Legionella 2010. ¿Qué sabemos de nuevo?

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FUNDACIÓ INSTITUT D'INVESTIGACIÓ EN CIÈNCIES DE LA SALUT
GERMANS TRIAS I PUJOL

LA BACTERIA

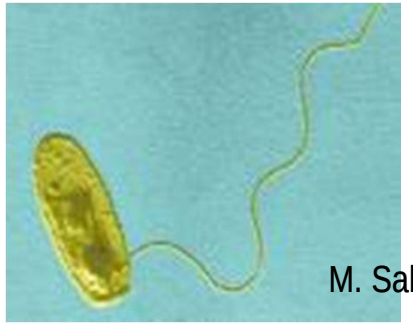
Género *Legionella* >50 especies y 70 serogrupos

……

Cerca del 70% de las neumonías por Legionella están causados por L. pneumophila sg 1

……

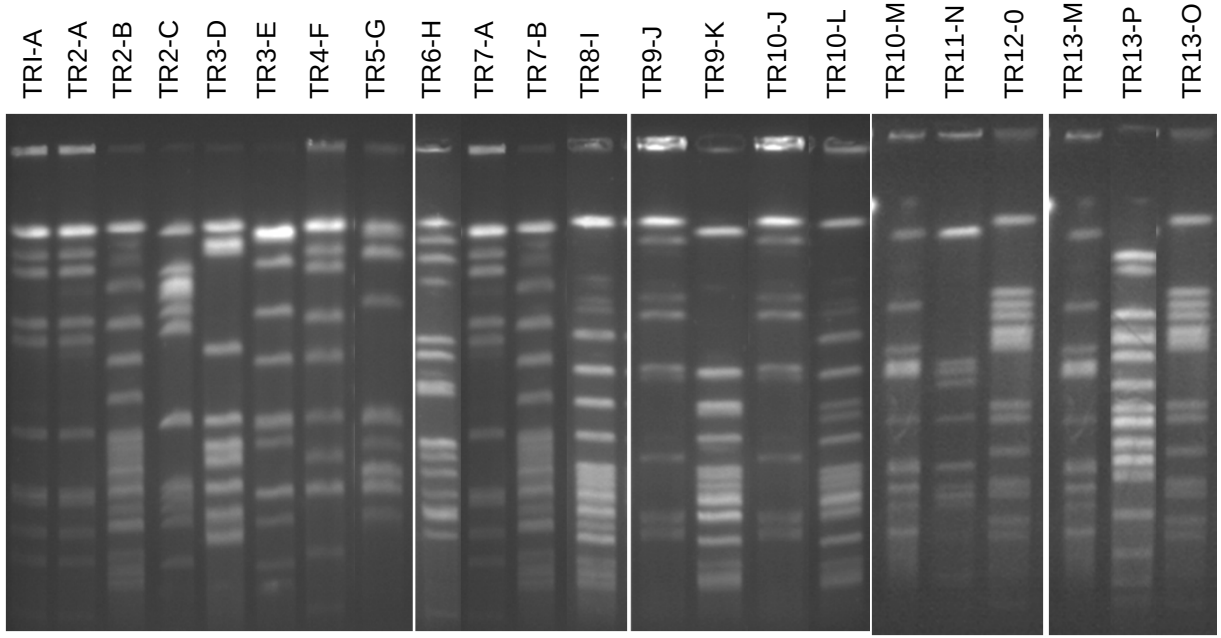
Genotipos de *L pneumophila sg 1* (variabilidad genotípica)



M. Sabrià, Yu VL. *Legionella*. Harrison´s Texbook of Internal Medicine. New York 2008

L pneumophila sg 1

Variabilidad genotípica

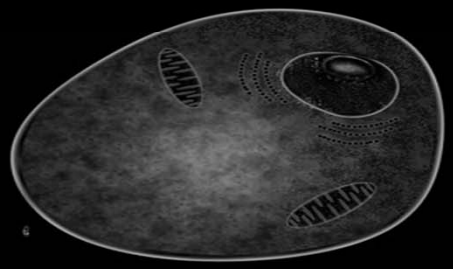


Virulenci distinta .....

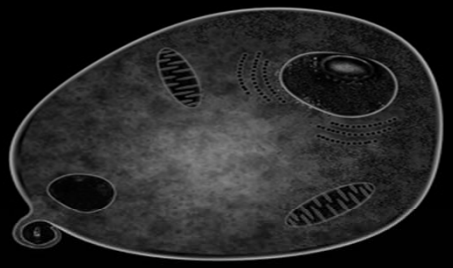
El potencial de agresividad de los diferentes subtipos de *L. pneumophila sg 1* no está establecido

Subtipo MAb2 de *L. pneumophila sg 1*

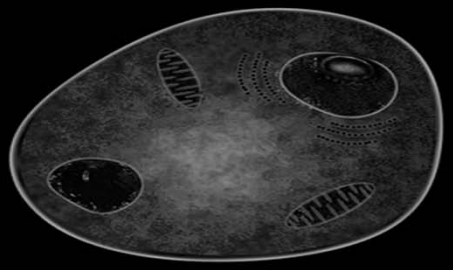
LA ECOLOGIA



1.



2.



3.

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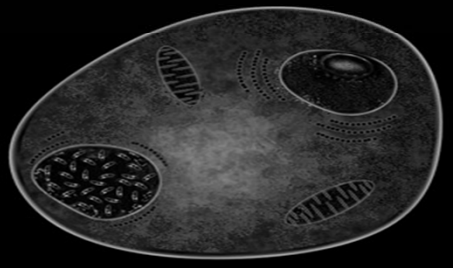
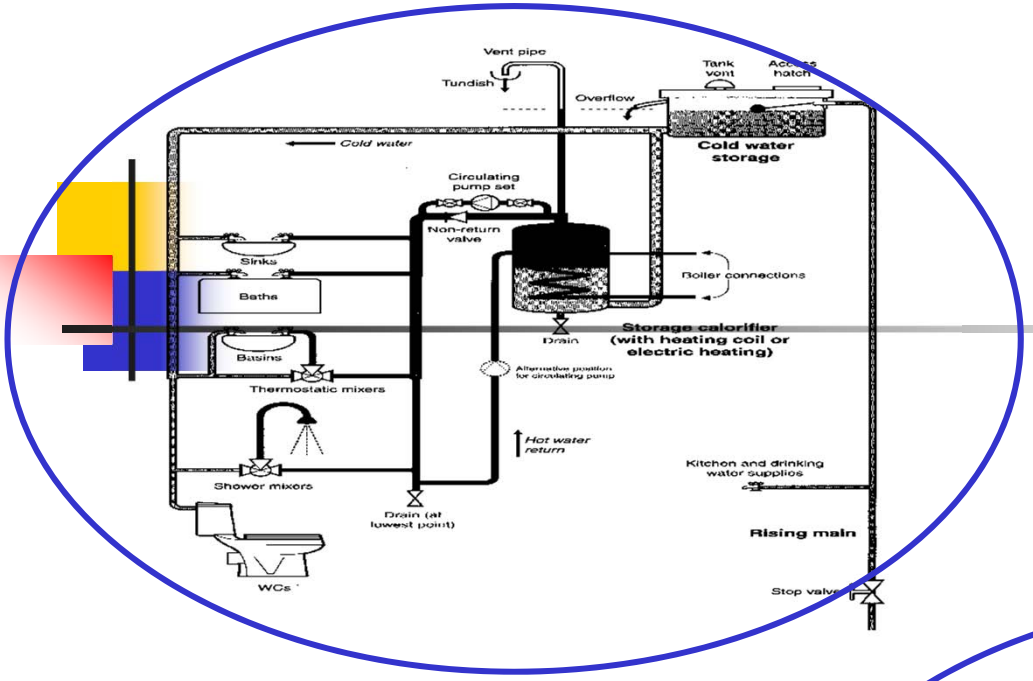


5.

6.



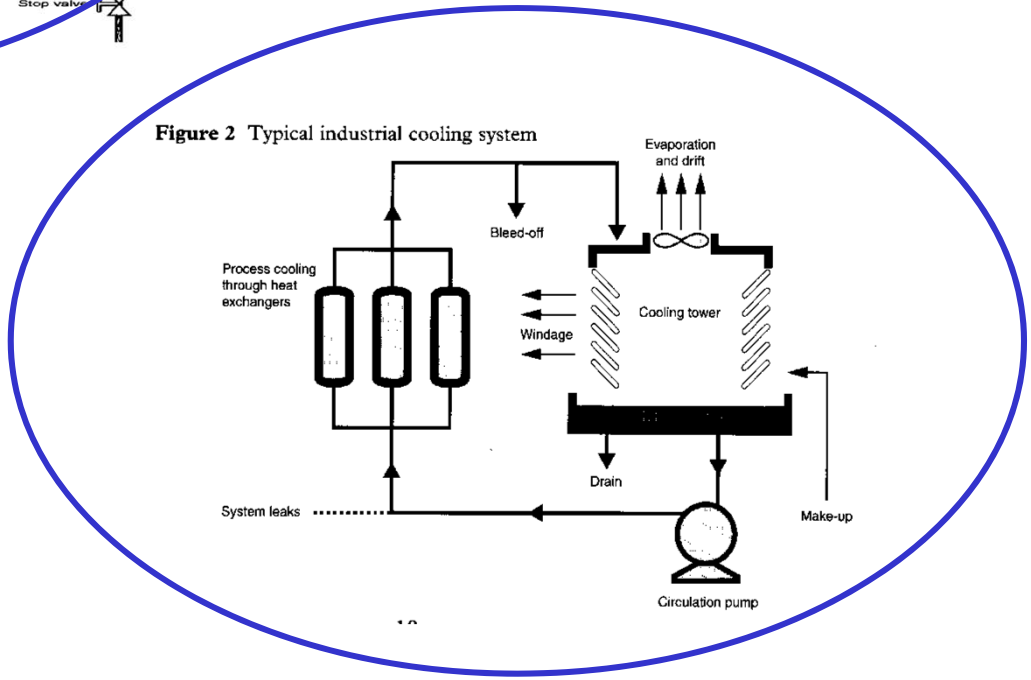
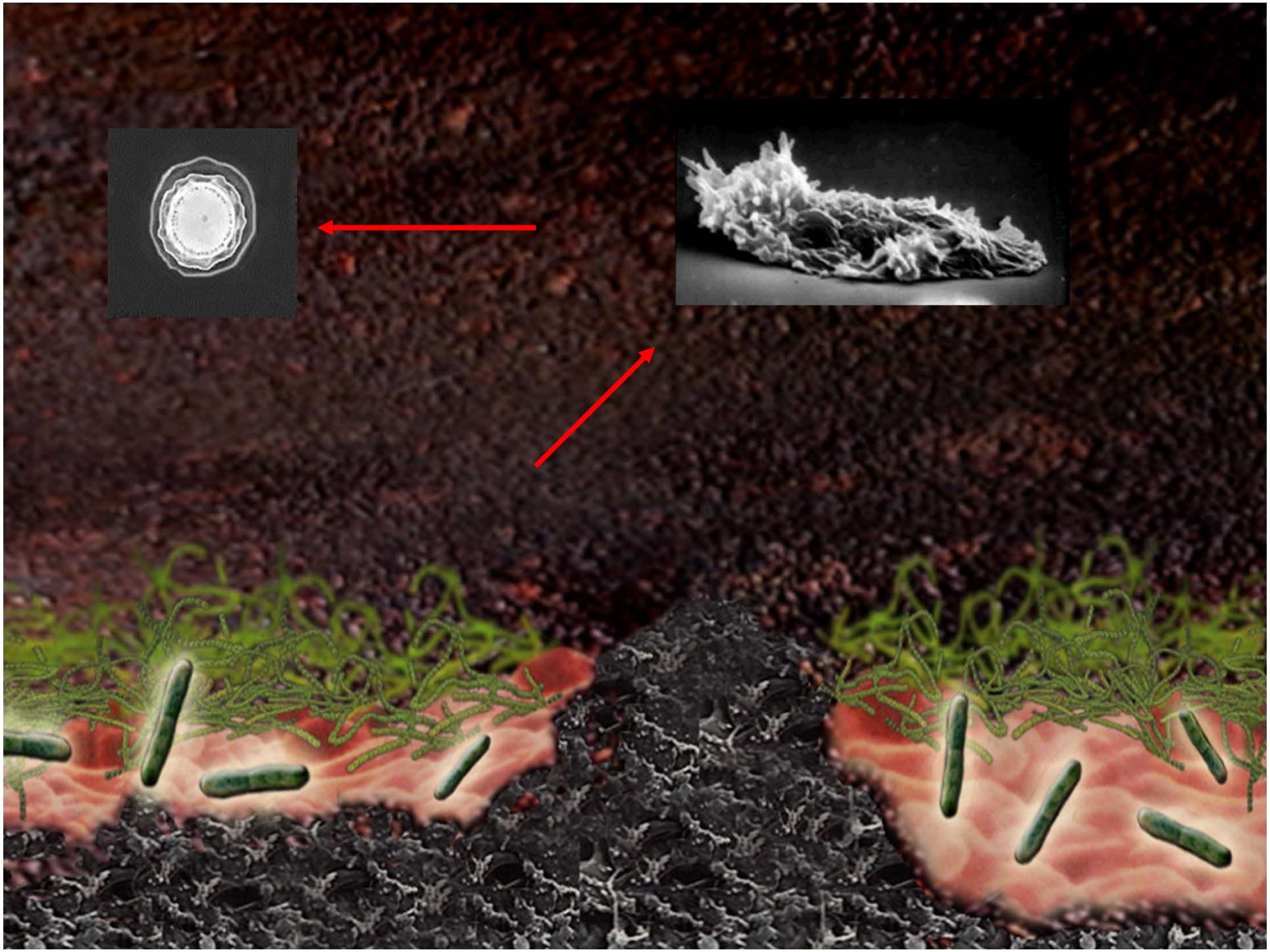


Figure 2 Typical industrial cooling system



ASPECTOS EPIDEMIOLOGICOS

LA ERA DE LA PIRULETA

1996-2006

1976-1995

Incidencia en Europa 1993-2008

Table 1 **Reported cases of legionnaires' disease and incidence rate per million population , 1993–2008 (*n*=53 494)**
Tableau 1 **Cas de maladie des légionnaires notifiés et taux d'incidence par million d'habitants, 1993-2008 (*n* = 53 494)**

Year – Année	No. of cases – Nombre de cas	No. of countries contributing data[a] – Nombre de pays ayant fourni des données[a]	Population (millions) – Nombre d'habitants (en millions)	Rate per million – Taux par million
1993	1242	19	300	4.1
1994	1161	20	346	3.4
1995	1255	24	339	3.7
1996	1563	24	350	4.5
1997	1360	24	351	3.9
1998	1442	28	333	4.3
1999	2136	28	398	5.4
2000	2156	28	400	5.4
2001	3470	29	455	7.6
2002	4696	32	466	10.1
2003	4578	34	468	9.8
2004	4588	35	550	8.3
2005	5700	35	554	10.3
2006	6280	35	566	11.1
2007	5907	33	523	11.3
2008	5960	34	506	11.8

Source: European Working Group for Legionella Infections (EWGLI). – Groupe de travail européen sur les infections à *Legionella* (EWGLI).

[a] England and Wales, Northern Ireland and Scotland counted as 3 distinct countries. – L'Angleterre et le pays de Galles, l'Irlande du Nord et l'Écosse ont été comptés comme 3 pays différents.

INCIDENCIA EN EUROPA

World Health Organization
Legionnaires disease in Europe **2008** http://www.who.int/wer
y European Working Group for Legionella Infection (EWGLI: **2004**)

2004 → 2008

Belgium (10.4)
1.5 → 12.9

Denmark (5.4)
19 → 23.3

The Netherlands (16.3)
13 → 20.5

Switzerland (7.4)
20 → 28.6

Spain (41.1)
23 → 27.3

Scotland
8 → 5.1

England
0. 66

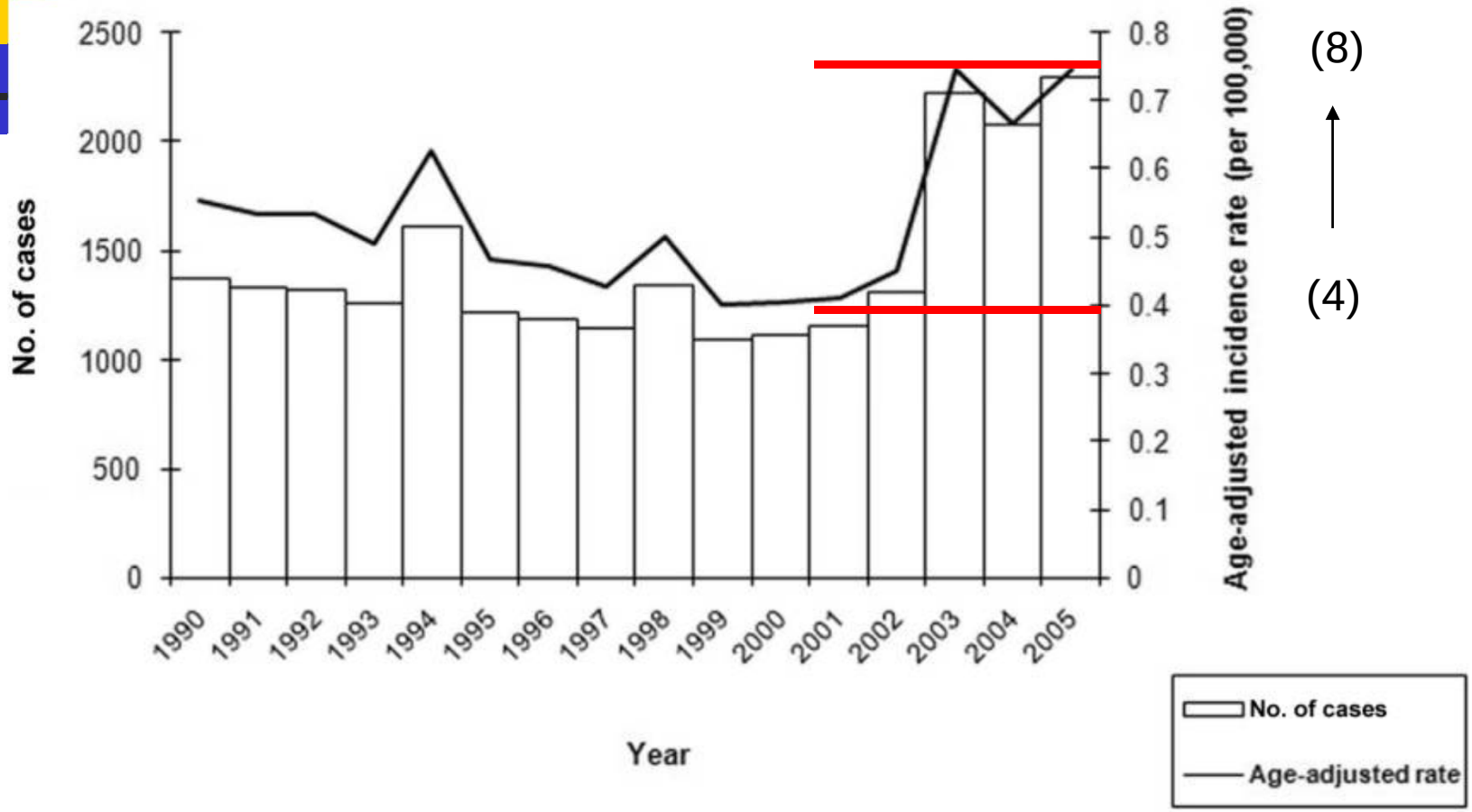
France (60.2)
19 → 19

Bulgaria (0.8) , Latvia (0.6) , Lithuania (0.6) , Poland (0.5)
>1/1000.000

Turkey
>0.1/100.000

Por 1000.000 habitantes

INCIDENCIA EN USA



(8)

(4)

Neil K.
Increasing incidence of legionellosis in the United States, 1990-2005: Changing epidemilogical Trends.
CID 2008;47:591-9

Table 5 **Outbreaks of Legionnaires' disease and associated cases by category of infection, 2007–2008**
Tableau 5 **Flambées de maladie des légionnaires et cas associés par catégorie d'infection, 2007-2008**

Category – Catégorie	2008	
	Outbreaks – Flambées	Cases – Nombre de cas
Nosocomial – Nosocomiale	15	50
Community – Communauté	49	113
Travel (abroad) – Voyage à l'étranger	30	69
Travel (home) – Déplacement dans le pays de résidence	37	157
Other – Autre	1	2
Total	**132**	**391**

Source: European Working Group for Legionella Infections (EWGLI). – Groupe de travail européen sur les infections à *Legionella* (EWGLI).

14,2
CASOS/ BROTE

2.3
CASOS/ BROTE

2003: 20/284

FUENTES, RIESGO, TRANSMISIÓN

FUENTE

Legionella longbeachae **pneumonia associated with potting mix** *(abonos)*
(Australia)
Med J Aust 1994

Legionnaires disease **associated with potting soil**
(USA.CA, OR,WA).
JAMA 2000

A cluster of Legionnaires' disease caused by *Legionella longbeachae* ***linked to potting compost***
in Scotland, 2008-2009.
Eurosurveillance 2010

Legionella spp was detected in ***46% of the commercial potting soils*** *collected in Switzerland 2006-2007.* Casati 2009

Legionella spp were recovered from ***6 / 8 centers collecting or processing gardening waste*** *for compost production* (Switzerland) Casati
Clinical Microbiology Infection 2009

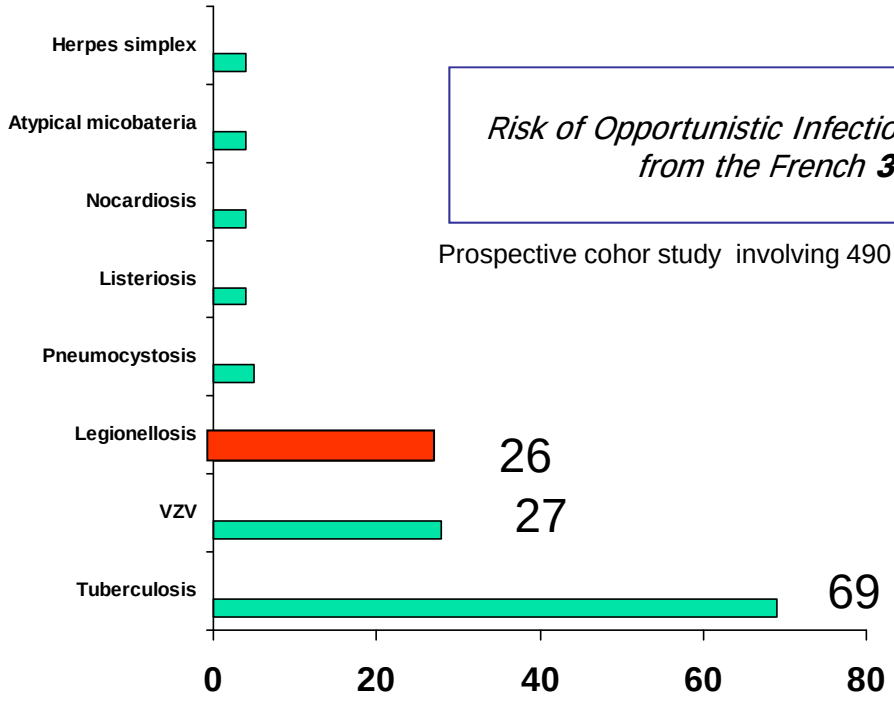
Legionella spp. were recovered from ***6 / 22 samples of potting soil*** *from the Athens area.* Velonakis. Clinical Microbiology Infection 2009

Different species and serogroups have been reported in these studies (not only *L. longbeachae*)

Release of **bioaresol** may occur during composting procedures or when using the compost/potting soil products (farmers-gardeners)

RIESGO

- Beigel. *Severe Legionella following infliximab therapy for Crohn´s disease.* Imm Bowell Dis 2009
- Mancini. *Tuberculosis and L. pneumophila in a patient receiving anti-TN therapy.* Clinical Infectious Diseases 2007



N. NICOLAS.
Risk of Opportunistic Infections on TNFr-x Blockers. . A case Control Analysis from the French ***3-Year Prospective*** *RATIO Registry*
ICAAC 2009

Prospective cohor study involving 490 clinical departaments and 31 pharmacovigilance regional centers

Biological therapy

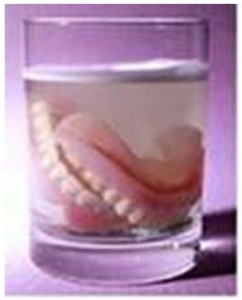
TRANSMISIÓN

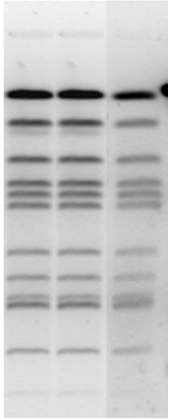
Blatt SP. Nosocomial Legionnaires' disease: aspiration as a primary mode of disease acquisition *Amer J Med 1993*

Kamimura M.Legionella pneumonia caused by aspiration of hot spring water after sarin exposure *Nihon Kokyuki Gakkai Zasshi. 1998*

Patients with HALD 122 Bedridden patients: 34

Intrinsic risk factors		
Depression of consciousness	18	54.5
Swallowing disorders	20	60.6
Manoeuvres favouring aspiration		
Orotracheal intubation*	12	36.3
Nasogastric tube in place	15	45.5
Enteral nutrition	14	42.4
Dental prosthesis**	2	6.06
Mouth secretion cleaning***	6	24.4





Enfermedad. pronostico

Sporadic and epidemic community legionellosis: two faces of the same illness

N. Sopena*, L. Force#, M.L. Pedro-Botet*, P. Barrufet#, G. Sauca¶, M. García-Núñez*, G. Tolchinsky*, J.A. Capdevila# and M. Sabrià*

Eur Respir J 2006; 29: 1–5

	Sporadic cases	Outbreak cases	p-value
	138	113	
Complications	75 (54.3)	37 (32.7)	0.001#
Respiratory failure	70 (50.7)	37 (32.7)	0.006#
Mechanical ventilation	15 (10.9)	4 (3.5)	0.03#
Acute renal failure	10 (7.4)	0	0.002#
Septic shock	11 (8)	2 (1.8)	0.04#
Evolution			
Cure	128 (92.6)	111 (98.2)	
Death related to pneumonia	8 (5.9)	2 (1.8)	0.1
Recurrence	2 (1.5)		

Universitat Autònoma de Barcelona

CONTROL AMBIENTAL. Microbiologico

Torres de refrigeración

Fluctuación

La fluctuación temporal de los recuentos de *Legionella* en torres de refrigeración hace que un muestreo poco frecuente (actualmente se recomienda cada 3 meses) no refleje el estado real del equipo.

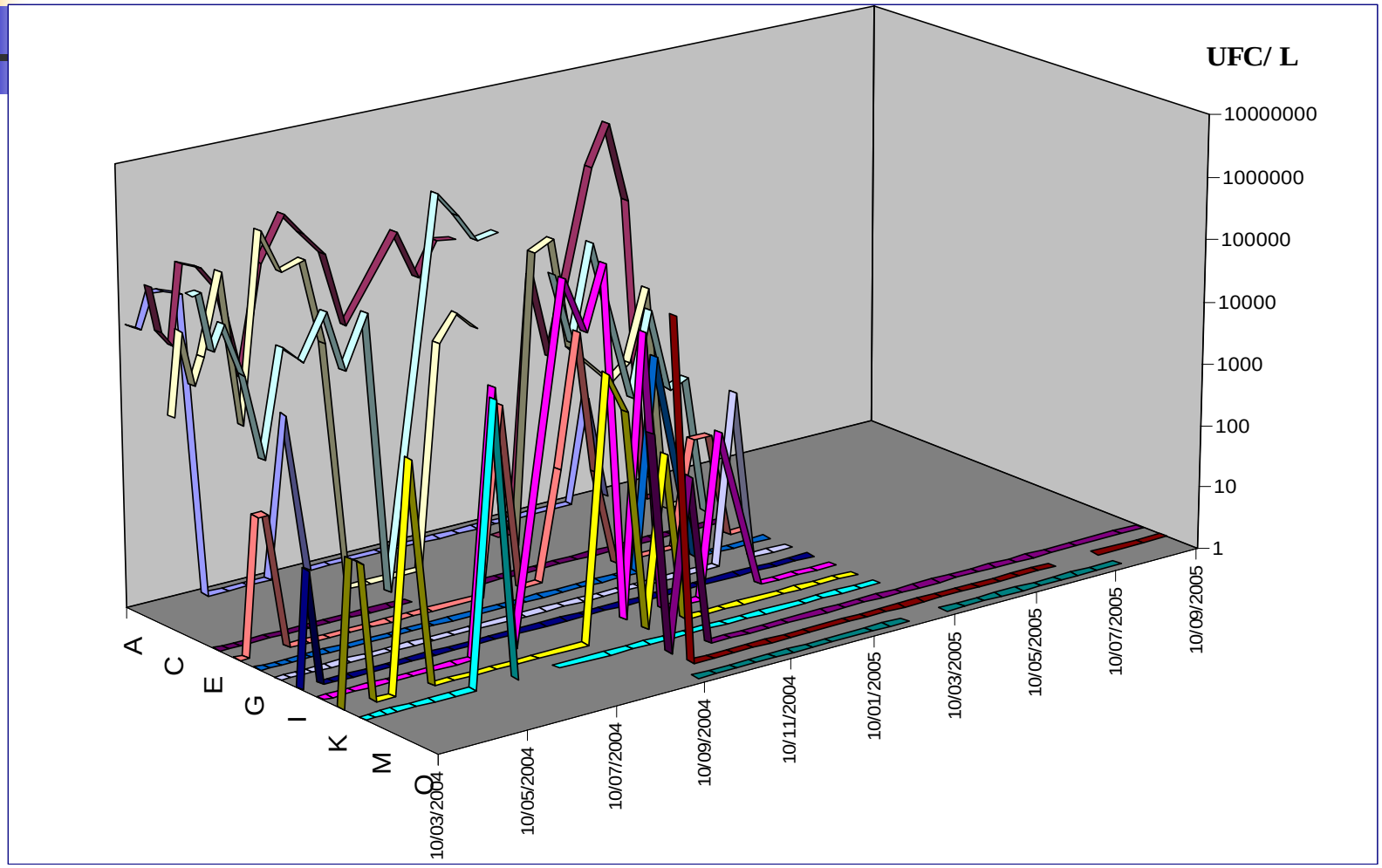
Bentham, R. H. **Routine sampling and the control of Legionella spp. in cooling tower water** systems. Curr. Microbiol. 41:271-275. **2000**

Carducci. Legionella in industrial **cooling towers: monitoring** and control strategies. J Appl Microbiol **2009**

S. Ragull et al.

Legionella pneumophila in cooling towers: fluctuations in counts, determination of genetic variability by pulsed-field gel electrophoresis (PFGE), and persistence of PFGE patterns.

Appl Environ Microbiol. August; 73(16): 5382–5384. 2007



Variabilidad genética

La presencia de subtipos cromosómicos idénticos en torres muy próximas obliga a ser prudente a la hora de responsabilizar del brote/caso a una instalación concreta

I. Sanchez et al. *Genotypic variability and persistence of Legionella pneumophila PFGE patterns in 34 cooling towers* from two different areas**. Environmental Microbiology (2008)**

Área grande (70 km. 27 Torres)

Ninguna de las 86 torres positivas estudiadas compartía perfiles moleculares

Área pequeña (1km. 7 Torres)

De los 16 subtipos cromosómicos OBSERVADOS, 11 eran exclusivos y 5 compartidos

SISTEMAS DISTRIBUCIÓN AGUA SANITARIA
Hospitales

Location	Hospitals	% With	Legionella Isolated
United Kingdom	40	70%	*Legionella pneumophila* serogroup 1
Quebec (CAN)	84	68%	*L.pneumophila*, serogrouo 1-8
West Pennsylvania (USA)	15	60%	*L pneumophila* serogroups 1-6
Catalonia (SPAIN)*	**20**	**85%**	***L. pneumophila* sg 1** ***L. pneumophila* sg 2-14**

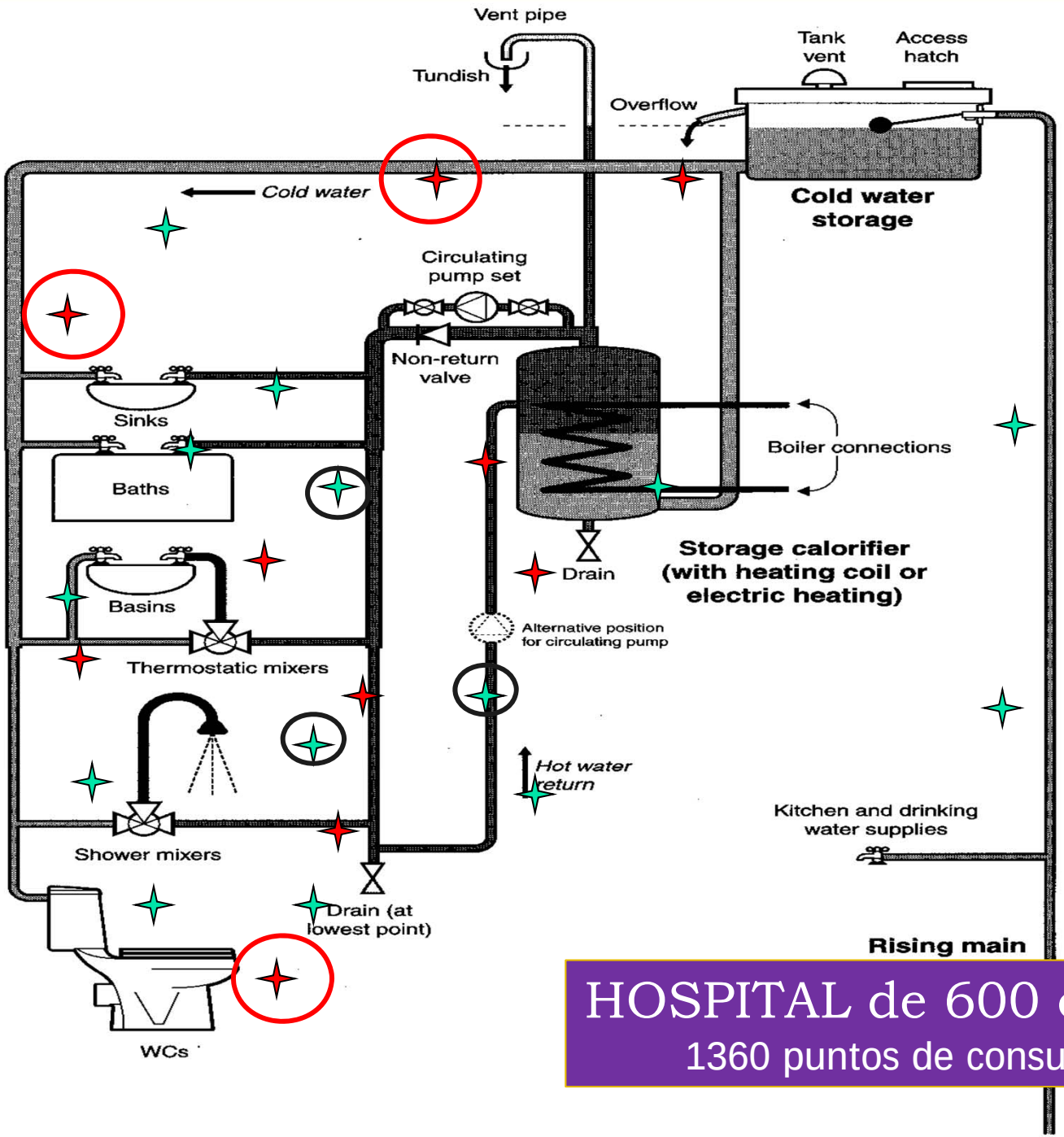
* M. Sabria, M. Garcia, M.L. Pedro-Botet.
Presence and Cromosomal Subtyping of Legionella in Potable Water in 20 hospitals of Catalonia. Spain.
Inf Control Hosp Epidemiol 2001;22:673-6

Legionnaires' Disease in a Newly Constructed Long-Term Care Facility.

Janet E. Stout PhD
Carole Brennen MSN
Robert R. Muder MD

- *L. pneumophila* serogroup 1 was recovered from the water distribution system within 1 month of operation
- 74% (61/82) of distal sites were positive during construction.
- In the first 2 years of occupancy, six cases of legionellosis were diagnosed.
- Copper-silver ionization systems were installed to control *Legionella* in the water system.

J Amer Geriatrics Soc 2000



HOSPITAL de 600 camas

1360 puntos de consumo

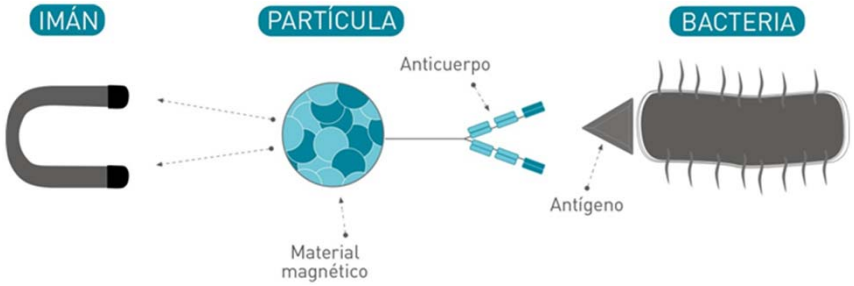
TECNICAS RÁPIDAS de EVALUACIÓN AMBIENTAL

G. Rodriguez et al. *L. pneumophila rapid detection by* ***immunosensing*** *in waters samples: assessment and validation* (submitted)

Lee JV et al. *international trial of* ***quantitative PCR*** *for monitoring for Legionella in artificial Water* (submitted).

Instrumentos de Alerta- Acción
(toma de decisiones en pocas horas)

KIT BIOALARM LEGIONELLA



ANALISIS INMUNOMAGNETICO

12 laboratorios ISO 17025
Participantes en un Ejercicio inter-colaborativo comparando Kit y cultivo (ISO11731).

50 Minutos
Tandas de hasta 10-14 análisis a la vez, por un sólo operario.

98 El porcentaje de eficiencia
Validación, con más de 500 ensayos con muestras de aguas.

An international trial of quantitative PCR for monitoring for *Legionella* in artificial water systems

J.V. Lee, S. Lai, M. Exner, J. Lenz, V. Gaia, S. Casati, P. Hartemann, C. Lück, B. Pangon, M.L. Ricci, M.Scaturro, S. Fontana, M. Sabria, I. Sánchez , and S Surman-Lee.

Health Protection Agency. United Kingdom
University of Bonn and Technische Universitat. Dresden Germany
Instituto Cantonale di Microbilogia. Switzerland
CHU. Nancy and CH Versailles. France
Instituto Superior di Sanita, Roma. Italy
Universidad Autónoma de Barcelona. Spain

Objetivo: definir niveles de alerta y acción en base los resultados de la qPCR En diferentes tipologias de agua.

Método analítico: Gene Systems Legionella (certificación AFNOR)

Conclusiones:

- Discrepancias entre los resultados de PCR y de cultivo
  (lo cual no significa Que el cultivo sea el método de control y protección de l salud pública idóneo)
- Establecimiento de niveles de alerta y de acción potencialmente útiles

PREVENCIÓN

Actuaciones a nivel ambiental

- **PRACTICAS DE BUEN MANTENIMIENTO DE LAS INSTALACIONES**

- **DESINFECCIÓN DEL** AGUA

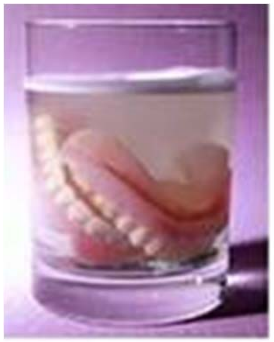
  . ASC : Temperatura > 50º C
  . AFCH: Hipoclorito sódico
  . TR: Biocidas

- **ANALISIS DE LEGIONELLA AMBIENTAL**
  (frecuencia, puntos de muestreo, rigurosidad)

- **DESINFECCION COMPLEMENTARIA**

Actuaciones a nivel clinico

Agua bidestilada para:



Higiene bucal con agua del grifo
“Toilette” bucal con agua del grifo (pacientes en coma)
Gargarismos con agua del grifo
Uso de agua del grifo para equipos de terapia respiratoria
Lavados y/o administración de agua del grifo para nutrición parenteral
Postizos dentales y agua del grifo

EQUIPO SANITARIO

EVITAR EXPOSICIÓN AL AGUA COLONIZADA

Desinfección continua (sistémica)

Eficacia en LAB
Eficacia en CAMPO
Eficacia CLINICA

Inocuidad

Agua sanitaria a 50º C
Agua sanitaria a 60ºC (C)
Pasteurización (C)

Peiro EF. *Evaluation of the effectiveness of the Pastormaster method for disinfection of Legionella in hospital water distribution system*. J Hosp Infect 2005;60:150

Cloración continua
- Hipoclorito sódico (X)
- Dioxido de cloro (C)

Srinivasan A. *A 17 month evaluation of a chlorine dioxide water treatment system to control Legionella species in a hospital water supply.* Infect Control Hosp Epidemiol 2003;24:575

Monocloraminas (A-B)
Cu/Ag Ionization (A)

Los grados de recomendación se han basado en la escala de la "Agency for Healthcare Research and Quality"

Desinfección focal

Filtros en el punto de uso

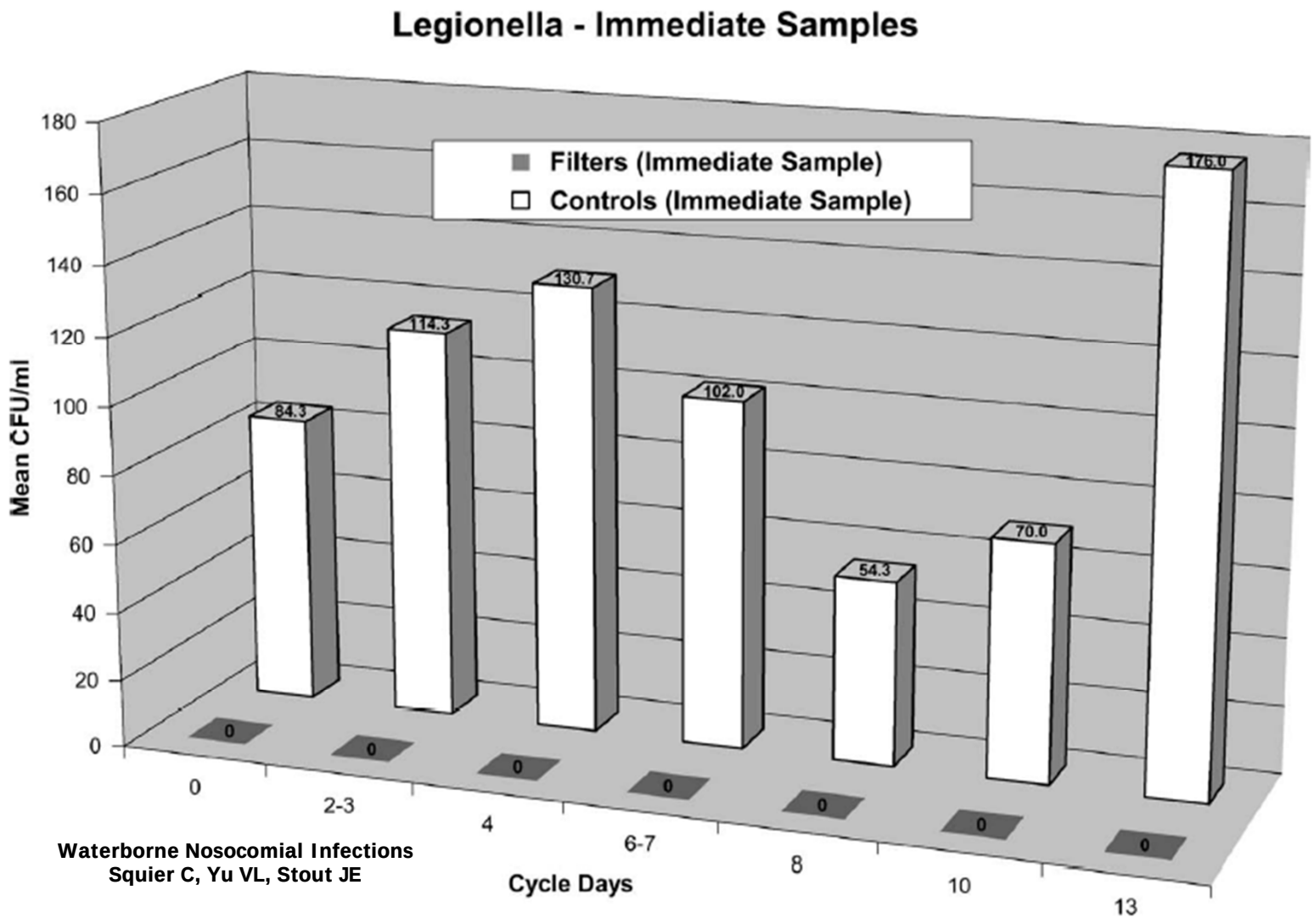
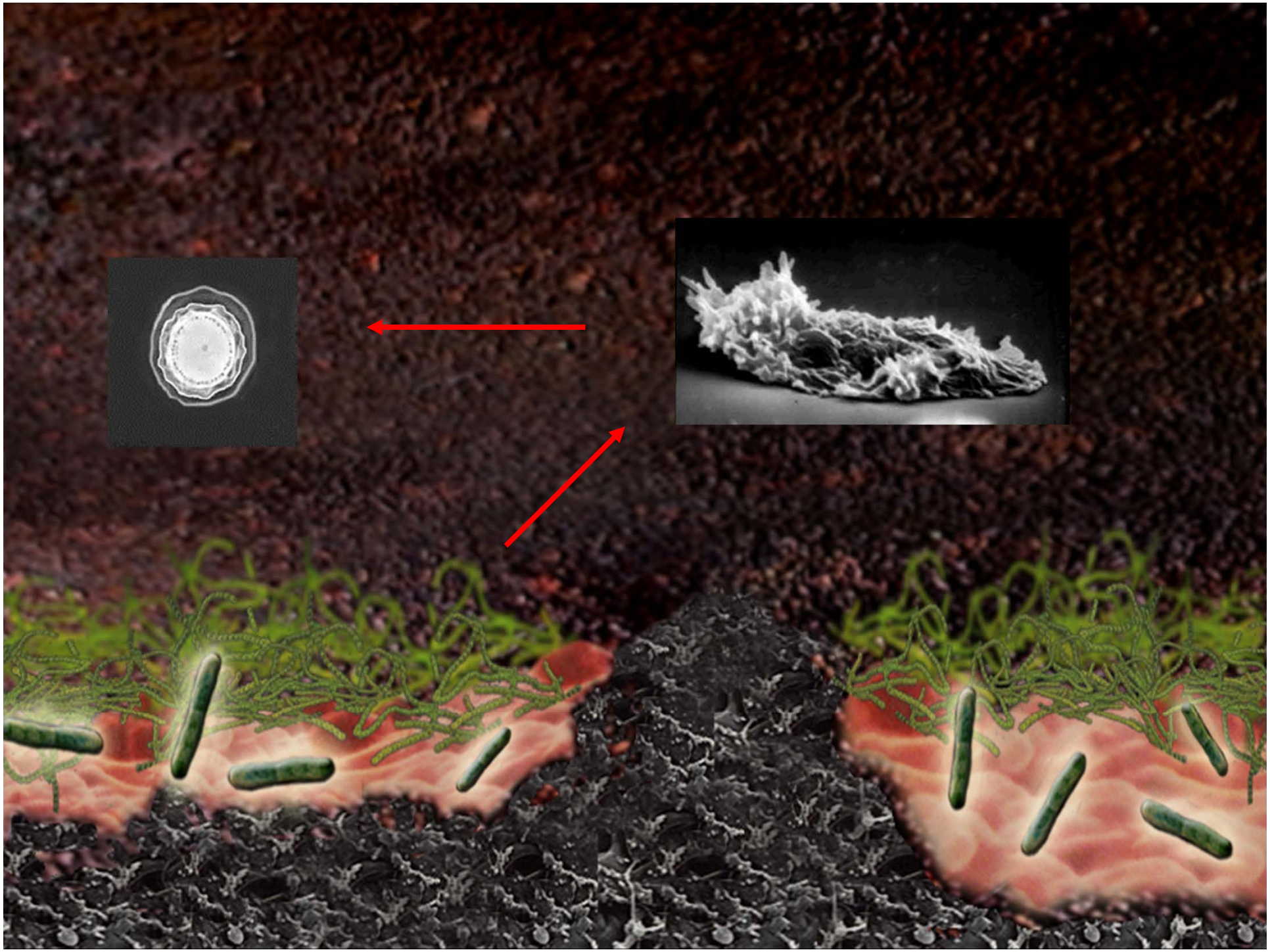


Fig 2. Recovery of *Legionella pneumophlia* (mean cfu/mL) from faucets with and without (controls) point-of-use filters through 13 days of use. Water samples were collected immediately after opening the faucet valve (immediate sample).



Development of a pilot-scale 1 for *Legionella* elimination in biofilm in hot water network: I evaluation

SHOCK TERMICO

M. Farhat[1,2], M.-C. Trouilhé[1], E. Briand[3], M. Moletta-Denat[1], E. Robine[1] and J. Frère[2]

1 Centre Scientifique et Technique du Bâtiment, Marne-la-Vallée cedex 02, France
2 Laboratoire de Chimie et de Microbiologie de l'Eau, Université de Poitiers, Poitiers, France
3 Direction Générale de la santé, Bureau EA2 – Environnements intérieurs, milieux de travail et accidents de la vie courante, Paris, France

Journal of Applied Microbiology **108** (2010) 1073–1082

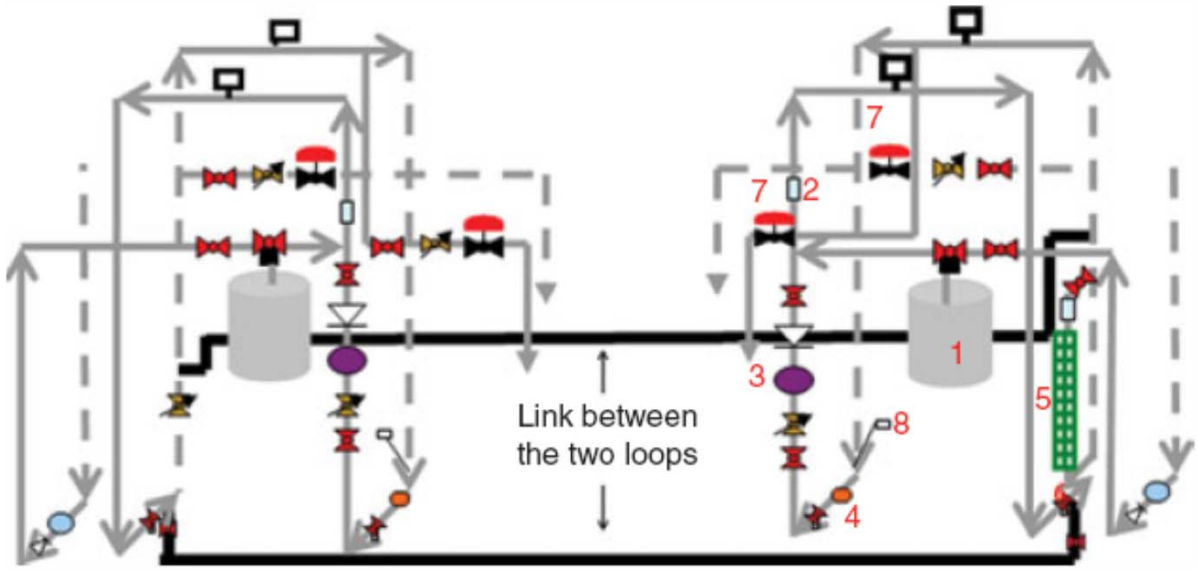
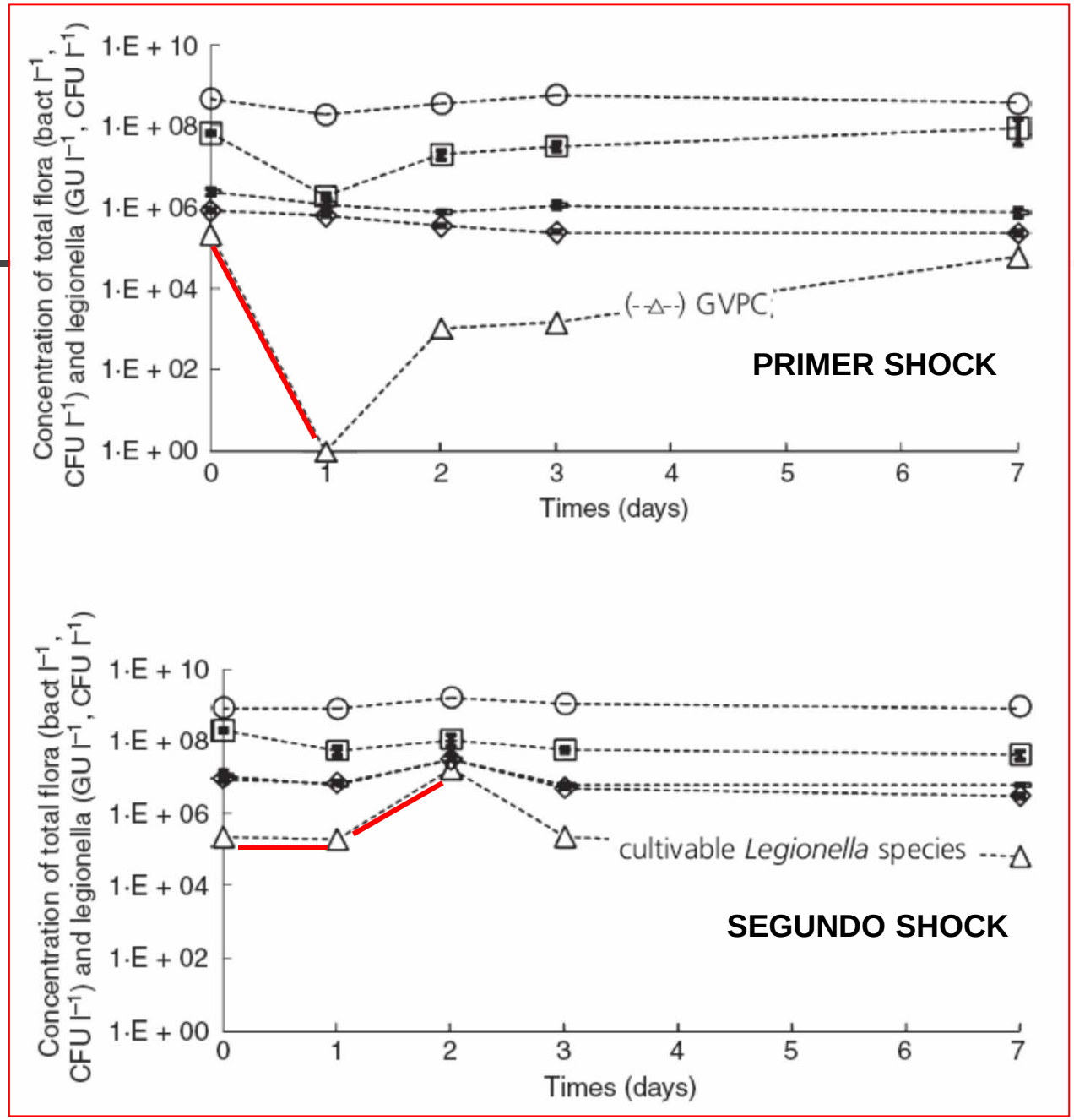


Figure 1 Schematic representation of the two identical loops (Control and Test Loops) of the pilot-scale unit. This loop is mainly composed of (1) hot water storage; (2) flow meter; (3) circulator; (4) heater; (5) biofilm sampling system; (6) sampling taps; (7) draining valves and (8) temperature sensor.



RECOLONIZACION Y ADAPTACION

Letters in
Applied Microbiology

Letters in Applied Microbiology 50 (2010) 24–29

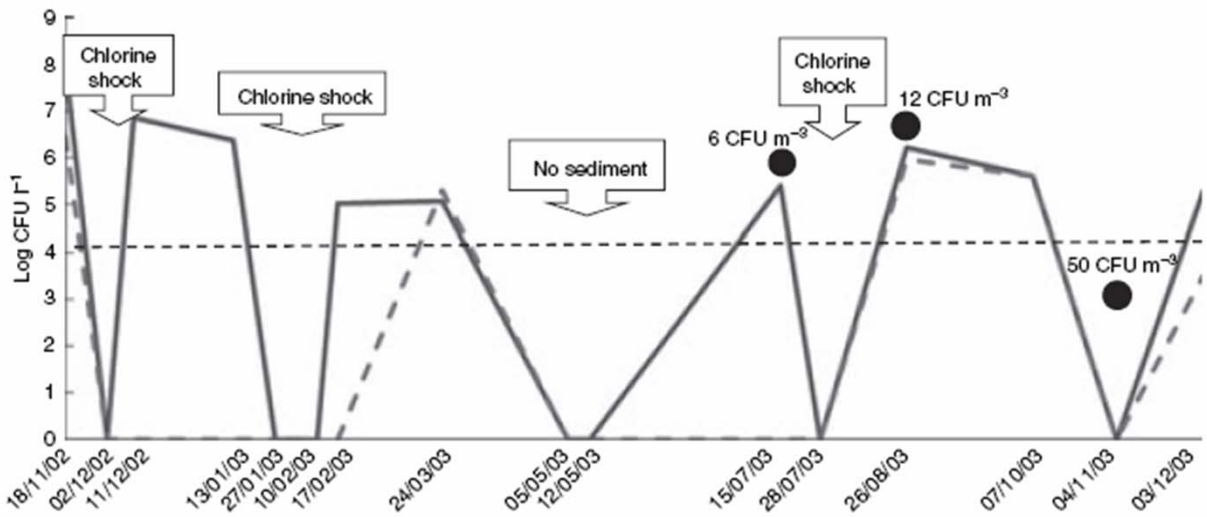
Legionella in industrial cooling towers: monitoring and control strategies

SHOCK DE CLORO

A. Carducci, M. Verani and R. Battistini

Biology Department, University of Pisa, Pisa, Italy

Chlorine shock dosing was able to eliminate bacterial contamination, but only for 10–15 days. Biocide shock dosing was also insufficient to control the prob-



The Efficacy of Heat and Chlorine Treatment against Thermotolerant Acanthamoebae and Legionellae

Scand J Infect Dis 36: 656–662, 2004

MICHAEL V. STOREY[1], JADWIGA WINIECKA-KRUSNELL[1], NICHOLAS J. ASHBOLT[2] and THOR-AXEL STENSTRÖM[1]

From the [1]Department of Parasitology, Mycology and Water Microbiology, Swedish Institute for Infectious Disease Control, SE-171 82 Solna, Sweden, and [2]Centre for Water and Waste Technology, University of New South Wales, UNSW-Sydney 2052, Australia

Table II. *The maximum value for thermal (°C), free and combined chlorine (mg/l) treatment at which A. castellanii and I4 Acanthamoeba cysts retained their viability at exposure times of 10, 30 and 60 min (n = 6)*

	A. castellanii			Acanthamoeba (I4)		
Treatment	10 min	30 min	60 min	10 min	30 min	60 min
Thermal (°C)	80	70	60	80	70	60
Free chlorine (mg/l)	100	10	<10	100	20	<10
Combined chlorine (mg/l)	100	50	20	100	50	20

The efficacy of biocides and other chemical additives in cooling water systems in the control of amoebae

M. Critchley[1] and R. Bentham[2]

1 CSIRO Materials Science and Engineering, Clayton VIC 3168, Australia
2 Department of Environmental Health, Flinders University, Adelaide SA 5001, Australia

Table 1 Minimum inhibitory concentration of biocides against amoebal trophozoites and cysts after 8 h exposure at 25°C (concentrations at mg l^{-1})

	Acanthamoeba sp.	*H. vermiformis*	*Vahlkampfia* sp.
Trophozoites			
Chlorine (residual)	1	1	1
Bromine (residual)	5	1	1
Isothiazolinones	150	50	25
Cysts			
Chlorine (residual)	5	5	2
Bromine (residual)	10	5	2
Isothiazolinones	200	150	100

systems operating at higher temperatures (above 30°C) may require longer biocide residence times for effective disinfection. The operating temperatures at the warmest parts of the system should also be considered.

Other chemical additives used in cooling water treatment may also compromise the efficacy of some biocide formulations. This is especially true for phosphate and molybdate additives and the efficacy of bromine and isothiazolinone. This study identified these two additives as

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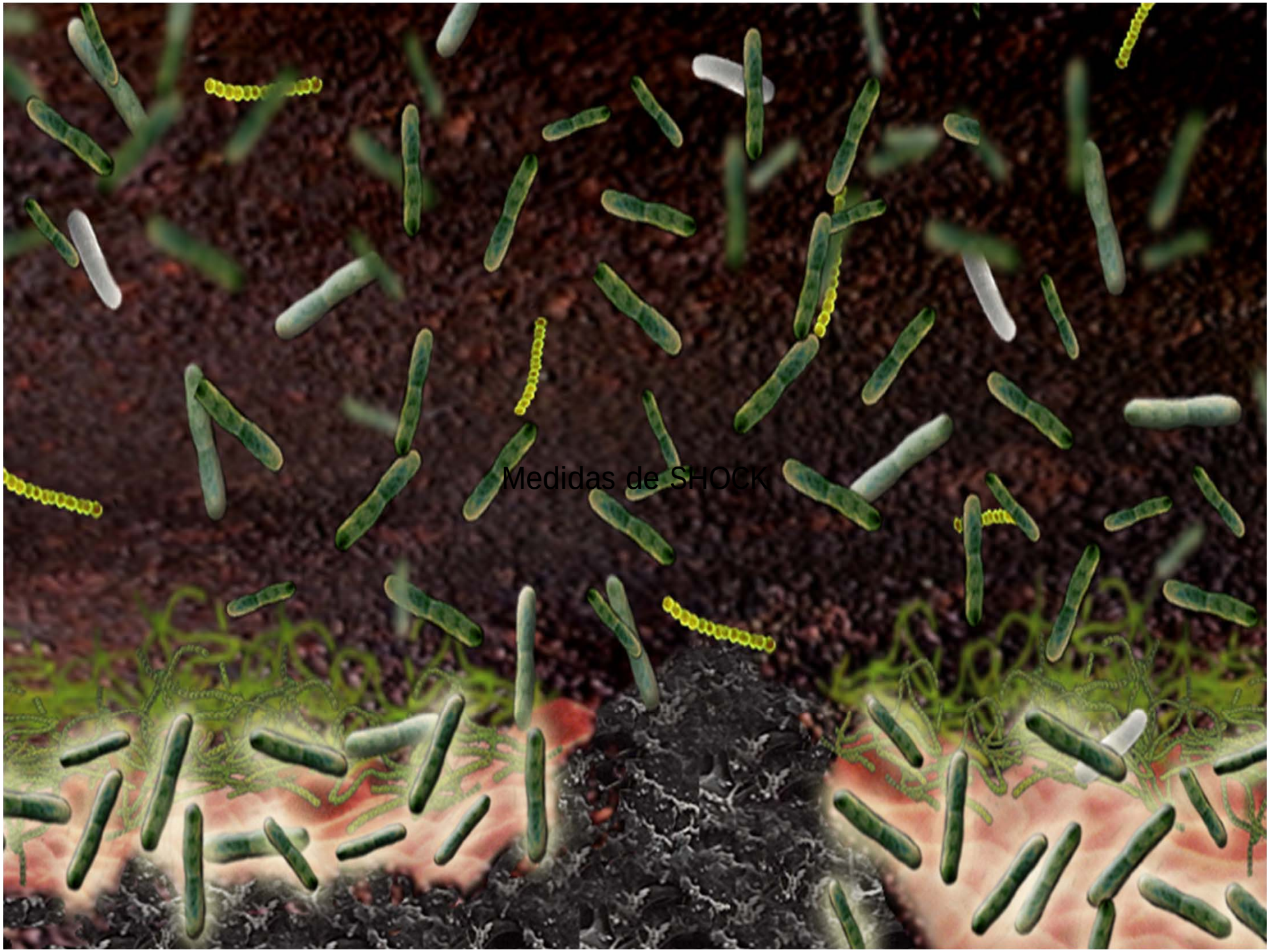
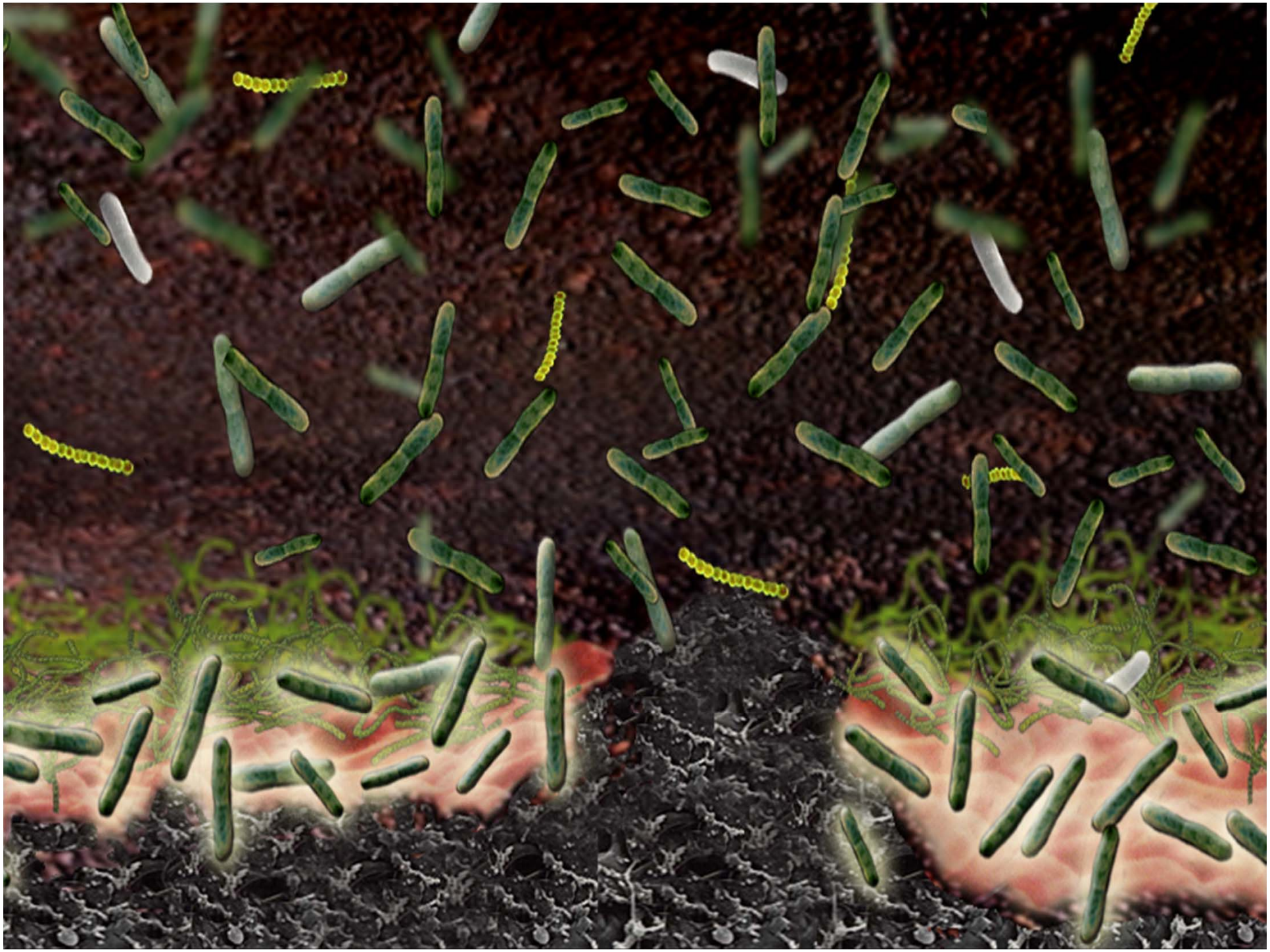

Medidas de SHOCK



CONCLUSIONES

La incidencia real de legionelosis no se conoce debido al INFRADIAGNÓSTICO de la enfermedad, pero la mayoria de paises comunican incidencias crecientes y/o estables en los últimos años

Exsite una amplia variabilidad genética de *Legionella pneumophila* sg 1 tanto en TR como en circuitos de AS que facilita la investigación epidemiológica

La evaluación microbiológica del riesgo de legionelosis define la acción a realizar: debe garantizarse el máximo rigor tanto en la recogida como en el procesamiento de la muestra.

La fluctuación en torres y el elevado número de puntos de consumo en grandes edificios, obliga a muestreos mas frecuentes y mas amplios respectivamente.

En hospitales colonizados por *Legionella* spp es prudente usar agua bidestilada para aquellas maniobras que impliquen un riesgo de aspiración

Los filtros, el dioxido de cloro y la ionización Cu/Ag han demostrado ser eficaces para la desinfección complementaria local y sistémica respectivamente en grandes edificios.

La elevada resistencia de los quistes amebianos a la acción del calor y biocidas hace que sea imposible en la actualidad hablar de erradicación de Legionella de un sistema mecánico acuoso.

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www.legionella-online.org

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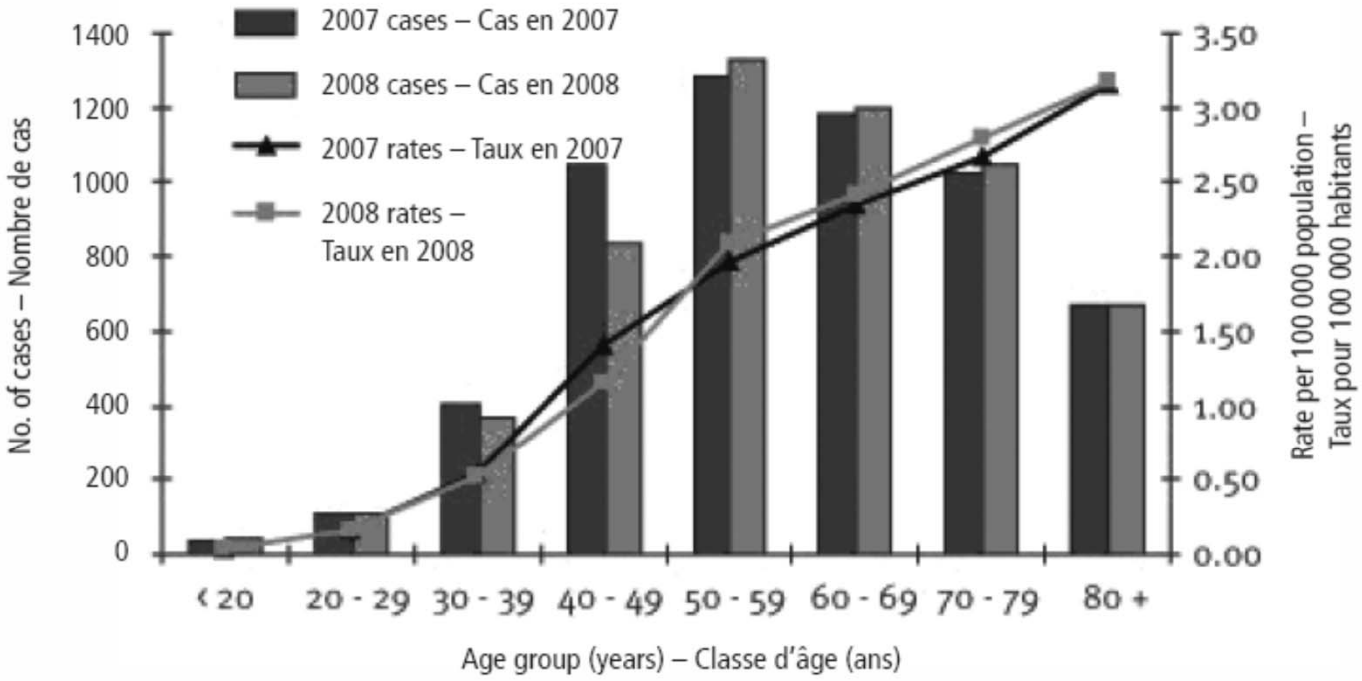
Figure 1 **Cases of Legionnaires' disease and age standardized rates per 100 000 population by age group, 2007–2008**
Figure 1 **Nombre de cas de maladie des légionnaires et taux standardisés sur l'âge pour 100 000 habitants, par classe d'âge, 2007-2008**



Source: European Working Group for Legionella infections (EWGLI). – Données du Groupe de travail européen sur les infections à *Legionella* (EWGLI)

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Use of Flow Cytometry To Monitor *Legionella* Viability[▽]

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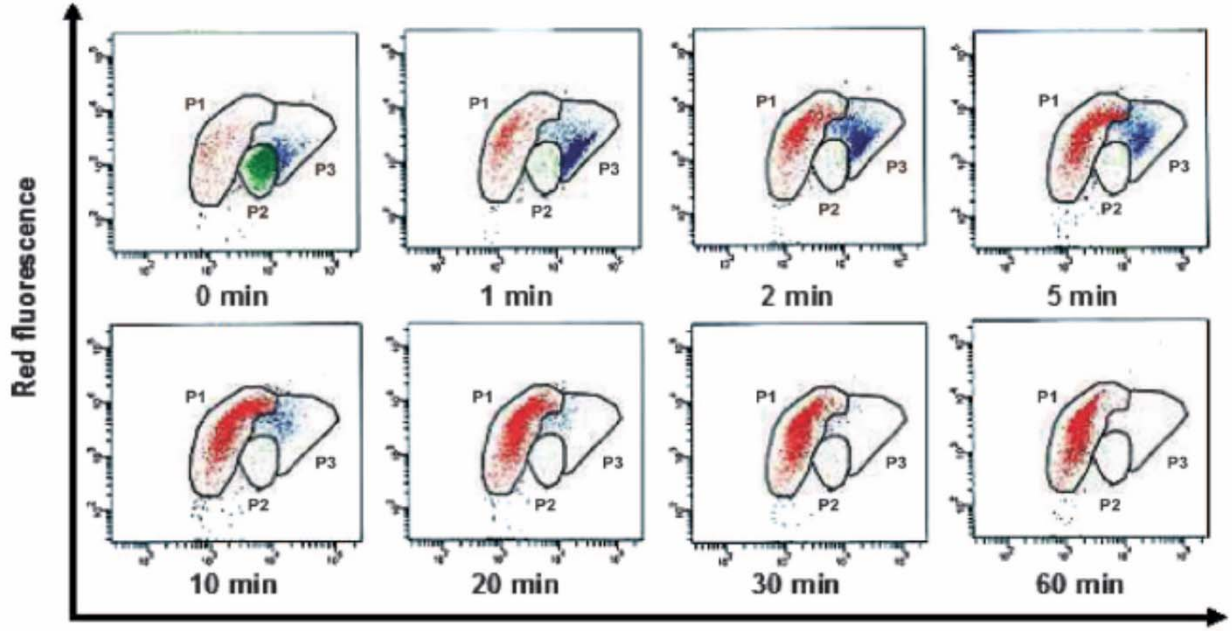
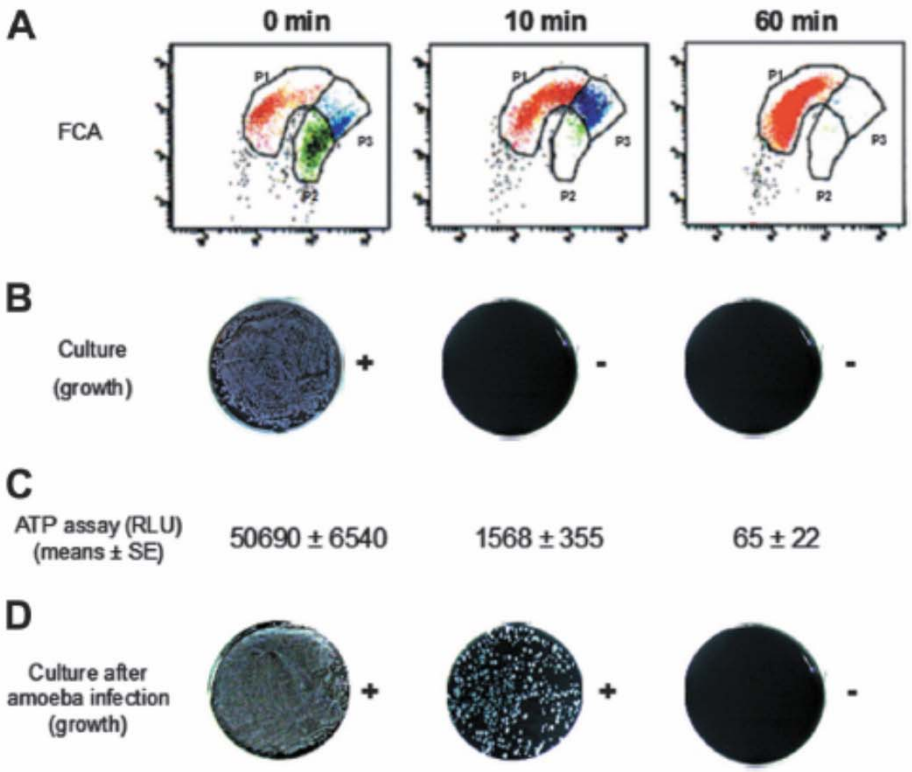
Legionella **viability was monitored during heat shock treatment at 70°C by a flow cytometric assay (FCA). After 30 min of treatment, for 6 of the 12 strains tested, the FCA still detected 10 to 25% of cells that were viable but nonculturable (VBNC). These VBNC cells were able to produce ATP and to be resuscitated after culture on amoebae.**


Red fluorescence
P1
P2
P3
0 min
1 min
2 min
5 min
10 min
20 min
30 min
60 min


A
0 min
10 min
60 min
FCA
P1
P2
P3
B
Culture
(growth)
+
-
-
C
ATP assay (RLU)
(means ± SE)
50690 ± 6540
1568 ± 355
65 ± 22
D
Culture after
amoeba infection
(growth)
+
+
-

An international trial of quantitative PCR for monitoring for *Legionella* in artificial water systems

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