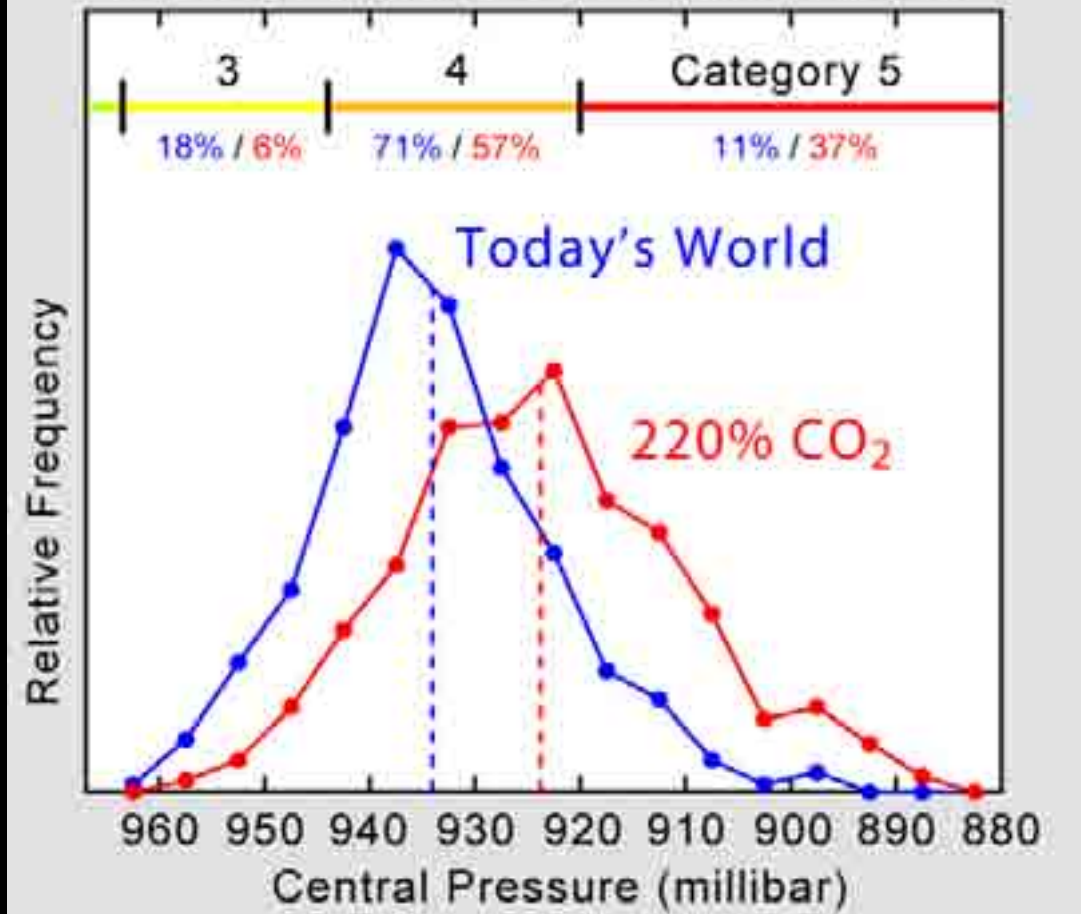


Figura 3. Precipitação média diária (mm/dia) de Novembro e Dezembro para os anos de 2020, 2050 e 2080, dos cenários A2 e B2, representada pela média de 6 modelos climáticos globais do Terceiro Relatório de Avaliação (TAR) do IPCC.

Achieved Storm Intensity Under Idealized Conditions



Heavy rainfall and diarrhea

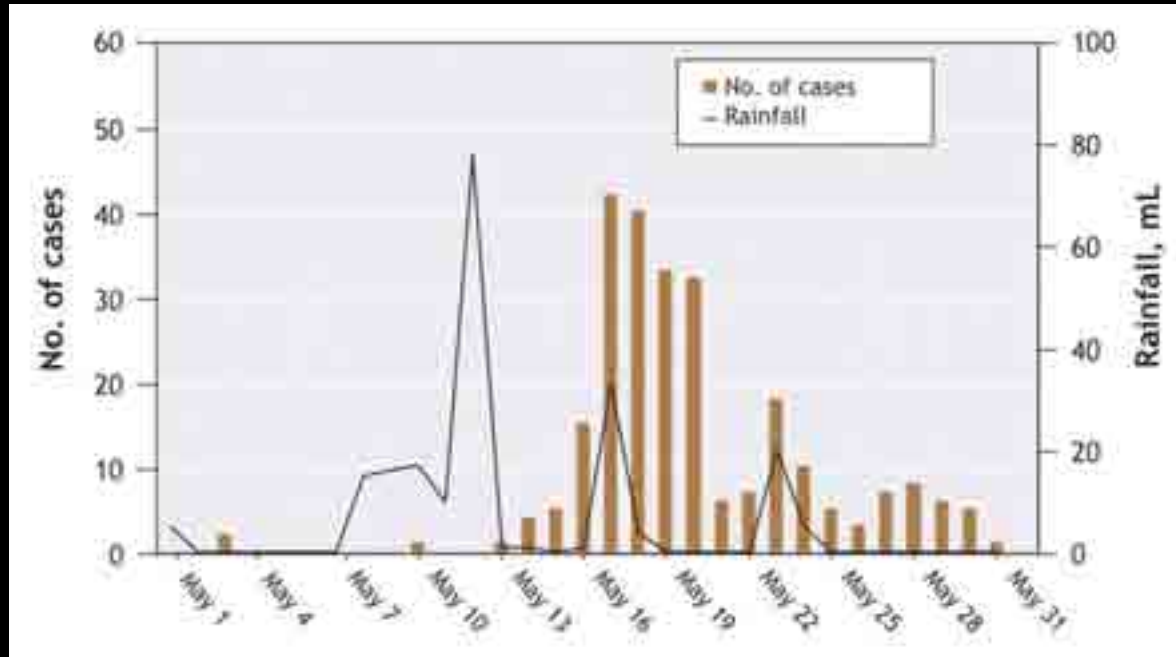


Figure 3: Association between precipitation and water-borne disease outbreaks. The graph shows the relation between unusually heavy rainfall and the number of confirmed cases of verotoxigenic *Escherichia coli* infection that occurred during a massive enteric disease outbreak in Walkerton, Ontario, in May 2000. The incubation period for verotoxigenic *E. coli* is usually 3–4 days, which is consistent with the lag between extreme precipitation events and surges in the number of cases. The figure is based on data published by Auld et al³⁶ and data published in *Report of the Walkerton Inquiry: the events of May 2000 and related issues. Part one.*³⁷

Laboratório de Microbiologia Molecular

(12.08.2010) > Spilki, Almeida, Cantarelli

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