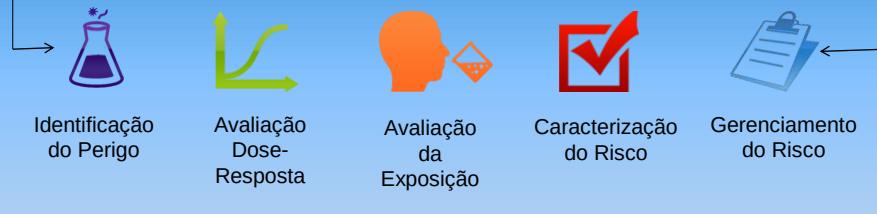
Introdução a Avaliação Quantitativa de Risco Microbiológico (AQRM)

Adelaide Cássia Nardocci
Departamento de Saúde Ambiental
Faculdade de Saúde Pública da USP

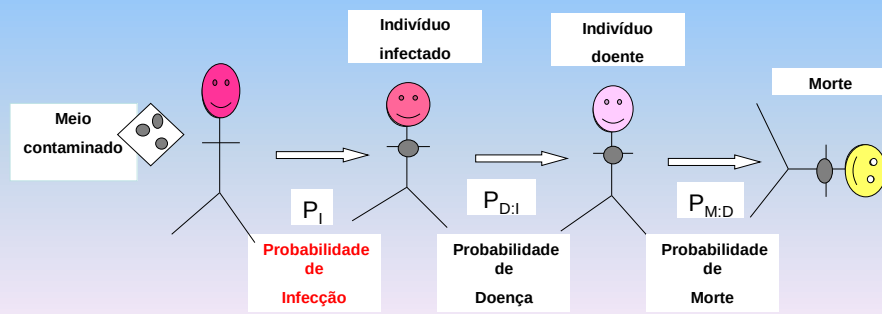
AQRM



Identificação do Perigo

É a primeira etapa da avaliação de risco microbiana quantitativa (QMRA). Após a formulação do problema que pode incluir a discussão sobre os locais, situações e problemas a serem abordados.

Compreende informações gerais sobre o agente microbiano (patógenos) e as consequências negativas para o hospedeiro contra a infecção e incorpora uma ampla gama de informações sobre os agentes infecciosos. Um microorganismo que pode infectar organismos hospedeiros pode causar infecções assintomáticas (sem doença).



Identificação do Perigo

Características microbiológicas do patógeno
- Taxonomia;
- Fases da vida;
- Os métodos de detecção, etc..

Informação epidemiológica
- Modo de transmissão;
- Momento da infecção: período de latência, período de incubação, tempo de comunicabilidade, duração da doença, etc.;
- Razão de letalidade;
- Razão de casos sintomáticos/assintomáticos;
- Endémica vs. doença epidémica

As informações clínicas
- Criterios de diagnóstico, tais como testes de laboratório para o patógeno e sua interpretação;
- Sinais e sintomas;
- Resultados clínicos;
- Efeitos sobre as pessoas vulneráveis (por exemplo, mulheres grávidas, crianças pequenas ou idosos);
- Natureza da imunidade (por exemplo, vs permanente temporária)
- Etc..

Tabela 1 - Total de doenças estimada nos Estados Unidos em função do agente infeccioso para 1985

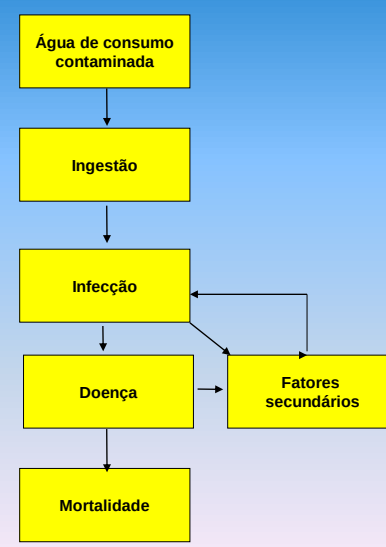
Agente	Casos	Mortes	% alimentos	% água
Campylobacter	2.1000.000	2100	100	15
Escherichia coli	200.000	400	25	75
Salmonella	2.000.000	2.000	96	3
Shigella	300.000	600	30	10
Vibrio (noncolera)	10.000	400	90	10
Crypstosporidium	50	25	NI	NI
Giardia	120.000	0	NI	NI
Hepatitis A	48.000	144	10	60
Rotavirus	8.000.000	800	NI	NI
Norwalk and related agents	6.000.000	6	NI	NI

Identificação de perigos

1. Interação agente patogênico e o hospedeiro é complexa

Fatores do hospedeiro: imunidade pré-existente; idade, condições nutricionais, resposta imune etc..

Fatores do agente: tipo e *strain* e sua capacidade vencer a resposta imune.



Entre as várias conseqüências de uma infecção:

- ❑ **possibilidade de doenças subclínicas** (assintomáticas) que são aquelas que resultam em sintomas não óbvios como febre, dor de cabeça ou diarréia.
- ✓ Isto é, indivíduos podem hospedar o agente patogênico e transmiti-lo a outros, sem que fiquem doentes. A razão de infecções clínicas e subclínicas varia de agente para agente, especialmente em vírus (Tabela 2).
- ✓ Em alguns casos, a probabilidade de desenvolvimento de doença clínica não apresenta relação com a dose recebida por um indivíduo via ingestão.
- ❑ o **desenvolvimento de doenças clínicas**, vários fatores interferem como idade, por exemplo. No caso da hepatite A, os sintomas clínicos podem variar de 5% em crianças menores que 5 anos até 75% em adultos.
- ✓ Por outro lado, crianças são mais prováveis de desenvolver gastrenterites retro virais.

Tabela 2– Razão entre os casos clínicos e subclínicos para infecção por vírus

Vírus	Freqüência de doenças clínicas (%)
Echovirus 12 (all)	50
Poliovirus 1	0.1-1
Hepatite A (adultos)	75
Rotavirus Adultos Crianças	 56-60 28
Astrovirus (adults)	12-50

Avaliação dose-resposta:

A etapa de avaliação Dose-Resposta estima a relação quantitativa entre o risco de resposta (infecção, doença ou morte) com relação a uma dose conhecida de um agente patogénico. A base desta etapa são os modelos Dose-Resposta derivados de funções matemáticas que descrevem a relação dose-resposta para agentes patogénicos específicos.

Obtenção de informações:

1. Estudos epidemiológicos : coorte e caso-controle.
2. Estudos experimentais.

PLAUSIBILIDADE DOS MODELOS DOSE-RESPOSTA

1. **Concentrações de muito baixas de organismos no meio de exposição**

✓ O que representa uma exposição a 0,1 organismos/L?

✓ Para concentrações muito baixas de organismos, parte da população não será efetivamente exposta...

2. **Capacidade dos microrganismos de se propagarem em locais apropriados dentro do hospedeiro susceptível.**

✓ Processos de infecção e doença resultam da superação, pelo agente infeccioso, de algumas barreiras, após um período de competição entre os mecanismos de ataque e defesa....no qual o ataque consegue superar algum valor crítico para indução do efeito (infecção).

Pressupostos básicos para a definição dos modelos:

1. A pessoa deve ingerir um ou mais organismos capazes de causar a doença...

2. Os organismos ingeridos irão sofrer decaimento ou serão impedidos de multiplicar-se pelas respostas do hospedeiro e somente uma fração do que foi ingerido conseguirá alcançar um local onde a infecção pode ter início.

Seja:

$P_1(j|d)$ - probabilidade de ingerir j organismos em uma exposição cuja dose média é d:

$P_2(k|j)$ a probabilidade de k organismos (≤j) sobreviver dentro do hospedeiro e desencadear a infecção.

Se os dois processos são independentes, a probabilidade absoluta de k organismos sobreviver e desenvolver a infecção é dada por:

$$P(k) = \sum_{j=1}^{\infty} P_1(j|d) P_2(k|j)$$

P_1 – reflete a variação no número real de organismos ingeridos de indivíduo para indivíduo em uma população;

P_2 – expressa fatores de interação hospedeiro-agente (probabilidade de sobrevivência do organismo dentro do hospedeiro).

Se K_{min} é o número mínimo de organismos necessários para iniciar uma infecção, a fração de pessoas expostas a uma dose média d que podem tornar-se infectados são:

$$P_I(d) = \sum_{k=k_{\min}}^{\infty} \sum_{j=1}^{\infty} P_1(j|d) P_2(k|j)$$

MODELO EXPONENCIAL

Parte das seguintes considerações:

1. Que a **distribuição dos organismos entre as doses é randômica** (i.e Poisson) ;
2. Que cada organismo tem uma probabilidade de sobrevivência idêntica, r;
3. Que k_{min} é igual a 1. Então:

$$P_1(j|d) = \frac{d_j}{j!} \exp(-d)$$

A probabilidade de sobrevivência dos agentes segue uma distribuição binomial...

$$P_2(k|j) = \frac{j!}{k!(j|k)!} (1-r)^{j-kr^k}$$

Substituindo em $P_I(d)$ e resolvendo, temos:

$$P_I(d) = 1 - \exp(-rd)$$

$$P_I(d) = 1 - \exp(-rd)$$

A **dose infectante média** (d= N_{50}) pode ser dada por (fazendo $P_I(d)=0,5$):

$$N_{50} = \frac{\ln(0,5)}{-r}$$

k_{min} # N_{50}

Modelo exponencial tem a propriedade de linearidade para baixas doses...Se rd<<1 ,
exp (-rd) ~1 – rd

$P_I(d) \cong rd$, para rd<<1

MODELO BETA-POISSON

Para alguns agentes e grupos de hospedeiros pode haver variação na probabilidade de sobrevivência (r)....esta variação pode ser considerada assumindo que r varia de acordo com uma distribuição de probabilidade:

$$P_2(k|j) = \int_0^1 \left[\frac{j!}{k!(j-k)!} (1-r)^{j-k} r^k \right] f(r)\,dr$$

Resolvendo....

$$P_I(d) = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha}$$

Se $P_I(d) = 0,5$:

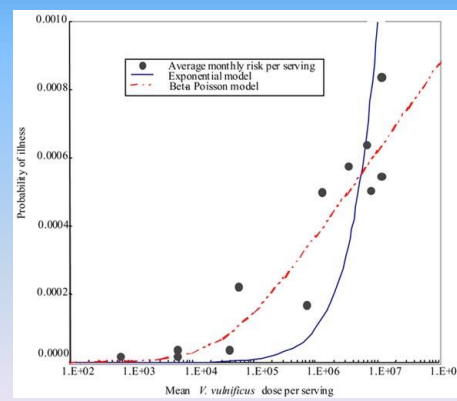
$$N_{50} = \beta(2^{1/\alpha} - 1)$$

$$P_I(d) = 1 - \left(1 + \frac{d}{N_{50}}(2^{1/\alpha} - 1)\right)^{-\alpha}$$

EXPONENCIAL X BETA-POISSON

1.O Modelo beta-Poisson produz curvas mais suaves (menor inclinação) que o modelo exponencial (α é sempre positivo)

2. A medida que $\alpha \rightarrow \infty$, o modelo beta-Poisson se aproxima do exponencial.



OUTROS MODELOS

Várias outras possibilidades podem ser assumidas:

1. Assumir que $k_{min} > 1$ (*threshold*);

2. Assumir uma distribuição de dose diferente de Poisson (distribuição binomial negativa);

   Com r constante;

   Com r variável;

3. Modelos empíricos (distribuição de tolerância em grupos vulneráveis) Ex.: Log-logistic, log-probit, Weibull.

1. Para doses muito baixas, os modelos podem diferir muito ($1x10^{-04}$);

2. A aplicabilidade dos modelos devem ser sempre RIGOROSAMENTE AVALIADA, seja do ponto de vista estatístico como de plausibilidade biológica.

Exemplos de modelos

Organismo	Melhor modelo	Modelo (Prob. De Infecção)	Parâmetros dos modelos
Echovirus 12	Beta- Poisson	$P = 1 - (1 + N/\beta)^{-\alpha}$	$\alpha = 0.374$ $\beta = 186,69$
Rotavirus	Beta- Poisson	$P = 1 - (1 + N/\beta)^{-\alpha}$	$\alpha = 0.26$ $\beta = 0.42$
Endamoeba coli	Beta- Poisson	$P = 1 - (1 + N/\beta)^{-\alpha}$	$\alpha = 0.128$ $\beta = 0.581$
Giardia Lamblia Poliovirus I Poliovirus I	Exponencial Exponencial Beta- Poisson	$P = 1 – \exp(-rN)$ $P = 1 – \exp(-rN)$ $P = 1 - (1 + N/\beta)^{-\alpha}$	$r = 0,02$ $r= 0,009102$ $\alpha = 0,1097$ $\beta = 1524$
Poliovirus III	Beta- Poisson	$P = 1 - (1 + N/\beta)^{-\alpha}$	$\alpha = 0.409$ $\beta = 0.788$

Fonte: Gerba, 2005

Avaliação de Exposição

Processo que determina o tamanho e a natureza da população exposta (dimensão e a extensão) e a rota de exposição, a concentração e distribuição dos microrganismos e a duração da exposição.

- ✓ Rotas (ou caminhos) da exposição
- ✓ Vias de exposição
- ✓ Medição ou estimativa da intensidade da exposição
- ✓ População exposta (população vulnerável)
- ✓ Frequência da exposição
- ✓ Duração da exposição
- ✓ Dose / Consumo

Cálculo da DOSE

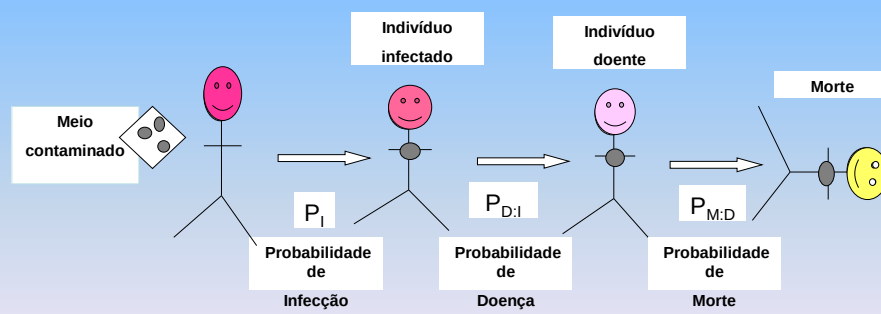
Concentração do patógeno no meio de interesse (água, ar, alimentos)

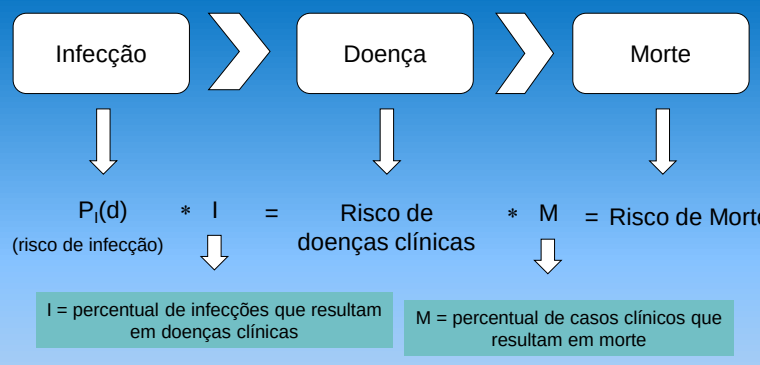
Fatores de exposição: quantidade ingerida /inalada, tempo de exposição, frequência de exposição, etc..

DOSE: ´quantidade diária de patógeno ingerida ou inalada'

CARACTERIZAÇÃO DO RISCO

Integra as etapas de avaliação de exposição e dose-resposta para a estimativa do risco





I = percentual de infecções que resultam em doenças clínicas

M = percentual de casos clínicos que resultam em morte

Vírus	Freqüência de doenças clínicas (%)	Taxa de fatalidade (%)
Poliovirus 1	0.1-1	0,90
Hepatite A (adultos)	75	0,30
Echovirus 9	15-60	0,27
Rotavirus Adultos Crianças	 56-60 28	0,01 (total)
Astrovirus (adultos)	12-50	

MÚLTIPLAS EXPOSIÇÕES

Considerando que o risco de segunda exposição é estatisticamente independente do risco a primeira exposição, então:

$$P_I = 1 - \prod_{j=1}^{i} (1 - P_j)$$

Se P_I é idêntica ao longo do ano (ou seja, o risco diário de infecção é o mesmo para todos os dias do ano), o **risco anual** é dado por:

$$P_A = 1 - (1 - P_I)^{365}$$

Da mesma forma, o **Risco para o tempo de vida** (70 anos) é:

$$P_{LT} = 1 - (1 - P)^{25.500}$$

Risco de Infecção, Doença, e Mortalidade para Rotavírus

Concentração de vírus por 100 litros	Risco	
	Diário	Anual
	Infecção	
100	$9,6 \times 10^{-2}$	1,0
1	$1,2 \times 10^{-4}$	$3,6 \times 10^{-2}$
0.1	$1,2 \times 10^{-4}$	$4,4 \times 10^{-2}$
	Doença	
100	$5,3 \times 10^{-2}$	$5,3 \times 10^{-1}$
1	$6,6 \times 10^{-4}$	$2,0 \times 10^{-1}$
0.1	$6,6 \times 10^{-5}$	$2,5 \times 10^{-2}$
	Mortalidade	
100	$5,3 \times 10^{-6}$	$5,3 \times 10^{-5}$
1	$6,6 \times 10^{-8}$	$2,0 \times 10^{-5}$
0.1	$6,6 \times 10^{-9}$	$2,5 \times 10^{-6}$

Exemplo 1: Avaliação do risco do rotavírus em água potável.

Identificação do patôgeno

⇩

Modelo dose-resposta
estudos de ingestão em humanos)

⇩

Exposição

(estudos de campo das concentrações na água)

⇩

Caracterização do risco

Rotavírus

⇩

Modelo Beta-Poisson(baseados em
$P = 1 - (1 + N/\beta)^{-\alpha}$, $\alpha = 0.26$ e $\beta = 0.42$

⇩

4 rotavirus/1000 litros

Assumindo a ingestão de 2 litros/dia

N = 0,008/dia

⇩

Risco de infecção/dia = 0,005

Risco de Infecção/ano = 0,8

Avaliação retrospectiva

RISCO → EXPOSIÇÃO

$$P_I = 1 - \ln(-rD)$$

$$P_I(d) = 1 - \left(1 + \frac{d}{N_{50}}(2^{1/\alpha} - 1)\right)^{-\alpha}$$

$$D(P_I) = -\frac{\ln(1 - P_I)}{r}$$

$$D(P_I) = N_{50}\frac{[(1 - P_I)^{-1/\alpha} - 1}{2^{1/\alpha} - 1}$$

$$D(P_I) = \beta((1 - P_I)^{-1/\alpha} - 1)$$

$$C(P_I) = \frac{D(P_I)}{V}$$

EXEMPLO

CENÁRIO: A vigilância sanitária registrou que 1% das pessoas que compraram vegetais frescos de um mercado orgânico foram infectados com *E. coli* O157. Estudos preliminares mostram que o modelo beta-Poisson ajusta melhor os dados de ingestão de *E. coli* O157, com parâmeteros α = 0,178 e β= 1,78x10^6 .

Tarefa: Estimar a concentração de *E.coli* 157 nos vegetais, assumindo que cada pessoa consome até 80g de vegetais.

$$D(P_I) = \beta((1 - P_I)^{-1/\alpha} - 1)$$

1. D(Pi) = 1,78E-06x((1-0,01)^(-1/0,178)-1) = 1E-05

$$C(P_I) = \frac{D(P_I)}{V}$$

C(Pi) = 1E-05/80g = 1250/g

Cuidado nos casos em que há mais de uma rota de exposição!!!

Avaliação da eficiência do tratamento da água

Cenário: Quantas reduções são necessárias para a proteção da saúde pública, considerando as seguintes concentrações de patógenos na água: rotavirus 10^4/100mL e *Cryptosporidium* 10^5/100 mL.

A EPA recomenda que o risco de infecção não deve ser maior que 1 caso em 10.000 pessoas expostas anualmente (1 x 10^{-4}). O risco de doenças de origem hídrica nos EUA é de 4x10^{-3}.

1º passo: Calcular o risco de infecção diária tolerável.

$$P_I = 1-(1-P_A)^{1/365}$$

PI = 1-(1-1x10^{-4})^(1/365) = 2,74 x 10^{-7}

2º passo: Calcular a dose diária associada ao risco de infecção de 2,74x10-7.

$$D(P_I) = \beta((1-P_I)^{-1/\alpha} - 1)$$

D(Pi) = 4,62x10-7 para rotavírus e 6,52x10-5 para *Cryptosporidium*

3º passo: Calcular a concentração de microrganismos que resulta no risco diário tolerável, assumindo que a ingestão diária de água é 2 litros.

$$C(P_I) = \frac{D(P_I)}{V}$$

C(Pi) = 2,31x10^{-7} pfu/L para rotavírus e 3,26x10^{-5} para *Cryptosporidium* .

4º passo: Calcular a redução desejável.

$\log_{10}$redução = $\log_{10}(C_{fonte})$-$\log_{10}$(C(Pi))

= 12 $\log_{10}$ redução para rotavirus
= 11 $\log_{10}$ redução para *Cryptosporidium*

Sites sobre “Microbial risk assessment”

1. Center for Advancing Microbial Risk Assessment – Michigan State University
   http://camra.msu.edu/

2. FoodRisk.org
   FoodRisk.org is operated by Joint Institute for Food Safety and Applied Nutrition (JIFSAN) in collaboration with the Center for Food Safety and Applied Nutrition from US Food and Drug Administration (CFSAN/FDA) and the Food Safety and Inspection Services from US Department of Agriculture (FSIS/USDA).
   http://foodrisk.org/

3. World Health Organization – WHO
   http://www.who.int/foodsafety/micro/about_mra/en/

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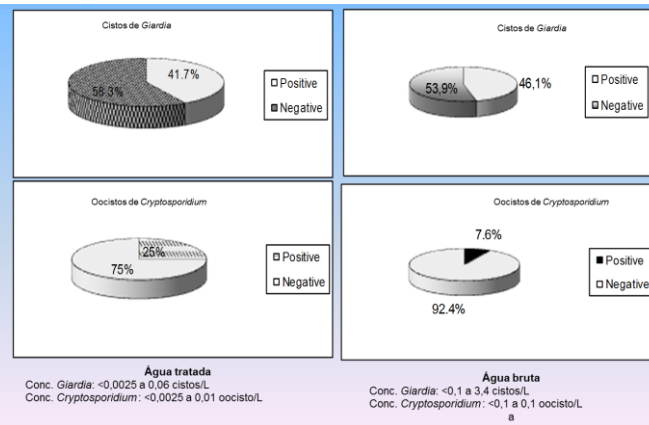
Detection of *Giardia* and *Cryptosporidium* cysts/oocysts in watersheds and drinking water sources in Brazil urban areas

Maria Tereza Pepe Razzolini, Thaís Filomena da Silva Santos and Veridiana Karmann Bastos



Água tratada
Conc. *Giardia*: <0,0025 a 0,06 cistos/L
Conc. *Cryptosporidium*: <0,0025 a 0,01 oocisto/L

Água bruta
Conc. *Giardia*: <0,1 a 3,4 cistos/L
Conc. *Cryptosporidium*: <0,1 a 0,1 oocisto/L

QUALIDADE MICROBIOLÓGICA DE ÁGUAS DE CONSUMO HUMANO EM ASSENTAMENTO PERIURBANO, MUNICÍPIO DE SUZANO (SP)

- **Três assentamentos irregulares: Vila Ipelândia, Vila Nova Ipelândia e V Divisão**
- **População: aproximadamente 2.000 habitantes**
- **Abastecimento de água tratada: reservatórios coletivos com capacidade de 5m³**
- **Reservatórios devem abastecer de 5 a 7 famílias, embora não haja controle.**

Qualidade das fontes de água de consumo

Thermotolerants coliforms
E. coli
Enterococcus
P. aeruginosa
Aeromonas
C. perfringens
Giardia / *Cryptosporidium*
(apenas nos poços)

Porcentagem de amostras positivas nos reservatórios coletivos e poços

Micro-organismo	Reservatórios coletivos	Poços
E. coli	4.5% (n=89) (2,0 a 5,1x10⁴NMP/100mL)	39.5% (n=177) (2,0 a 8,6x10⁴NMP/100mL)
Enterococcus	24.7% (n=89) (1,0 a 79 UFC/100mL)	80.2% (n=177) (1,0 a >200 UFC/100mL)
C. perfringens	14.5% (n=40) (<2,2 a 16 NMP/100mL)	77.8% (n=45) (<2,2 a 9,2NMP/100mL)
Aeromonas	17.2% (n=35) (<0,3 a 1,2x10²NMP/100mL)	21.9% (n=32) (<0,3 a 2,4x10²NMP/100mL)
P. aeruginosa	0.0% (n=89)	0.0% (n=177)
Giardia	NR	62.5% (n=16) (<0,1 a 36,1 cistos/L)
Cryptosporidium	NR	0.0% (n=16)

NR – Não realizado

Cloro residual <0,1 mg/L em 69 (78%) reservatórios examinados

Considerações

Os resultados deste estudo confirmam a vulnerabilidade dos sistemas de abastecimento de água em áreas de assentamento irregular.

As condições de coleta e armazenamento da água e a utilização de água de poços domiciliares, muitos em condições precárias, figuram como fatores de risco à saúde.

A presença de *Aeromonas*, patógeno emergente, em águas tanto dos reservatórios quanto dos poços, sinaliza um importante aspecto de risco à saúde dos residentes da área de estudo que consomem essas águas.

Publicações

Brazilian Journal of Microbiology (2011) 42:
ISSN 1517-8382

QUALITY OF WATER SOURCES USED AS DRINKING WATER IN A BRAZILIAN PERI-URBAN AREA

Maria Tereza Pepe Razzolini*, Wanda Maria Risso Günther, Francisca Alzira dos Santos Peternella, Solange Martone-Rocha, Veridiana Karmann Bastos, Thaís Filomena da Silva Santos, Maria Regina Alves Cardoso

... Pública, Universidade de São Paulo, São Paulo, SP, Brasil.

Brazilian Journal of Microbiology (2010) 41: 694-699
ISSN 1517-8382

***AEROMONAS* PRESENCE IN DRINKING WATER FROM COLLECTIVE RESERVOIRS AND WELLS IN PERI-URBAN AREA IN BRAZIL**

Maria Tereza Pepe Razzolini*, Wanda Maria Risso Günther, Solange Martone-Rocha, Heloísa Duarte de Luca, Maria Regina Alves Cardoso

Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo, SP, Brasil.

Submitted: June 29, 2009; Returned to authors for corrections: September 03, 2009; Approved: March 16, 2010.

15th International Symposium on Health-Related Water Microbiology
31 May – 5 June 2009 Naxos island, Greece

Quantificação de cistos de *Giardia* e oocistos de *Cryptosporidium* em águas de abastecimento público e avaliação do risco de infecção associada à ingestão de água em município da Região Metropolitana de São Paulo (RMSP) – em andamento

Frequência de amostras positivas

Giardia – 82,6% (38/46)

Cryptosporidium – 39,1% (18/46)

Concentrações
Giardia **-** <0,1 a 8,6 cistos/L
Cryptosporidium **-** <0,1 a 0,9 oocisto/L

Presença de *G. intestinalis* (A e B) e *C. parvum* e *C. hominis* – contaminação antrópica

2014 Water Microbiology Conference:
Microbial contaminants from watersheds to human exposure

SURVEILLANCE OF ENTERIC VIRUS IN SOURCE WATERS FOR DRINKING WATER SUPPLY IN METROPOLITAN REGIONS OF SÃO PAULO STATE, BRAZIL.

Sato MIZ[1], Garcia SC[1], Bonanno VMS[1], Razzolini MTP[2], Nardocci AC[2], Lauretto MS[3], Hachich EM[1]
CETESB - Environment Company of São Paulo State, São Paulo. 05429-130. SP. Brazil
[1] Environmental Agency of São Paulo State – CETESB; [2] School of Public Health of University of Sao Paulo; [3] School of Arts, Science and Humanities of University of Sao Paulo-Brazil
16th International Symposium on Health-Related Water Microbiology
28 Sept – 23 Sept 2011 Rotorua, New Zealand

The mean concentration of viruses at the 4 locations was 2.59 (SD 3.37) PFU/L, and this value was multiplied by the frequency of Echovirus (about 40%)

The CI95% of annual estimated infection risk is $7.6x10^{-6}$ to $4.1x10^{-4}$ and the median is $5.5x10^{-5}$ for adults and for children, the CI95% ranged from $1.9x10^{-7}$ to $1.2x10^{-4}$ and the median is $4.9x10^{-6}$.

The sensitivity analysis showed that the driver of risk for adults was the concentration of Echovirus (98.2%) followed by the ingestion rate (1.8%) while for children it was the ingestion rate (63.5%) and the pathogen concentration (36.3%).

Risk assessment of *Giardia* infection by consumption of drinking water

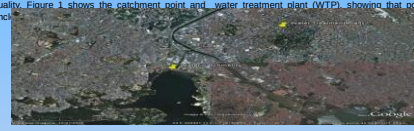
Maria Tereza Pepe Razzolini[1], Elayse Maria Hachich[2], Maria Inês Zanoli Sato[2], Marcelo de Souza Lauretto[3], Adelaide Cássia Nardocci[1] [1]Environmental Health Department of School of Public Health of University of São Paulo, [2]CETESB – São Paulo State Environmental Company, [3]School of Arts, Science and Humanities of University of São Paulo
Corresponding author: razzolini@usp.br

INTRODUCTION

Giardia cysts presence in drinking water poses public health concerns considering its potential to cause gastroenteritis including chronic effects outcomes, persistence in environment and resistance to chlorine used as a disinfectant in water treatment plants.
Although São Paulo State is the most developed area in Brazil, data from the Epidemiological Surveillance Center of São Paulo State reveal the occurrence of acute diarrheal disease in the population is 1.2% and for children under 5 years old this number reaches 5%, based on data from 2009.

OBJECTIVE

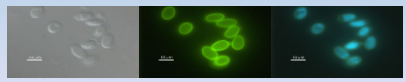
Evaluating the risk of *Giardia* infection by the consumption of drinking water from an important system of watershed catchment in São Paulo state (Brazil) responsible for delivering drinking water, after conventional treatment, for about 3.8 millions of people. It is enclosed in a metropolitan area which is undergoing a quick population expansion with inadequate sanitation arrangements. Almost 60% of watershed area is occupied by human activities and only 37% of the area maintains the native vegetation, endangering the source water quality. Figure 1 shows the catchment point and water treatment plant (WTP), showing that points are encl



METHODS

DETECTION OF *Giardia* CYSTS

The sampling of raw water was carried out on a monthly basis in accordance with the USEPA – Method 1623, 2005, totaling 13 samples. The detection of *Giardia* cysts was performed by USEPA – Method 1623, 2005 which consists of concentration, immunomagnetic separation and enumeration. The results were expressed in cysts/L.



RISK ASSESSMENT PROCEDURE

The daily and annual risk for children (< 5 years old) and for adults (≥ 21 years old) was estimated using the exponential dose-response model. It was estimated that the conventional treatment employed by the Water Plant can reduce 3.0 logs of *Giardia* and all cysts were viable and infectious. Table 1 summarizes the approach used for risk estimates. The Monte Carlo analysis was performed using Crystal Ball® software, considering 10,000 simulations from the previous distributions assumptions.

Table 1 Procedure for calculating risk from infection.

Parameters	Values	Distributions
Raw water quality – cysts per liter (C_w)	Mean 0.39 (Min 0.1 – Max 3.4)	Triangular
Treatment effect (TE) (%)	3.0 logs	
Drinking water quality (C_D)	$C = x.(1-TE)$	
Consumption of drinking water (V) (L/d)	Adults: Mean 1.21 SD 0.29 Children: Mean 0.44 SD 0.92	Lognormal
Dose-response (r)	Mean 0.01982 (95% CI 0.009798 – 0.03582)	Triangular
Daily risk of infection (P_i)	$1- \exp(-r \times C_D \times V)$	
Annual risk of infection (P_a)	$1-(1-P_i)^{365}$	

RESULTS

The recovery rate and relative standard deviation (RSD) obtained by the method was 33.6% and 15.2%, respectively, in accordance with the EPA´s recommendations.

Giardia cysts were present in 46.1% of the samples in concentrations ranging from the theoretical detection limit (0.1cysts/L) to 3.4 cysts/L(arithmetic mean of 0.39 cyst/L).

Table 2 shows the level of risk for *Giardia* infection for children and adults exposed.

Table 2 Procedure for calculating risk from infection *Giardia*

	Probabilidade (95% CI)	
	Daily	Annual
Children	$4.2x10^{-7}$ to $4.7x10^{-7}$	$1.5x10^{-4}$ to $1.6x10^{-4}$
Adults	$8.4x10^{-6}$ to $9.3x10^{-6}$	$3.2x10^{-3}$ to $2.9x10^{-3}$

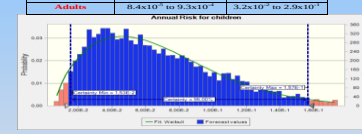


Figure 2: The annual probability of infection (95% CI) for adults and children

CONCLUSIONS

These findings are in accordance with the epidemiological data which shows a rate for acute diarrheal disease of 5% for children and for adults it was 10 times greater than 1.2%. It is also noteworthy that this rate is under estimated due to a number of cases not reported by the medical centers, especially for adults who do not frequently seek medical attention.

It should be emphasized that QMRA is useful to establish technical requirements for water treatment procedures leading to public policies adressed to risk reduction of infection disease from water ingestion, especially by children. QMRA application should be considered for all Brazilian regions taking into account their especific epidemiological condition.

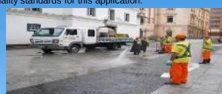
Financial Support: FAPESP (06/00280- 0)

Giardia and Enterovirus Risk Infection in Urban Usage of Reclaimed Domestic Wastewater

Lauretto, M[1]; Nardocci A.[2]; Razzolini, M. T. P.[2];Hachich, E. M.[3]; Sato, M. I. Z.[3]
[1]School of Arts, Sciences and Humanities/USP; [2]School of Public Health/USP; [3] CETESB – Sao Paulo State Environmental Company, São Paulo, Brazil.
Corresponding author: marcelo.lauretto@gmail.com

INTRODUCTION

The reuse of treated municipal wastewater for urban purposes has become very important in the last decades as a consequence of the great stress on the natural water cycle. In metropolitan regions, like São Paulo City, where there is a high demand for water, the urban reuse of wastewater is increasingly important. The washing of streets is one of the most common urban reuse in the city; however no Brazilian regulation establishes quality standards for this application.



OBJECTIVE

The purpose of the present study was to evaluate *Giardia* sp and *Enterovirus* infection risk for workers during the process of street washing with treated municipal wastewater.

METHODS

Sampling was performed in three Wastewater Treatment Plants (WTPs) at Metropolitan Region of Sao Paulo, Brazil, from February to December 2009. WTP 1 and 3, use conventional activated sludge followed by filtration (membrane: WTP1; sand WTP3) and chlorine disinfection and WTP 2 uses anaerobic reactor, activated sludge, sand filtration and chlorine disinfection. *Giardia* sp was detected by IFA (immunofluorescence microscopy assay) using the USEPA 1623 Method. For enteroviruses detection the samples were concentrated by adsorption to and elution from electronegative membrane filters (Standard Methods). Viruses were quantified by plaque assay using human rhabdomyosarcoma cells (RD). Enterovirus and *Giardia* concentrations were adjusted by Gamma distributions, via maximum likelihood method. Since some Enterovirus concentrations were below the detection limit (0.025 pfu/L), the modified maximum likelihood method for left-censored data (Owen 1980), implemented in R Package "fitdistrplus" was applied (Delignette-Muller et al, 2010). Daily and annual risks of *Giardia* sp and Enterovirus infection were estimated using Ex[...] analysis was performed us[...]butions assumptions.

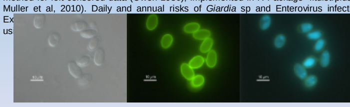


Photo Credit: H.D.A Lindquist, U.S. EPA

The procedure to calculate the risk of infection by *Giardia* and enterovirus for workers during the process of street washing with treated municipal wastewater is in table 1.

Table 1 Procedure for calculating risk from infection.

Description	Notation	Value/ assumeddistrib	Obs / Reference
Worker Exposure Parameters			
Human respiration intake (m^3/h)	R	0.830	(2)
Daily exposure time (hours/day)	T	2	
Work days per year	W	242	11 months, 22days/month
Plume model parameters			
Water flow rate (L/s)	F	Normal(a)(4167, 833)	Normal(15m^3/h, 3 m^3/h)
Efficiency of aerossolization	A	Triang(b)(0.001, 0.003, 0.018)	(1)
Impaction factor	I	Triang(0.8, 1.0, 1.2)	(3)
Distance between worker and waterflow(m)	x	Triang(4.0, 5.0, 6.0)	
Distance between worker and spray plume centerline in cross-wind direction(m)	y	0,0	(2)
Worker's height (mouth/nose) (m)	z	1.6	
Height of plume formation (m)	H	Triang(0.4, 0.5, 0.8)	
Wind velocity (m/s)	u	Triang(2.0, 3.0, 4.0)	(2)
standard deviation in y-axis	s_y	1.437	sy: a x^b , a=0.36, b=0.86
standard deviation in z-axis	s_z	0.878	s_z: c x^d, c=0.22, d=0.86
Pathogenconcentrationparameters			
Enterovirus	q	Gama (0.1158, 0.5948)	Fitted via Maximum Likelihood method with left-censored data (Owen, 1980)
Giardia sp	q	Gama (0.7273, 0.0456)	Fitted via Maximum Likelihood method
Dose-response parameters			
Beta-Poisson model (Enterovirus)(c)			
Alpha	alpha	1,06	(4)
N_{50}	N_{50}	921,94	
Exponential Model (*Giardia*)			
R	r	0.01982	(5)
Annual risk of infection (P_a)	$1-(1-Pi)^{365}$		

(a) Normal distribution notation: Normal(mean, std dev) (b) Triangular distribution notation: Triang(min, mode, max) (c) *Echovirus parameters* (1) Camann (1980) (2)Hoglund et al (2002) (3) Petterson&Ashbolt (2005) (4) Haas et al (1999) (5) Rose et al (1991)

RESULTS

Giardia sp and Enteroviruses were detected in 100% (0.25 to 109.6 cysts/L) and 50% (0.025 to 1.0 uff/L), respectively, of the treated wastewater samples from the three WTPs. Figure 1 shows the observed and adjusted (gamma) distributions for the analyzed pathogens.

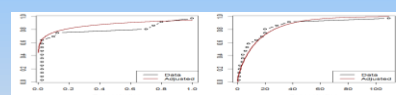


Figure 1 Observed and adjusted (gamma) distributions for *Enterovirus* (left) and *Giardia* (right) concentrations (uff/L and cysts/L)

	Median	95% Probability Interval
Enterovirus	5.71×10^{-5}	3.60×10^{-5}– 8.60×10^{-5}
Giardia	8.34×10^{-2}	6.99×10^{-2}– 9.95×10^{-2}

wastewater.

CONCLUSIONS

These results revealed that an elevated percentage of the wastewater samples were positive for *Giardia* sp (100%) and *Enterovirus* (50%) in concentrations from 0.25 to 109.6 cysts/L and 0.025 to 1.0 uff/L, respectively, demonstrating that the treatments could not efficiently remove *Giardia* sp and *Enterovirus*. The risk analysis showed a median probability of infection for *Giardia* superior to the tolerable risk set by USEPA (10^{-4}) while for *Enterovirus* such risk was about 1 log lower than 10^{-4}. The results obtained bring up the urgent need to set minimal standard of quality for the reuse of these wastewaters in order to protect the workers' health as well passers-by and neighborhoods during the street washing process.

Determination of *Enterovirus*, *Giardia* and *Cryptosporidium* Limits in Reclaimed Domestic Wastewater for Unrestricted Urban Reuse

Lauretto, M[1]; Nardocci A.[2]; Razzolini, M. T. P.[2]; Hachich, E. M.[3]; Sato, M. I. Z.[3]
[1]School of Arts, Sciences and Humanities/USP; [2]School of Public Health/USP; [3] CETESB – Sao Paulo State Environmental Company, São Paulo, Brazil.
Corresponding author:
marcelo.lauretto@gmail.com

INTRODUCTION

Strategies to minimize public health risks associated with the reuse of treated municipal wastewater have been a challenge for authorities in developing countries. In metropolitan regions, like Sao Paulo city, where there is a high demand for water and water resources are each day more scarce, the urban reuse of wastewater is already a reality. Street washing is one of the most common urban reuse in the city of Sao Paulo; however there is no Brazilian regulation establishing quality standards for this use.

Photo Credit: Sabesp Image Book Photo Credit: prefeitura.sp.gov.br

OBJECTIVES

Our purpose was to develop an approach to determine maximum concentration limits for *Enterovirus*, *Giardia* and *Cryptosporidium* in treated wastewater, based on tolerable infection risks for workers involved in the street washing activity.

METHODOLOGY

The approach used for risk assessment is described in Table 1. The determination of pathogen concentrations limits were calculated considering different scenarios of tolerable annual risk (T_a). For a given T_a, the corresponding tolerable daily risk (T_d) and concentration (C) were computed by simple inversion of equations presented in Table 1, substituting P_a by T_a and P_d by T_d: $T_d = 1 - (1 - T_a)^{1/WD}$, Dose = $P^{-1}(T_d)$ and C = Dose / (D x F x T x A x IF x R). Monte Carlo simulations resulted in a collection $C_1, C_2 \ldots C_K$ of concentrations, each one yielding a different risk distribution. In order to choose the most suitable of these concentrations, besides the parameter T_a, we also took into account the desired confidence level (q) such that $P_a^q(C) < T_a$. Given the pair (T_a, q), which we denoted by T_a^q, the upper limit for the q-quantile concentration, denoted by C^q, was the maximum value of C among $C_1, C_2 \ldots C_K$ such that $P_a^q(C) < T_a$. For example, if $T_a^{0.5} = 1x10^{-4}$ (meaning that the median tolerable risk 1×10^{-4}), the upper limit for the median concentration should be $C^{0.5} = max_C \mid P_a^{0.5}(C) < 1 \times 10^{-4}$. For each scenario, we defined $T_a^{0.5}$ and $T_a^{0.95}$ in order to compute the upper bounds for 0.5 and 0.95-quantiles of pathogen concentrations in treated wastewater ($C^{0.5}$ and $C^{0.95}$). Three scenarios were considered, in descending order of conservativeness:

(1) $T_a^{0.5} = 1 \times 10^{-4}$ / $T_a^{0.95} = 5 \times 10^{-4}$;
(2) $T_a^{0.5} = 1 \times 10^{-4}$ / $T_a^{0.95} = 1 \times 10^{-3}$;
(3) $T_a^{0.5} = 5 \times 10^{-4}$ / $T_a^{0.95} = 5 \times 10^{-3}$.

RESULTS

Table 1 - Procedure for calculating risk of infection.

Description	Notation	Value	Obs / Reference
Worker exposure parameters			
Human respiration intake (m^3/h)	R	0.830	(2)
Daily exposure time (hours/day)	T	2	-
Work days per year	WD	242	11 months, 22days/month
Plume model parameters			
Water flow rate (15m^3/h)	F	15±3	Normal distribution(a)
Efficiency of aerossolization	A	0.001, 0.003, 0.018	Triangular distribution(b) (1)
Impaction factor	IF	0.8, 1.0, 1.2	Triangular distribution (3)
Distance worker and water flow (m)	d	4.0, 5.0, 6.0	Triangular distribution
Distance worker and spray plume centerline in cross-wind direction (m)	y	0,0	(2)
Worker's height (mouth/nose) (m)	z	1.6	
Height of plume formation (m)	H	0.4, 0.5, 0.8	Triangular distribution
Wind velocity (m/s)	u	2.0, 3.0, 4.0	Triangular distribution (2)
Dispersion	D		Gaussian plume model (2)
standard deviation in y-axis	s_y	1.437	s_y: a x^b , a=0.36, b=0.86
standard deviation in z-axis	s_z	0.878	s_z: c x^d , c=0.22, d=0.86
Dose-response parameters			
Enterovirus - Beta-Poisson(c)			
Alpha	alpha	1,06	(4)
N_{50}	N_{50}	921,94	
Giardia - Exponential Model	r	0.01982	(5)
Cryptosporidium - Exponential Model	r	0.00467	(6)
Daily risk of infection	(P_d)		
Dose	CxDxFxTxAxIFxR		C= PFU/L or (oo)cysts/L
Exponential Model	$P(d) = 1 - \exp(-r \times Dose)$		
Beta-Poisson Model	$P(d)= 1 - [1+Dose((2^{(1/\alpha)} - 1))/N_{50}]^{-\alpha}$		
Annual risk of infection (P_a)	$P_a(C)=1 - [1-P_d(C)]^{WD}$		K=100,000 Monte Carlo simulations
Quantile estimation of $P_a(C)$	$P_a^q(C)$		

(a)Normal distribution notation: Normal(mean, std dev) ; (b) Triangular distribution notation: min, mode, max; (c) *Echovirus parameters* ;(1) Camann (1980); (2) Hoglund et al (2002); (3) Petterson&Ashbolt (2005); (4) Haas et al (1999); (5) Rose et al (1991) ; (6) Haas et al (1996).

Table 2 - Limits of pathogens concentration according to the three tolerable risks scenarios considering 50% and 95% percentiles

Pathogens	Limits for pathogen concentrations ($C^{0.5}$ / $C^{0.95}$)		
	A	B	C
***Enterovirus* (PFU/L)**	0.375 / 0.750	0.375 / 1.501	1.877 / 7.520
***Giardia* (cysts/L)**	0.020 / 0.040	0.020 / 0.080	0.101 / 0.403
***Cryptosporidium* (oocysts/L)**	0.085 / 0.171	0.085 / 0.341	0.427 / 1.709

A = $T_a^{0.5} = 1x10^{-4}$ / $T_a^{0.95}= 5x10^{-4}$; B = $T_a^{0.5} = 1x10^{-4}$ / $T_a^{0.95} = 1x10^{-3}$, C = $T_a^{0.5} = 5x10^{-4}$ / $T_a^{0.95} = 5x10^{-3}$

CONCLUSIONS

These data should be considered preliminary, since some issues regarding model assumptions, particularly the Gaussian plume model, must be further addressed. Nonetheless, the proposed approach tried to offer intuitive quality standards to comply with risk criteria, considering the uncertainties involved in this process. This study aimed to be a contribution to the urgent need of setting minimum standard for quality of treated wastewater to protect workers' health as well passers-by and neighborhoods during the street washing process.

2014 Water Microbiology Conference, Chapel Hill, North Carolina

International Journal of Environmental Health Research
2011, 1–13, i*First* article

Risk of *Giardia* infection for drinking water and bathing in a peri-urban area in São Paulo, Brazil

Maria Tereza Pepe Razzolini[a]*, Mark H. Weir[b], Maria Helena Matte[a], Glavur Rogerio Matte[a], Licia Natal Fernandes[a] and Joan B. Rose[b]
[a]*School of Public Health of University of São Paulo, São Paulo, Brazi;* [b]*Center for Water Sciences, Michigan State University, E. Lansing, Michigan, USA*

Peri-urban settlements irregularly established in the Protection Water Catchment Area in the MRSP (south-eastern Brazil), were identified as areas of concern when poor sanitary conditions were observed. This area has approximately 2,000 inhabitants. Some treated water is supplied by truck-tanks and transferred to people to look for alternative water sources by digging shallow wells (depth of 3–5 m) in their backyards. These wells are used as drinking water sources and for household activities such as cooking, cleaning and bathing. The sewage is disposed in septic tanks, in basic latrines or directly into water bodies. It should be emphasized that neither the wells nor the septic tanks were built following any sanitary criteria.

A total of 16 water samples were collected from wells during four months spanning winter and spring (July, September, October and November 2008). Sampling was performed according to the US EPA – Method 1623 (2005). Ten liters of water were

Giardia *genotyping*

Giardia genotyping of three samples of well water was undertaken. Sample concentration (10 l) was carried out according to Araújo et al. (2006), which consists of filtration and centrifugation steps. Millipore membranes and a manifold

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Table 3. *Giardia* cysts concentration detected in well water samples from a peri-urban area in the metropolitan region of São Paulo, 2008.

Date of collection	Wells	Concentration of *Giardia* cysts/l[1]
07/28/2008	Well 1	<0.1
07/28/2008	Well 2	<0.1
07/28/2008	Well 3	5.0
09/01/2008	Well 4	2.8
09/01/2008	Well 5	28.0
09/29/2008	Well 6	33.2
09/29/2008	Well 7	36.1
10/13/2008	Well 8	18.6
10/13/2008	Well 9	15.0
10/13/2008	Well 10	10.0
10/13/2008	Well 11	4.0
11/02/2008	Well 12	2.5
11/10/2008	Well 13	<0.1
11/10/2008	Well 14	<0.1
11/24/2008	Well 15	28.0
11/24/2008	Well 16	<0.1
Mean		9.7
Standard Deviation		12.67
CI 95th (Lower/Upper)		4.16/15.24.

[1]Detection limit=0.1 cysts/l.

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[a]*School of Public Health of University of São Paulo, São Paulo, Brazi;* [b]*Center for Water Sciences, Michigan State University, E. Lansing, Michigan, USA*

Table 4. Daily and annual probability of *Giardia* infection by drinking (WI) and bathing, WI(B), in contaminated well water.

	Daily probability of infection (P_i)				Annual probability of infection (P_a)			
	WI		WI(B)		WI		WI(B)	
	Adult	Children	Adult	Children	Adult	Children	Adult	Children
Mean	$3.20\ (10^{-1})$	$1.76\ (10^{-1})$	$3.08\ (10^{-3})$	$7.12\ (10^{-3})$	$9.99\ (10^{-1})$	$9.99\ (10^{-1})$	$6.76\ (10^{-1})$	$9.20\ (10^{-1})$
Lower 95th	$1.74\ (10^{-1})$	$9.10\ (10^{-2})$	$1.53\ (10^{-3})$	$3.52\ (10^{-3})$	$9.99\ (10^{-1})$	$9.99\ (10^{-1})$	$4.28\ (10^{-1})$	$7.24\ (10^{-1})$
Upper 95th	$5.02\ (10^{-1})$	$2.94\ (10^{-1})$	$5.57\ (10^{-3})$	$1.28\ (10^{-3})$	$9.99\ (10^{-1})$	$9.99\ (10^{-1})$	$8.70\ (10^{-1})$	$9.91\ (10^{-1})$

WI = Wateringestion, WI(B) = Water ingestion during bathing.

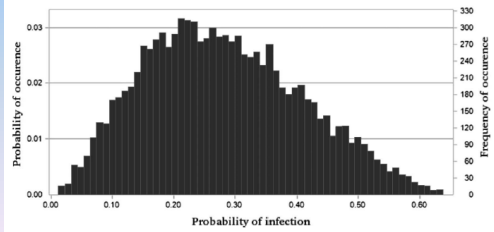

Figure 2. Risk to children from combined exposure (drinking and bathing) to water contaminated with *Giardia* cysts.

International Journal of Environmental Health Research
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Conclusions

The results of this study demonstrate the vulnerability of shallow well water supplies in irregular settlements where people are exposed to waterborne pathogens. The daily risk as a probability of *Giardia* infection reflects a median risk to the population which is too high and not acceptable. Overall it can be shown that the highest risk is to the members of the population who are ingesting the larger volumes and using the wells as drinking water, thus it is can be recommended that this activity should be discontinued Public policies which provide an educational campaign, and access to water with improved quality will reduce the risk of infection and promote health for the people living in these areas.

Acknowledgements

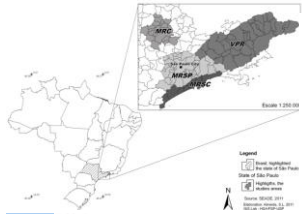
We would like to thank FAPESP – Fundação de Amparo a Pesquisa do Estado de São Paulo (06/05011-7), CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico (200007/2009-2) for its financial support and also Center for Advancing Microbial Risk Assessment (CAMRA).

Science of the Total Environment 442 (2013) 389–396

Assessing the infection risk of *Giardia* and *Cryptosporidium* in public drinking water delivered by surface water systems in Sao Paulo State, Brazil

Maria Ines Z. Sato [a,*], Ana Tereza Galvani [a], Jose Antonio Padula [a], Adelaide Cassia Nardocci [b], Marcelo de Souza Lauretto [c], Maria Tereza Pepe Razzolini [b], Elayse Maria Hachich [a]

[a] CETESB — Companhia Ambiental do Estado de Sao Paulo, Av. Prof. Frederico Hermann Jr., 345, São Paulo, SP 05459-900, Brazil
[b] Faculdade de Saude Publica, Universidade de Sao Paulo, Av. Dr Arnaldo 715 1° andar, Sao Paulo, SP 01246-904, Brazil
[c] EACH — Escola de Artes, Ciencias e Humanidade, Universidade de Sao Paulo, R. Arlindo Bettio, 1000, São Paulo, SP 03828-000, Brazil



The water sampling sites were situated in nine different watersheds at eastern region of the State covering the Metropolitan Region of Sao Paulo (MRSP), Metropolitan Region of Campinas (MRC), Metropolitan Region of South Coast (MRSC) and Vale do Paraiba region (VPR), which together comprise 58% of the Sao Paulo state and 13% of the Brazilian population (Fig. 1). The main characteristics of each region selected are summarized in Table 1.

Table 2
Protozoan parasites monitoring results.

Protozoan	Number of samples tested	Number and percentage of samples by protozoan concentration range				Maximum concentration
		<0.1	0.1–1,0	1.1–10.0	>10.0	
Giardia	cysts/L					
VPR	48	19 (39.6)	16 (33.3)	12 (25.0)	1 (2.1)	22
MRC	82	36 (43.9)	21 (25.6)	22 (26.8)	3 (3.6)	53
MRSP	56	33 (58.9)	8(14.3)	11 (19.6)	4(7.1)	97
MRSC	20	16 (80.0)	2 (10.0)	2 (10.0)	0	1.7
All regions	206	104 (50.5)	47 (22.8)	47 (22.8)	8 (3.9)	–
Cryptosporidium	oocysts/L					
VPR	48	48 (100,0)	0	0	0	<0.1
MRC	82	68 (82.9)	13 (15.8)	1 (1.2)	0	6
MRSP	56	52 (92.8)	3(5.3)	1(1.9)	0	2.5
MRSC	20	19 (95.0)	1 (5.0)	0	0	0.3
All regions	206	187(90.8)	17 (8.2)	2(1.0)	0	–

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[a] CETESB — Companhia Ambiental do Estado de Sao Paulo, Av. Prof. Frederico Hermann Jr., 345, São Paulo, SP 05459-900, Brazil
[b] Faculdade de Saude Publica, Universidade de Sao Paulo, Av. Dr Arnaldo 715 1° andar, Sao Paulo, SP 01246-904, Brazil
[c] EACH — Escola de Artes, Ciencias e Humanidade, Universidade de Sao Paulo, R. Arlindo Bettio, 1000, São Paulo, SP 03828-000, Brazil

Table 4
Mean and standard deviation of adjusted gamma distribution concentrations per regions considering the three scenarios.

Regions/scenarios	Adjusted[a]		DL[b]		Half DL	
	Mean	SD	Mean	SD	Mean	SD
Giardia (cysts/L)						
All regions	1.6464	4.1446	1.7014	2.6729	1.6768	2.8428
VPR	1.3706	2.7502	1.4114	2.0037	1.3941	2.1680
MRC	2.1203	4.8869	2.1466	3.3468	2.1099	3.5661
MRSP	1.7864	5.5409	1.8278	3.0670	1.8121	3.2761
MRSC	[c]	[c]	0.2494	0.2555	0.2088	0.2649
Cryptosporidium (oocysts/L)						
All regions	0.0709	0.3549	0.1601	0.1288	0.1142	0.1205
MRC	0.1299	0.4637	0.2093	0.2046	0.1684	0.2035
MRSP	[c]	[c]	0.1575	0.1236	0.1104	0.1150
MRSC	[c]	[c]	0.1099	0.0310	0.0626	0.0316

[a] Adjusted — values adjusted *via* maximum likelihood with left-censoring.
[b] DL — the theoretical detection limit value was assumed for censored data.
[c] Regions with less than five positive samples were not considered for this scenario.

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Table 5
Annual probability (mean, median and probability interval limits for 95% of confidence) of *Giardia* infection by ingestion of drinking water for adults and children.

Regions/scenarios	Adults				Children			
	Mean	Median	LPI[d] 95%	UPI[e]95%	Mean	Median	LPI 95%	UPI95%
All regions								
Adjusted[a]	1.94%	1.92%	1.42%	2.59%	0.55%	0.54%	0.33%	0.87%
DL[b]	1.99%	1.98%	1.65%	2.38%	0.57%	0.56%	0.40%	0.81%
Half DL	1.90%	1.89%	1.53%	2.32%	0.55%	0.53%	0.37%	0.92%
VPR								
Adjusted	1.66%	1.65%	1.30%	2.10%	0.46%	0.44%	0.30%	0.77%
DL	1.69%	1.68%	1.40%	2.03%	0.50%	0.49%	0.34%	0.72%
Half DL	1.67%	1.66%	1.37%	2.01%	0.47%	0.46%	0.33%	0.68%
MRC								
Adjusted	2.47%	2.46%	1.87%	3.16%	0.73%	0.68%	0.43%	1.25%
DL	2.48%	2.47%	2.03%	2.98%	0.73%	0.70%	0.50%	1.27%
Half DL	2.42%	2.41%	1.97%	2.94%	0.73%	0.71%	0.48%	1.11%
MRSP								
Adjusted	2.17%	2.13%	1.47%	3.15%	0.55%	0.52%	0.31%	0.92%
DL	2.18%	2.17%	1.77%	2.64%	0.66%	0.63%	0.44%	1.07%
Half DL	2.22%	2.21%	1.77%	2.75%	0.63%	0.59%	0.40%	1.07%
MRSC[c]								
DL	0.29%	0.29%	0.25%	0.33%	0.08%	0.08%	0.06%	0.12%
Half DL	0.24%	0.24%	0.21%	0.28%	0.07%	0.07%	0.05%	0.10%

[a] Adjusted — values adjusted *via* maximum likelihood with left-censoring.
[b] DL — the theoretical detection limit value was assumed for censored data.
[c] Regions with less than five positive samples were not considered for adjusted scenario.
[d] Lower Probability Interval.
[e] Upper Probability Interval.

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Table 6
Annual probability (mean, median and probability interval limits for 95% of confidence) of *Cryptosporidium* infection by ingestion of drinking water for adults and children.

Regions/scenarios	Adults				Children			
	Mean	Median	LPI[d] 95%	UPI[e] 95%	Mean	Median	LPI 95%	UPI 95%
All								
Adjusted[a]	0.10%	0.10%	0.05%	0.17%	0.02%	0.02%	0.01%	0.05%
DL[b]	0.22%	0.22%	0.20%	0.25%	0.06%	0.06%	0.05%	0.08%
Half DL	0.15%	0.15%	0.13%	0.18%	0.05%	0.05%	0.04%	0.08%
MRC								
Adjusted	0.18%	0.18%	0.11%	0.26%	0.05%	0.04%	0.02%	0.09%
DL	0.29%	0.29%	0.25%	0.33%	0.08%	0.08%	0.06%	0.11%
Half DL	0.23%	0.23%	0.20%	0.28%	0.06%	0.06%	0.05%	0.09%
MRSP[c]								
DL	0.22%	0.22%	0.19%	0.24%	0.06%	0.06%	0.05%	0.09%
Half DL	0.15%	0.15%	0.13%	0.18%	0.05%	0.04%	0.03%	0.06%
MRSC[c]								
DL	0.15%	0.15%	0.14%	0.16%	0.04%	0.04%	0.03%	0.05%
Half DL	0.09%	0.09%	0.08%	0.10%	0.03%	0.02%	0.02%	0.04%

[a] Adjusted — values adjusted *via* maximum likelihood with left-censoring.
[b] DL — the theoretical detection limit value was assumed for censored data.
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[d] Lower Probability Interval.
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The rate of acute diarrheic disease (ADD) reported by CVE (2010) is about 1% to 2% and 3% to 7%, for the total population and for children, respectively, in the four regions evaluated (Table 1). The annual risks predicted of *Giardia* infection in VPR, MRC and MRSP regions for adults and children are consistent with such rate but lower (one order of magnitude) for MRSC region. Zmirou-Navier et al. (2006),

The Quantitative Microbiological Risk Assessment conducted to evaluate the safety of drinking water in four densely populated regions of Sao Paulo State, Brazil demonstrated that the infection risks of *Giardia* and *Cryptosporidium* are superior to the adopted target of 10^{-4} and emphasizes the need to implement the Water Safety Plans as recommended by WHO (2011). As the majority of the Water Treatment Plants supply cities with more than 100,000 habitants, performance targets for the treatment should be established to achieve the required level of public health risk. Sanitary and health measures need to be implemented urgently to reduce the circulation of these protozoa in the environment. Government policies aiming to improve the urban occupation and the collection and treatment of domestic sewage should be implemented in order to reduce the discharge of raw or poorly treated sewage effluents in source waters.

Acknowledgments

Financial support to this research was provided by the Water Resource Fund of Sao Paulo State (FEHIDRO) and the Environmental Company of Sao Paulo State (CETESB).