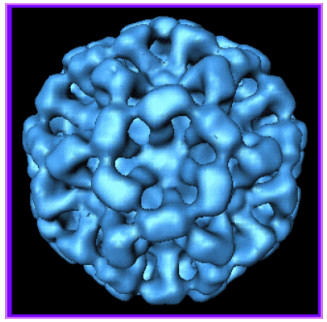
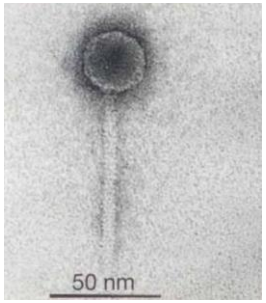
Detection and survival of enteric viruses in water



C. Gantzer



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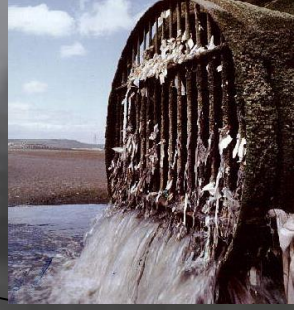
Laboratory of physical chemistry and microbiology for the environment (LCPME)
Faculté de Pharmacie
5 rue Albert Lebrun
54000 Nancy (France)

General context : Fecal pollution of water

http://tpeeaupotable.ifrance.com/ma%20photo/egout1.jpg

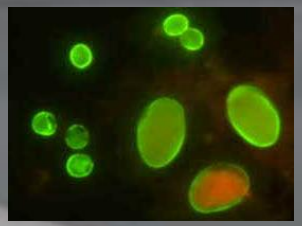


Parasites :

Giardia

Cryptosporidium

…



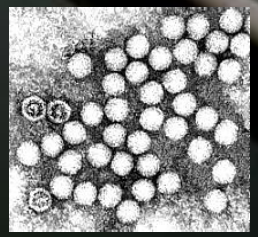
http://www.vetparasitology.ugent.be/page1/page1.html

Bacteria :

Salmonella

…

http://upload.wikimedia.org/wikipedia/commons/e/ee/Salmonella_typhimurium.png



Enteric viruses pathogenic for human

http://www.worsleyschool.net/science/files/virus/page.html

The problem was identified more than 70 years ago…

Infected human

Virus inactivation/ removal

High viral excretion in stools :
Up to 10^{10} / g

Food

Drinking water/ environment

Low infective dose :
10-100 infectious units

Human

Virus inactivation/ removal

4 log reduction
1/10000 = risk

The main targets :

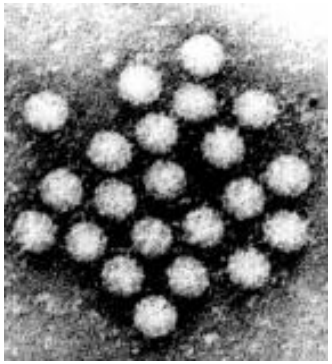
- *Norovirus*

- Hepatitis viruses (A and E)

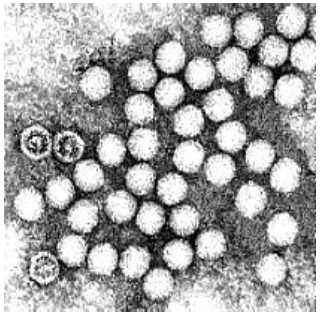
- Rotavirus/Astrovirus/Adenovirus /Enterovirus/Aichivirus…

- Other emerging viruses (SRAS, H5N1…)

20-30 nm
ssRNA

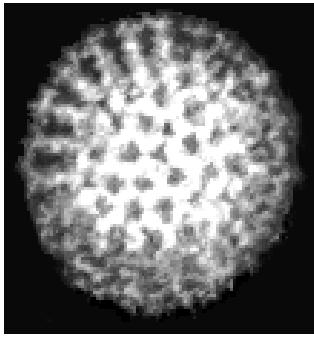


http://virology-online.com/viruses/Diarrhoea5.htm



http://www.worsleyschool.net/science/files/virus/page.html

70-80 nm
dsRNA or DNA



http://pathmicro.med.sc.edu/mhunt/RNA-HO.htm

Table 1 – Examples of the concentration of viruses found in sewage, freshwater and seawater by qPCR. Results are expressed in genome copy logs (GC logs).

Virus	Type of sample	Collection site	Concentration	% Positive samples	Quantification method	Reference
Adenovirus	Sewage (raw)	Spain	4–7 GC logs/100 ml	100%	qPCR	Bofill-Mas et al. (2006)
	Sewage (secondary effluent)		3 GC logs/100 ml	100%		
	Biosolids		4–7 GC logs/100 g	100%		
	River water		1–4 GC logs/l	90%		Albinana-Gimenez et al. (2009b)
	Seawater		1–3 GC logs/l			Calgua et al. (2008)
	Sewage (raw)	USA	4–5 GC logs/100 ml		qPCR	Fong et al. (2010)
	Sewage (tertiary effluent)		3–4 GC logs/100 ml			
Adenovirus 40, 41	River water	Japan	3–5 GC logs/l	61%	qPCR	Haramoto et al. (in press)
JC Polyomavirus	Sewage (raw)	Spain	5 GC logs/100 ml	100%	qPCR	Bofill-Mas et al. (2006)
	Biosolids		3–5 GC logs/100 g	100%		
	River water		0–3 GC logs/l	90%		Albinana-Gimenez et al. (2009b)
	Sewage (raw)	Brazil	4–7 GC logs/100 ml	96%	qPCR	Fumian et al. (in press)
	Sewage (secondary effluent)		4–5 GC logs/100 ml	39%		
	River water	Japan	2–3 GC logs/l	11%	qPCR	Haramoto et al. (in press)
Astrovirus	Sewage (raw)	France	5–7 GC logs/100 ml	100%	qRT-PCR	Le Cann et al. (2004)
Enterovirus	Sewage (raw)	France	7 GC logs/100 ml		qPCR	Schvoerer et al. (2001)
Hepatitis A virus	Sewage (raw)	Spain	4 GC logs/100 ml		qPCR	Rodriguez-Manzano et al. (2010)
Hepatitis E virus	Sewage (raw)	Spain	3 GC logs/100 ml		qPCR	Rodriguez-Manzano et al. (2010)
Norovirus	Sewage (raw)	United Kingdom	6 GC logs/100 ml		qPCR	Laverick et al. (2004)
GII	Sewage (effluent)	Brazil	2–3 GC logs/l		qPCR	Victoria et al. (in press)
GI			2 GC logs/l			

Girones *et al.* (2010)

Some criteria for the universal ideal indicator :

- Present at the same time as the pathogenic organisms (fecal pollution) and more abundant if possible. Absent in unpolluted water.

- No multiplication

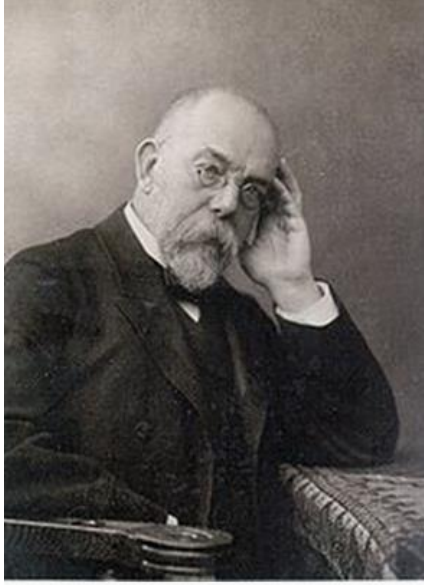
- Easily detectable with simple method

- Not pathogenic

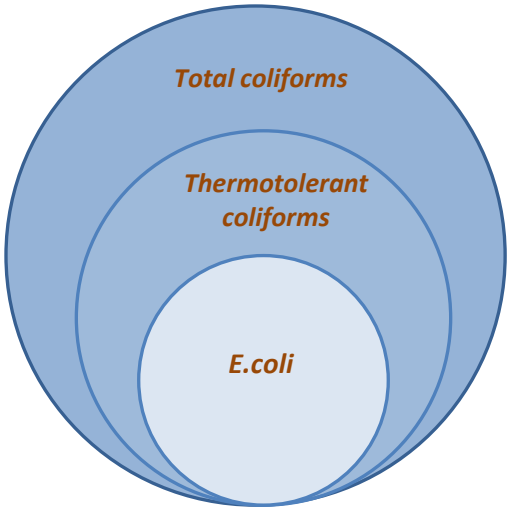
- More resistant than the corresponding pathogen in environment but also with respect to water treatment

Fecal bacteria indicators

1881 : total cultivable bacteria…



Portrait de Robert Koch (1843-1910).



Enterococci + other bacteria…

Regulation for drinking water, bathing water, oysters…

Limits of fecal bacteria as viral indicator :

Survival lower than pathogens in environment : virus et parasites

Resistance to treatment lower than pathogens

Different behavior : soil migration, filtration…

E. coli and enterococci are indicators of :
Fecal pollution which do not take into account virus survival in environment and virus behavior. They cannot be used as model for estimating virus treatment efficiency.

Recent fecal pollution

E. coli
Enterococci

Presence
or
High level

Probability of the presence of infectious viruses

+ + + +

Treatment or survival in environment

Absence
or
Low level

+ + +

+ +

+

+ / -

-

?

How may we discriminate such situations ?

Some examples : Detection of pathogenic viruses in tapwater without the presence of fecal indicators

In South Africa

Grabow *et al.* (2004) : 11-16% positif samples for infectious *Enterovirus*.

ELSEVIER

Water Research 38 (2004) 2699–2705

WATER RESEARCH

www.elsevier.com/locate/watres

Detection of enteroviruses in treated drinking water

J.C. Vivier*, M.M. Ehlers, W.O.K. Grabow

Department of Medical Virology, Institute of Pathology, University of Pretoria, P.O. Box 2034, Pretoria 0001, South Africa

Received 16 May 2001; received in revised form 25 August 2001; accepted 26 September 2001

Some examples : Detection of pathogenic viruses in tapwater without the presence of fecal indicators

In South Korea

Lee and Kim (2002) : between 40 et 50% + (0,002 – 0,03 NPPUC/L) for infectious *Adenovirus* and *Enterovirus*

PERGAMON

Water Research 36 (2002) 248–256

WATER RESEARCH

www.elsevier.com/locate/watres

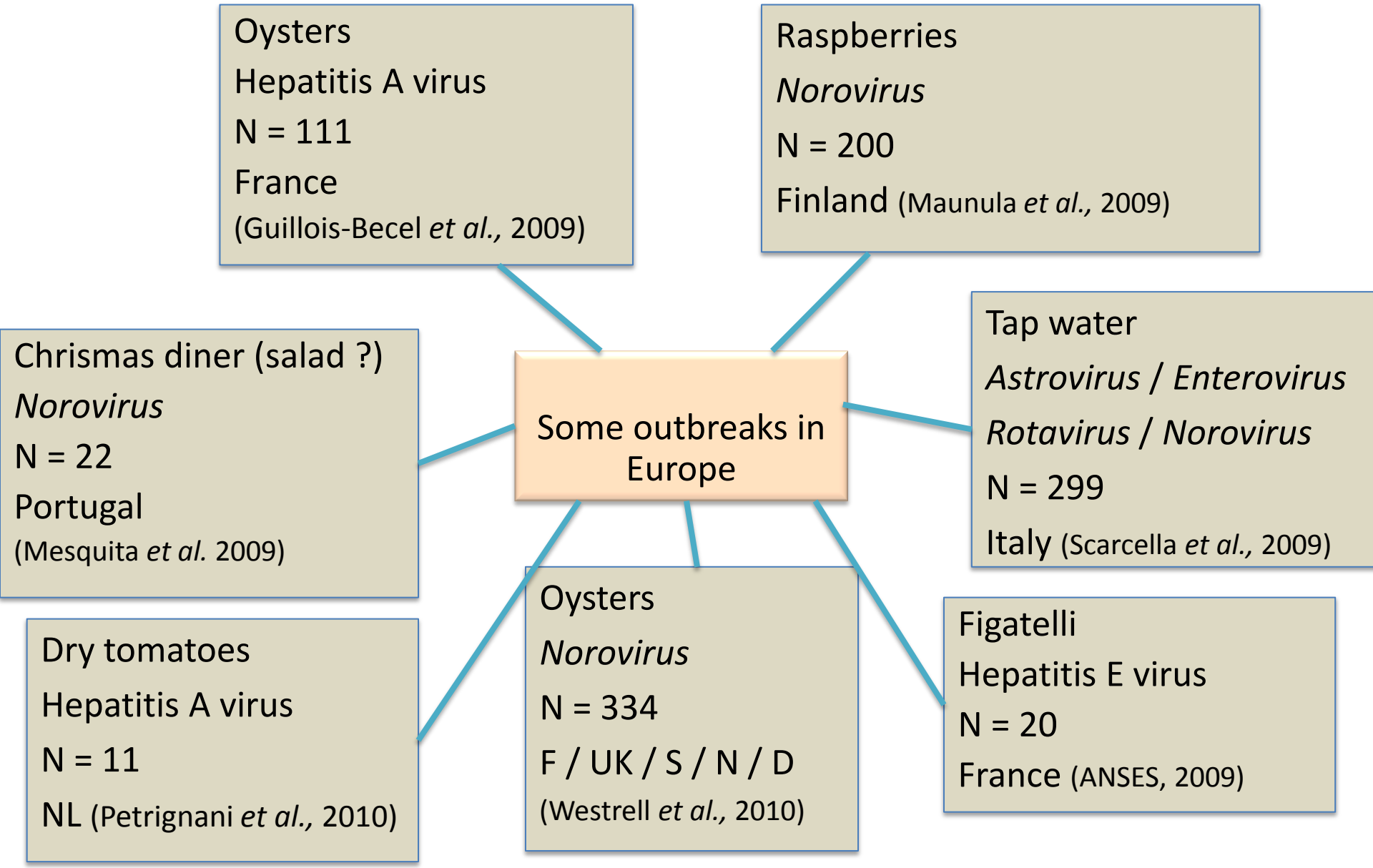
Detection of infectious enteroviruses and adenoviruses in tap water in urban areas in Korea

Seung-Hoon Lee, Sang-Jong Kim*

School of Biological Sciences, College of Natural Sciences, Seoul National University, Shilim-dong, Kwanak-Gu san 56-1, Seoul 151-742, South Korea

Received 5 July 2000; received in revised form 22 January 2001; accepted 24 April 2001

FAO et WHO (2008) : an increase of food or water outbreaks



Eurosurveillance (http://eurosurveillance.org)

Why do we observe such outbreaks ?

Viruses are most resistant than bacteria which are currently used as indicators (*E. coli*, *enterococci*)

Microbiological criteria

> limits = high viral risk

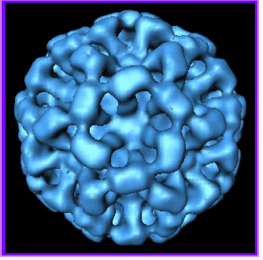
< limits = some outbreaks may still be described due to viruses

« Some criteria may be defined for enteric viruses in mollusc and water as soon as the analytical tools will be developed. (Règlement 2073/2005/CE)

Two questions for prevention of viral outbreaks :

1. Are tools for detecting viruses enough developed to define criteria and regulations ?

2. How can we select a model to describe virus survival (environment or during treatment) ?



1. Diagnostic tools

Viral targets : Norovirus, HAV (HEV?)
Matrices : oysters, raspberries, salads, water, surfaces.

Cell culture not usable

Molecular tools (RT-PCR) : only way

Define detection method

Food Environ Virol (2010) 2:146–155
DOI 10.1007/s12560-010-9042-5

ORIGINAL PAPER

International Standardisation of a Method for Detection of Human Pathogenic Viruses in Molluscan Shellfish

David Lees · CEN WG6 TAG4

Lees (2010)

Now we have a standardized method

Extraction/concentration of viruses from the matrice

Dissection, digestion with proteinase K (Jothikumar *et al.* 2005) : oysters
Others : elution, filtration…

Extraction of nucleic acid

Guanidine isothiocyanate et magnetic beads

Real time RT-PCR

Primers in conserved regions (HAV, Norovirus GI et GII)
Controls (control +/- for process, inhibition)

But…

Presence of viral genome is not a proof of the presence of infectious virus

Example : Poliovirus 1, mineral water, 35°C (Gassilloud *et al.* 2003)

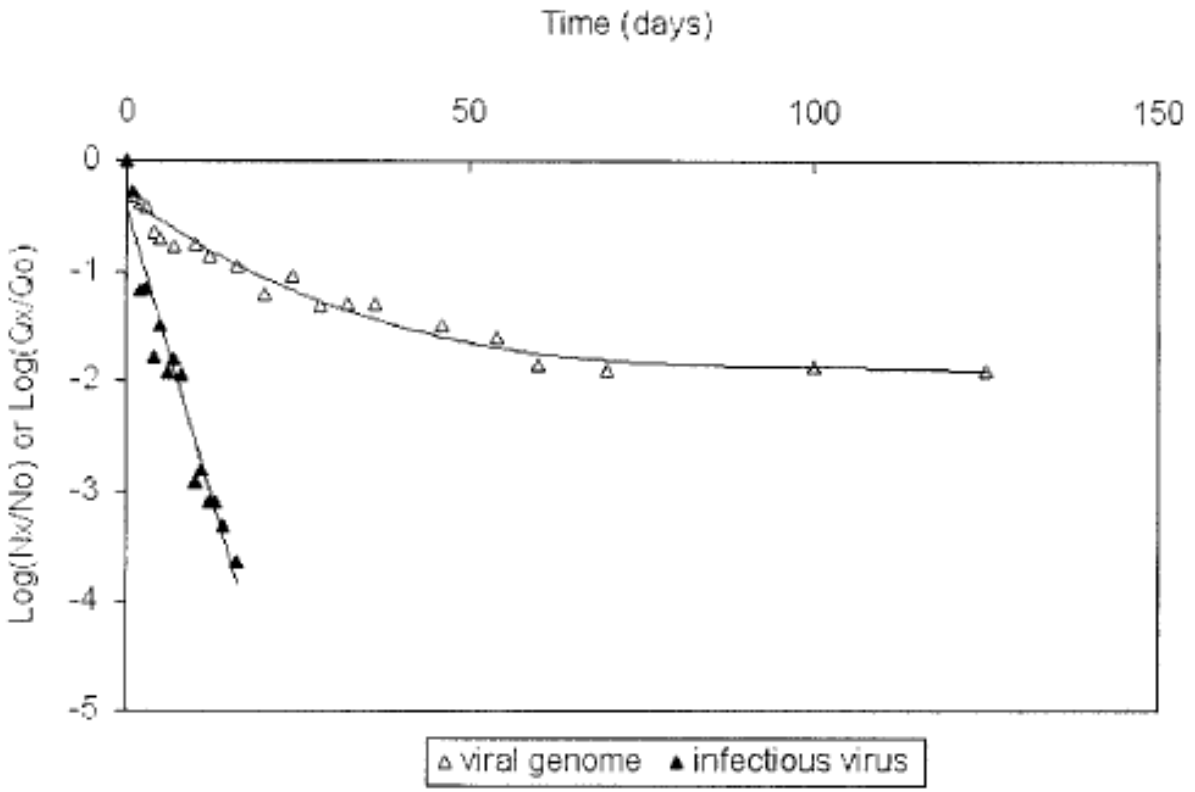
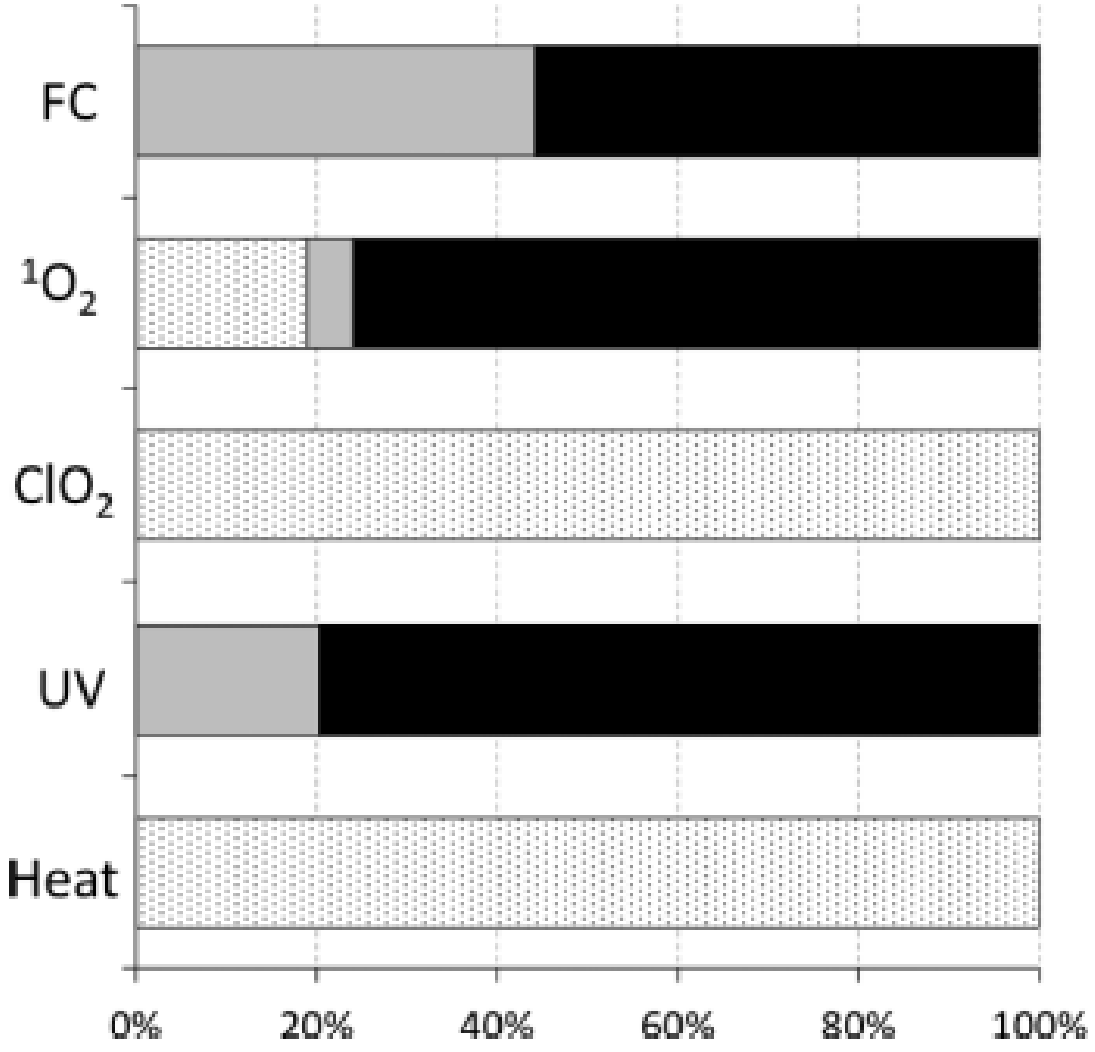


FIG. 3. Persistence of the PV1 genome (viral genome) and infectious PV1 (infectious virus) in mineral water at 35°C over time as described by equations 2 and 1, respectively.

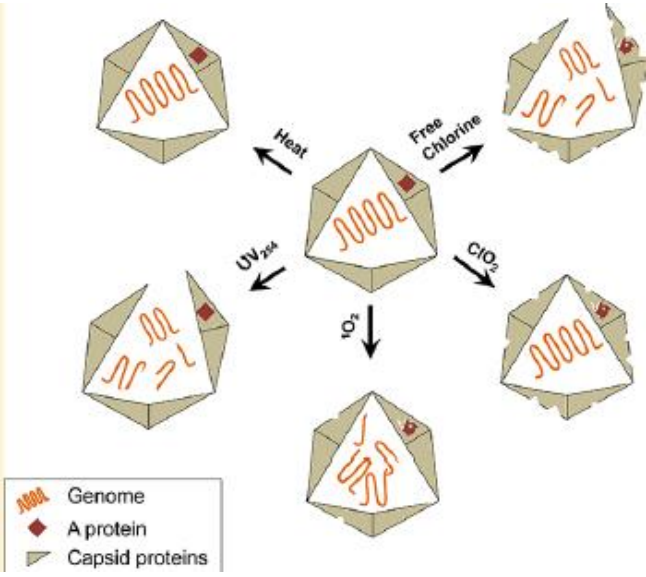
Same results for a lot of situations : ClO_2 ; Ozone ; UV… (Simonet *et al.* 2006 ; Sano *et al.* 2010…)

Relation between genome and infectivity depend on the inactivation mechanisms

Loss of the capacity to bind to the cell receptor (binding), to inject genome inside the cell (injection) and to replicate the genome (replication). Model : MS2 phage .



Wigginton *et al.* 2012



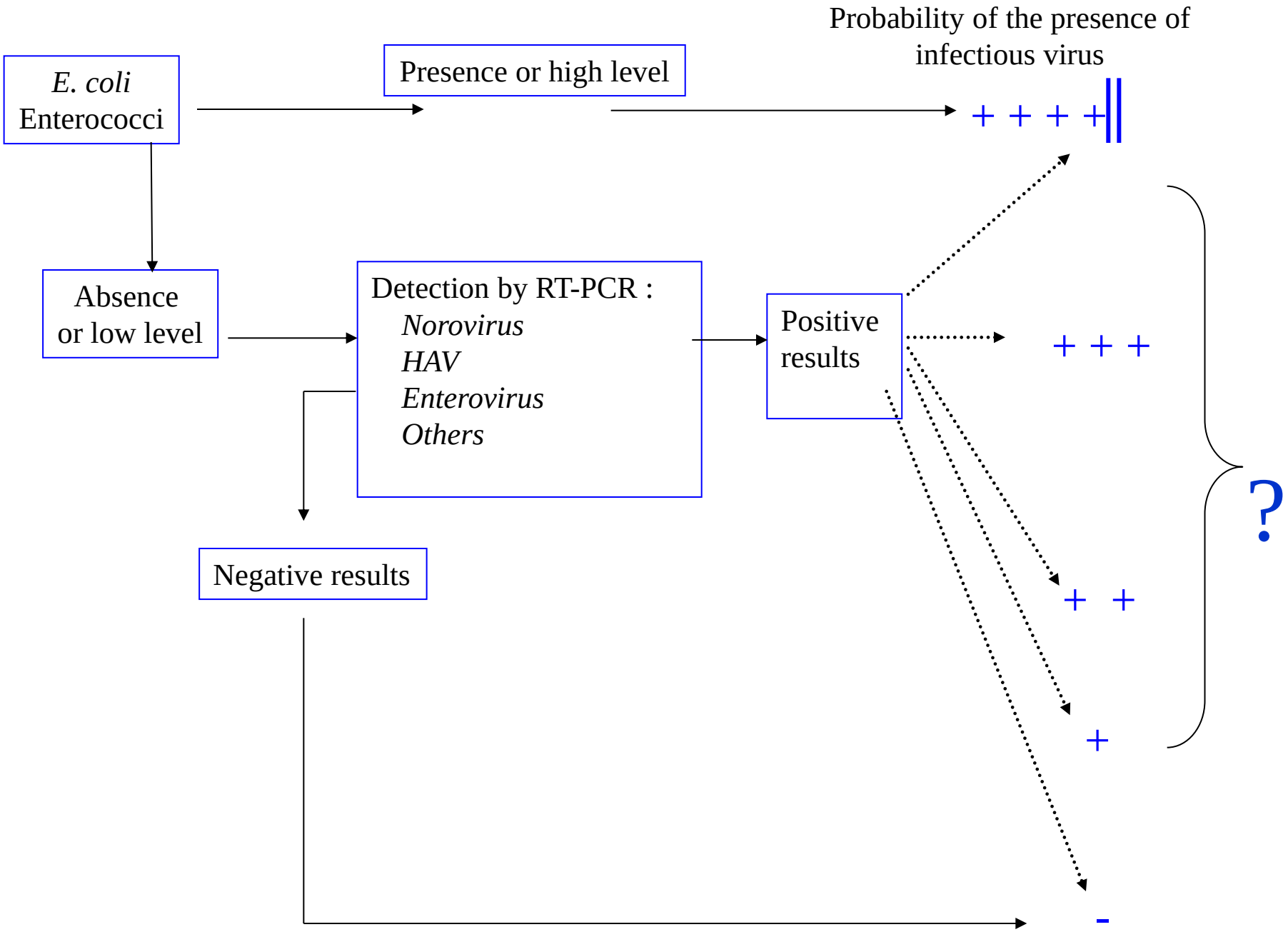
How can we interpret the presence of viral genome in term of viral risk ?

- Detection of viral genome is an indicator of the presence of a viral pollution which may be recent or old. Their presence is not always correlated with the presence of infectious virus.

- Absence of viral genome (if the right control are made) correspond to the absence of the corresponding infectious virus.

- Absence of viral genome may not give any information about the global fecal pollution and the presence of other viruses.

- Molecular tools largely underestimate impact of treatments.


Probability of the presence of infectious virus
E. coli Enterococci
Presence or high level
+ + + +
Absence or low level
Detection by RT-PCR :
Norovirus
HAV
Enterovirus
Others
Positive results
+ + +
+ +
+
?
Negative results
-

Prevalence of Norovirus genome is sometimes very high !

- Oyster (production zone)

*76.2% (n= 844) (GB) (Lowther *et al.* 2012)

60% > 100 cg/g ; 30% >1000cg/g some with 10 000cg/g !

*3.9% (4.4% HAV) (n= 390) (USA) (DePaola *et al.* 2010)

*de 9% à 23% NV (F) (Beuret *et al.* 2003 ; Le Guyader *et al.* 2000)

- Red fruits

*7% et 34% en France et Belgique (Baert *et al.* 2011)

- Salads

*0,8% à 12,4% (n=210) (Adria Normandie – Prevavir 2011)

What should be done ?

Use the standardized method to evaluate genome prevalence in different matrices (water, food)

Quantifying viral genome to evaluate viral genome pollution in different matrices

Try to make links between detection of viral genome and outbreaks

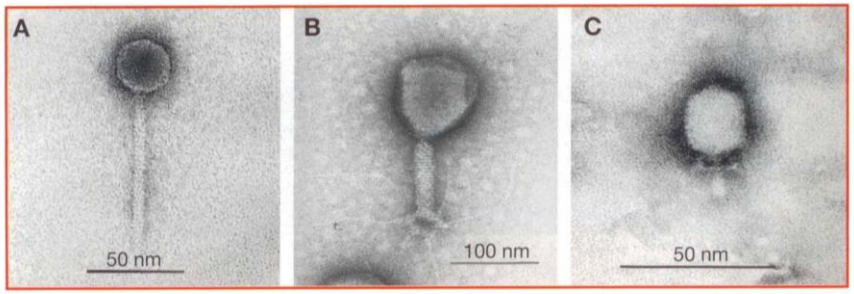
Develop studies to better understand viral inactivation mechanisms to discriminate infectious from non infectious viruses and define conditions which favor genome degradation

Don’t forget that other indicators can help ! Fecal bacteriophages, hygienic indicators…

Felix d'Herelle
1873-1949

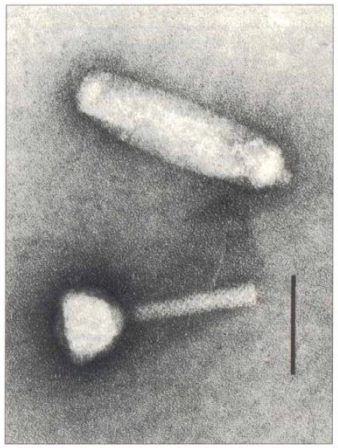
Frédérick Twort
1877 -1950

Are phages interesting in such context ?



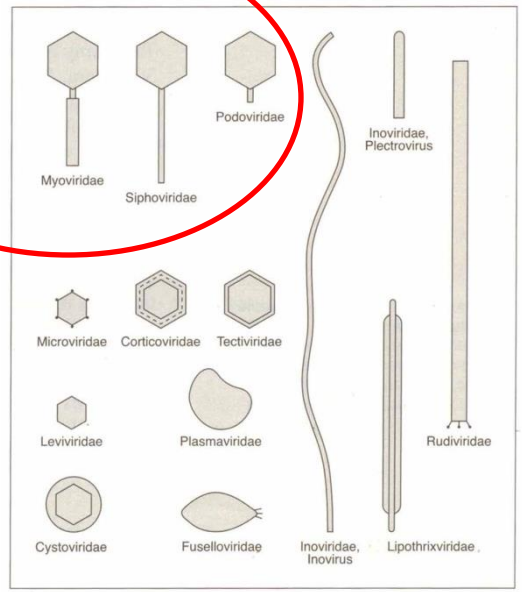
Photographies obtenues par microscopie électronique à transmission (avec la permission de H. Ackermann dans ICTVdB - The Universal Virus Database, version 4. http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/) des trois familles (*Siphoviridae* (A), *Myoviridae* (B) et *Podoviridae* (C)) de l'ordre des *Caudovirales*, retrouvées en milieux aquatiques.

Bacteriophages : the most abundant biological entities in environment



En bas, phage JX1 de *Janthinobacterium halosensibilis* (*Myoviridae*) et, en haut, phage 71A-6 de *Vibrio vulnificus* (*Podoviridae*). Acétate d'uranyle à 2 % ; le trait indique 100 nm.

97% : *Caudovirales*



3% : others

Ackermann H. *Virologie* (2001)

Phages are present : - in human stools

Bacteriophages	Frequence	Concentrations
Somatic coliphages	20%-90%	10^4-10^6 UFP/g
F- specific RNA phages	0%-57%	10-10^3 UFP/g
B. fragilis phages	0%-15%	10^2-10^8 UFP/g

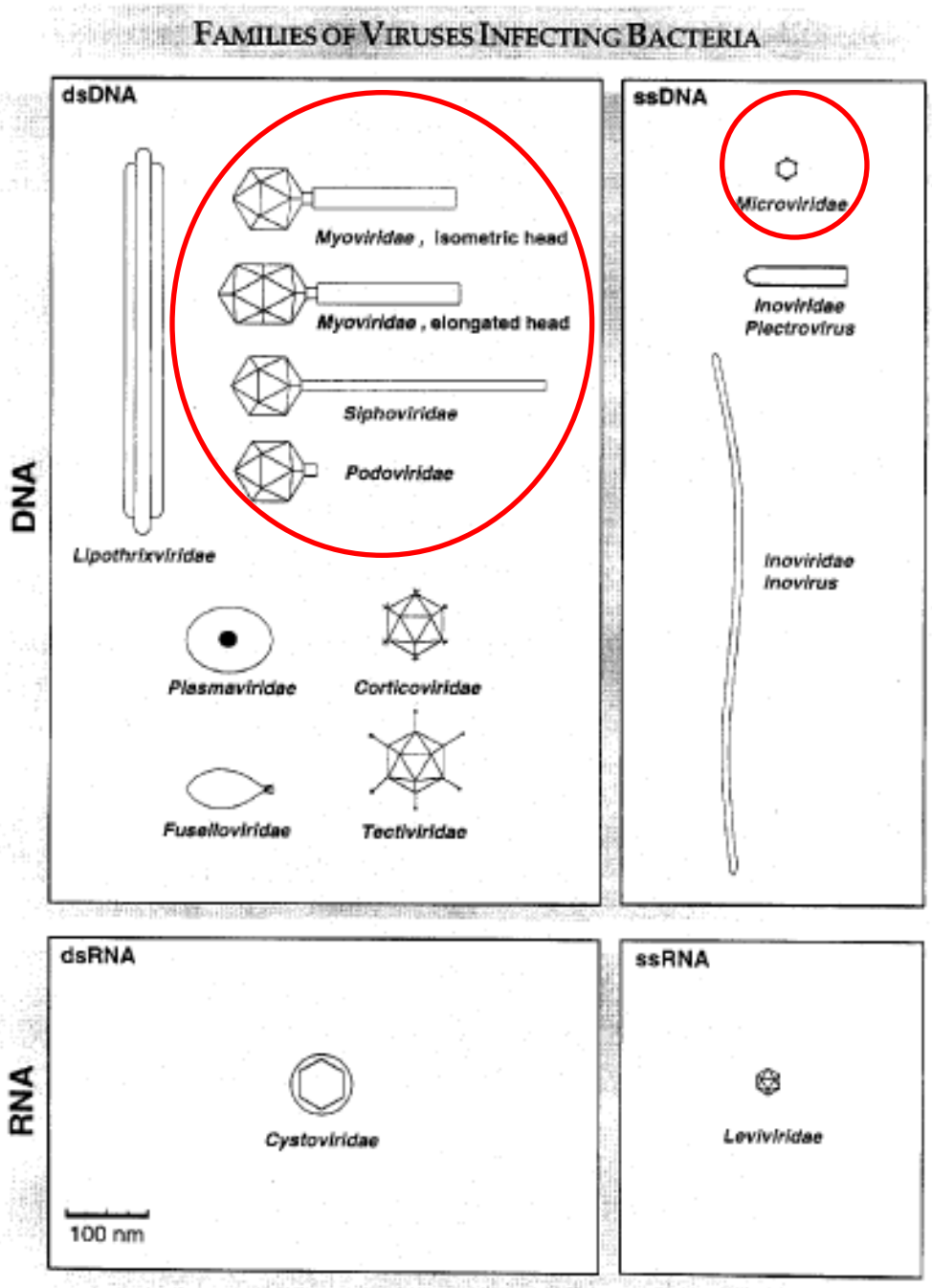
- in wastewater

Log_{10} PFU or CFU/ 100mL

	FC	FE	SRC	SOMCPH	FRNAPH	BFBRYCPH
Argentina (n = 36)						
Mean	6·74	5·86	5·37	5·78	4·85	4·07
Min.	5·66	4·44	4·60	4·95	3·54	2·00
Max.	8·30	7·20	6·50	6·67	6·15	4·98
Colombia (n = 38)						
Mean	7·05	5·87	5·63	5·75	5·24	3·75
Min.	5·23	4·45	4·48	3·15	4·00	1·00
Max.	8·36	6·90	6·60	7·00	6·34	5·23
France (n = 38)						
Mean	6·65	5·95	4·76	6·14	5·69	4·65
Min.	5·32	4·85	2·00	4·41	4·04	2·54
Max.	6·89	6·51	5·39	6·86	6·41	5·39
Spain (n = 35)						
Mean	7·24	6·27	5·60	7·17	5·87	4·71
Min.	6·19	5·61	3·48	6·34	4·36	3·95
Max.	7·93	7·00	6·71	7·95	6·89	5·89

FC : Fecal coliforms ; FE : Fecal enterococci ; SRC : Spores of sulphite-reducing clostridia ; SOMCPH : Somatic coliphages, FRNAPH : F-specific RNA phages, BFBRYCPH : B. fragilis (RYC) phage

Lucena et al. (2004)

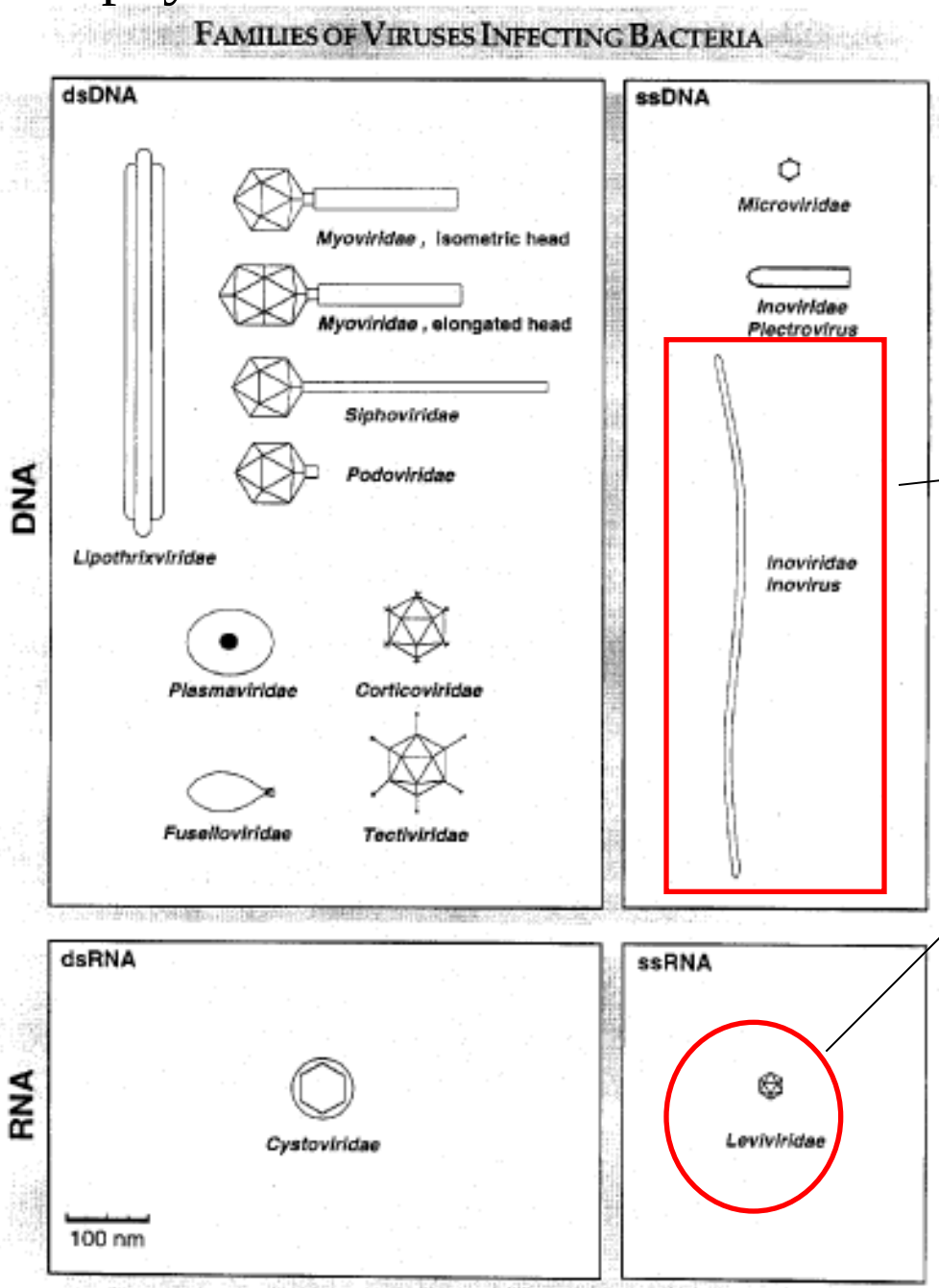


Somatic coliphages (*E. coli* WG5)

4 families : *Myoviridae*, *Siphoviridae*, *Podoviridae* and *Microviridae*

Murphy *et al.* 1995

Murphy et al. 1995

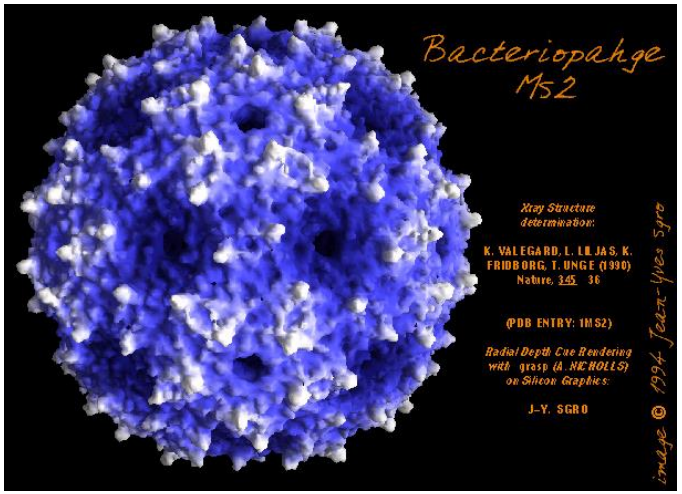


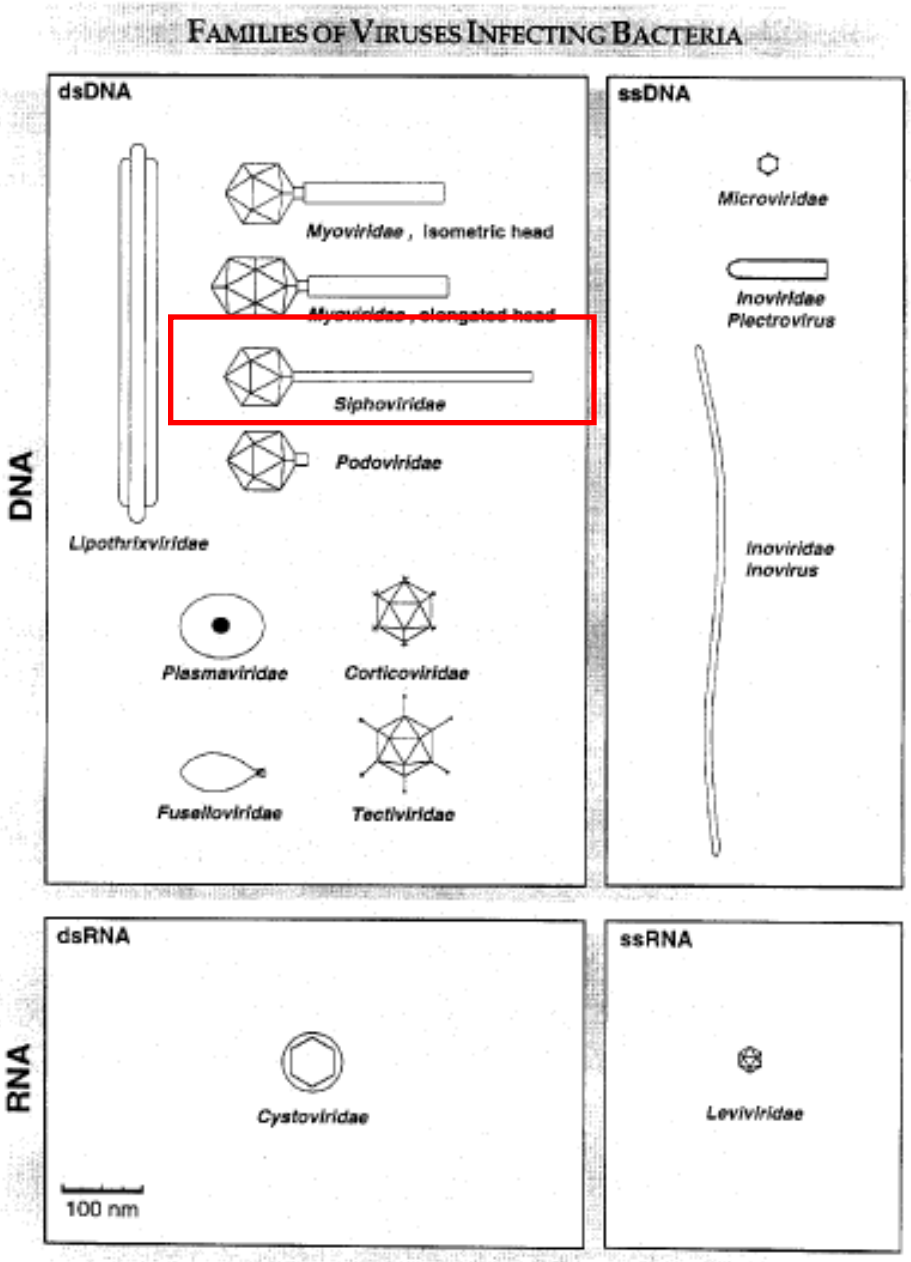
F-specific phages (*S. typhimurium* WG49 or *E. coli C*)

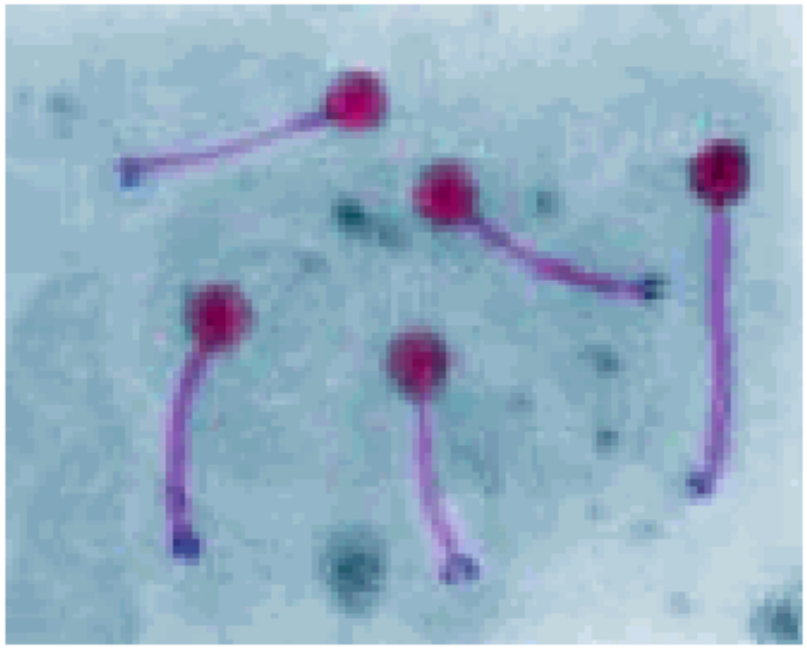
2 families : *Leviviridae* and *Inoviridae*

F-specific DNA phages

F-specific RNA phages







B. fragilis (HSP40 or RYC 2056)

1 family : *Siphoviridae*

Murphy et al. 1995

Journal of Applied Microbiology ISSN 1364-5072

REVIEW ARTICLE

Is the replication of somatic coliphages in water environments significant?

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Department of Microbiology, School of Biology, University of Barcelona, Barcelona, Spain

Keywords
coliphages, environment, replication, significance, water.

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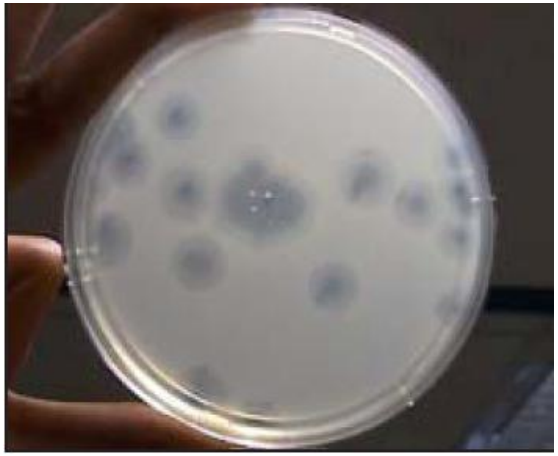
doi:10.1111/j.1365-2672.2008.03957.x

Summary

Somatic coliphages are amid several groups of bacteriophages that have been suggested as indicators in water quality assessment. One of the limitations frequently endorsed to somatic coliphages as indicators is that they can replicate in the water environment. This review intends to evaluate the significance of this potential replication. In view of: the threshold densities of somatic coliphages and host bacteria needed for productive infection to occur, the densities of both host cells supporting somatic coliphages replication and these phages in water environments, and the poor contribution of lysogenic induction to the free somatic coliphage numbers in water, it can be concluded that replication of somatic coliphages in waters is very unlikely. Consequently, the contribution of replication in the environment of somatic coliphages is expected to have a non-noticeable influence on the numbers of somatic coliphages detected in water environments. Thus, the replication in the environment should not be argued as a limitation to the use of somatic coliphages as indicators.

Phages are fecal indicators which are not pathogenic, may not replicate in environment are easily detectable with low cost method and for which the infectious character can be easily verified.



Standardized method

Some phage have a similar structure compared to pathogenic viruses(*Leviviridae*)

They have a similar survival in a lot of situations

Some of them may discriminated the origin of fecal pollution

Blanch et al. (2006): 20 parameters + 18 associations
N= 103 samples of wastewater from known origin (81 + 22)
100% good discrimination

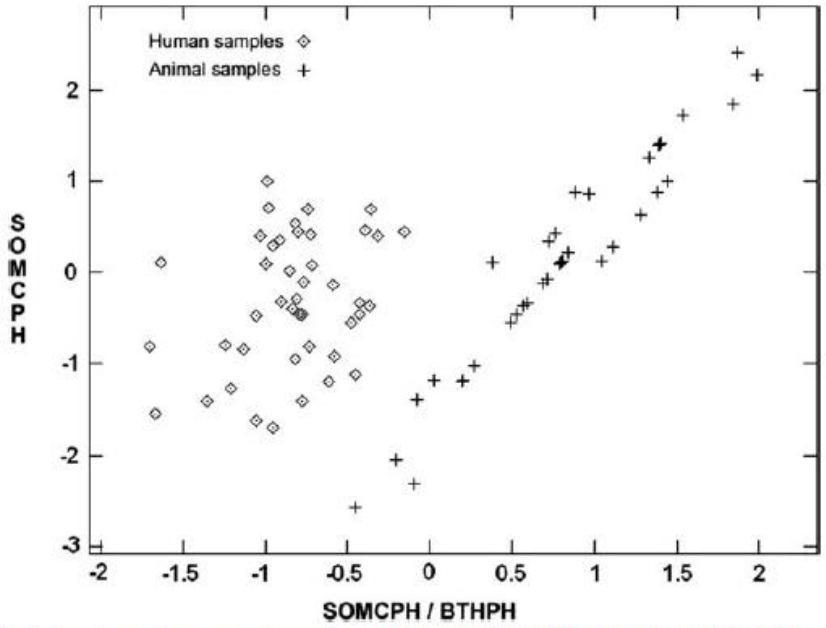
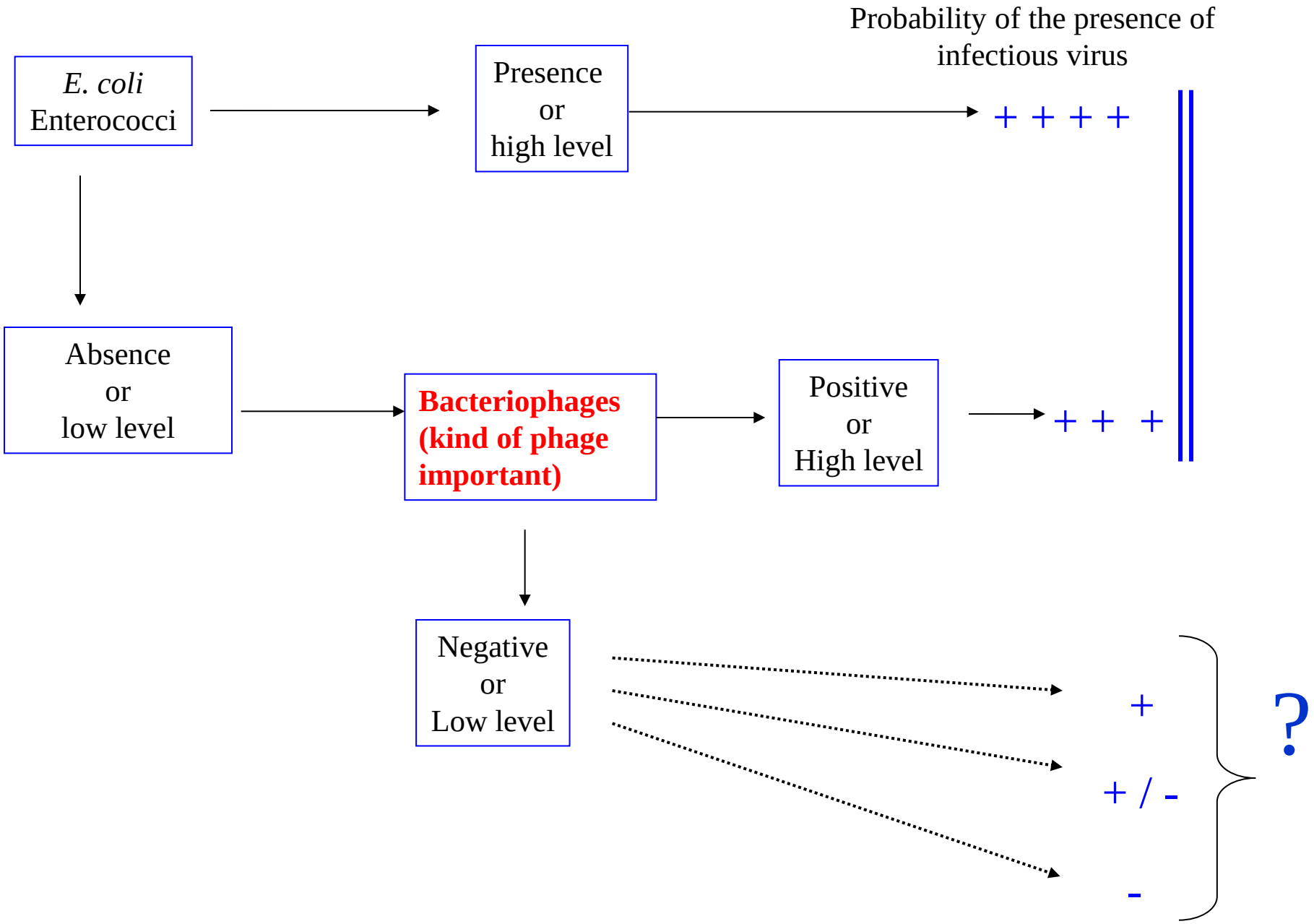


FIG. 1. Distribution of training observations according to the variables SOMCPH/BTHPH and SOMCPH. Values are standardized to zero mean and unit standard deviation.

but also F-specific RNA phage genotyping


Probability of the presence of infectious virus
E. coli Enterococci
Presence or high level
+ + + +
Absence or low level
Bacteriophages (kind of phage important)
Positive or High level
+ + +
Negative or Low level
+
+ / -
-
?

2. Choose a good model to estimate global enteric virus inactivation

Use a cultivable model

For *Norovirus*

- same family : FeCV ou MNV (Canon et al. 2006)

- same structure : MS2 phage , Enterovirus,… (Casteel et al. 2009)

- the most resistant :

The most resistant :

2.1 UV = MS2 phage (Hijnen *et al.* 2006)

2.2 heat = ΦX174 phage ≈ *Lactobacillus helveticus phages* ≈ *Lactococcus lactis phages* (Bertrand *et al.* 2012)

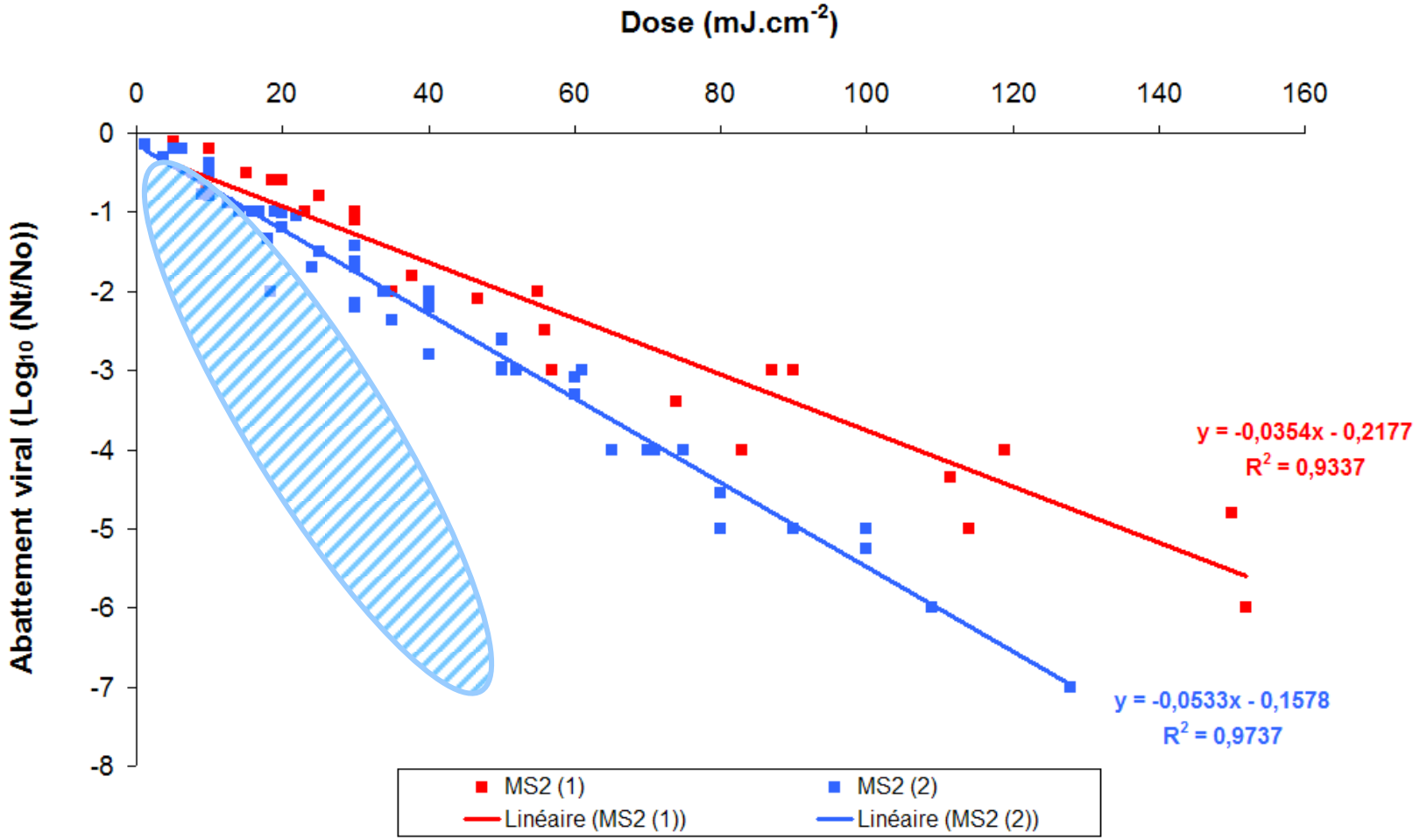
Choose the rigth model is important

2.3 Ex : MS2 vs GA vs Qβ phages = same family and structure is not sufficient

Example for UV : simple conditions

MS2 Phage : (20-30 nm ; RNA ≈3500 b)

MS2 phage : 14 publications for 64 Log reduction.



z-value between 19 and 29 mJ.cm^{-2}

Conclusions for UV in simple media

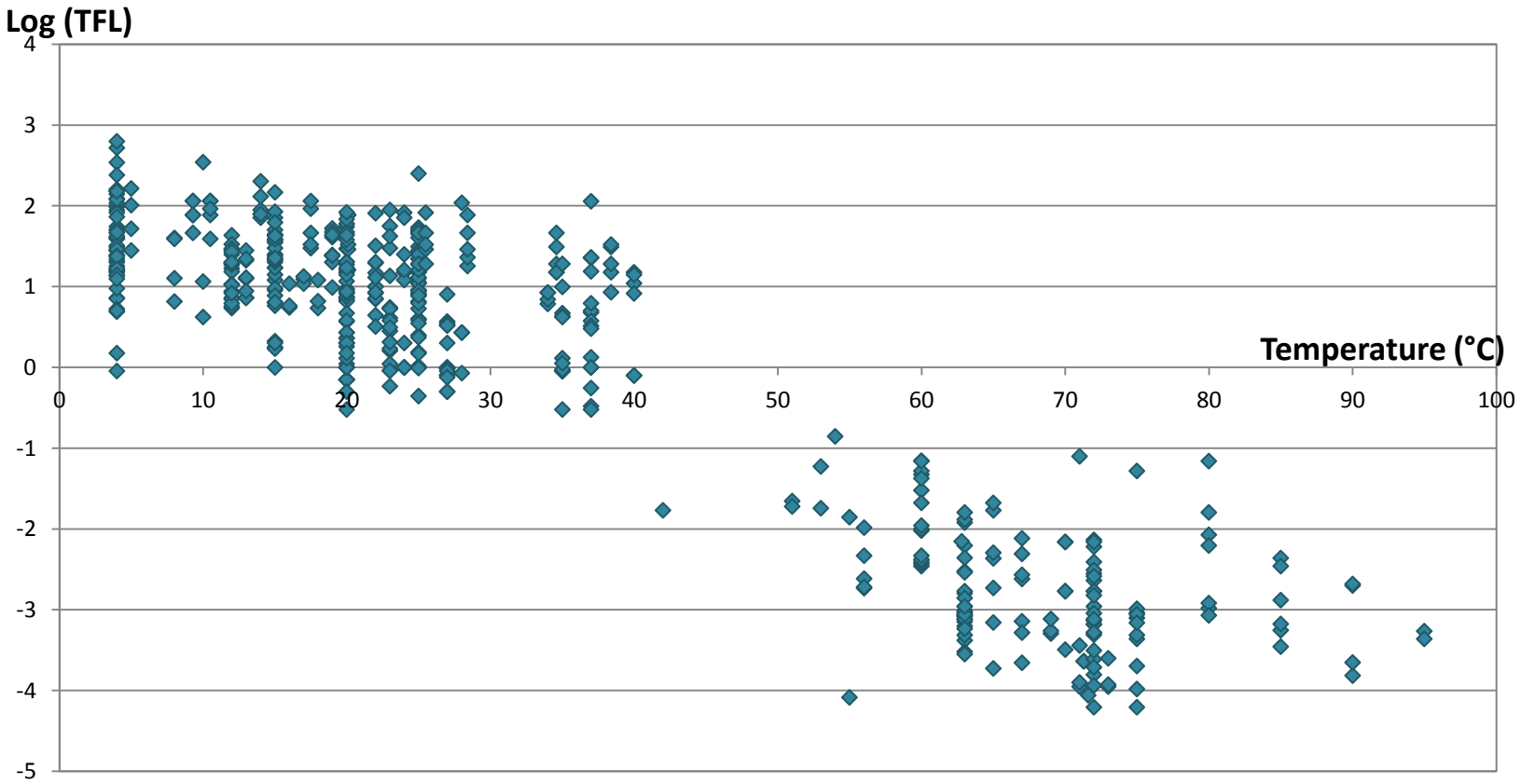
(Hijnen *et al.* 2006 : review) + J. Simonet (thesis 2007) + COST 929

UV sensitivity :

ϕX174 phage **>**enteroviruses ≈ hepatitis A virus ≈ animal caliciviruses > rotaviruses > MS2 phage > adenoviruses (41)

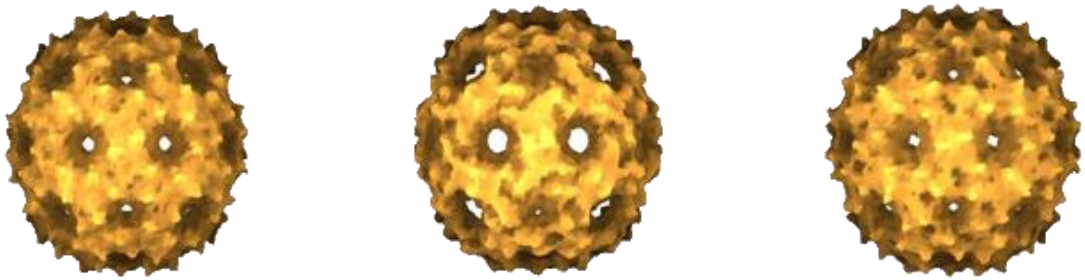
Resistance to temperature

Inactivation/dégradation du genome= 652 TFL de73 publications



heat = ΦX174 phage ≈ *Lactobacillus helveticus phages* ≈ *Lactococcus lactis phages* (Bertrand *et al.* 2012)

Description of RNA F-specific bacteriophages



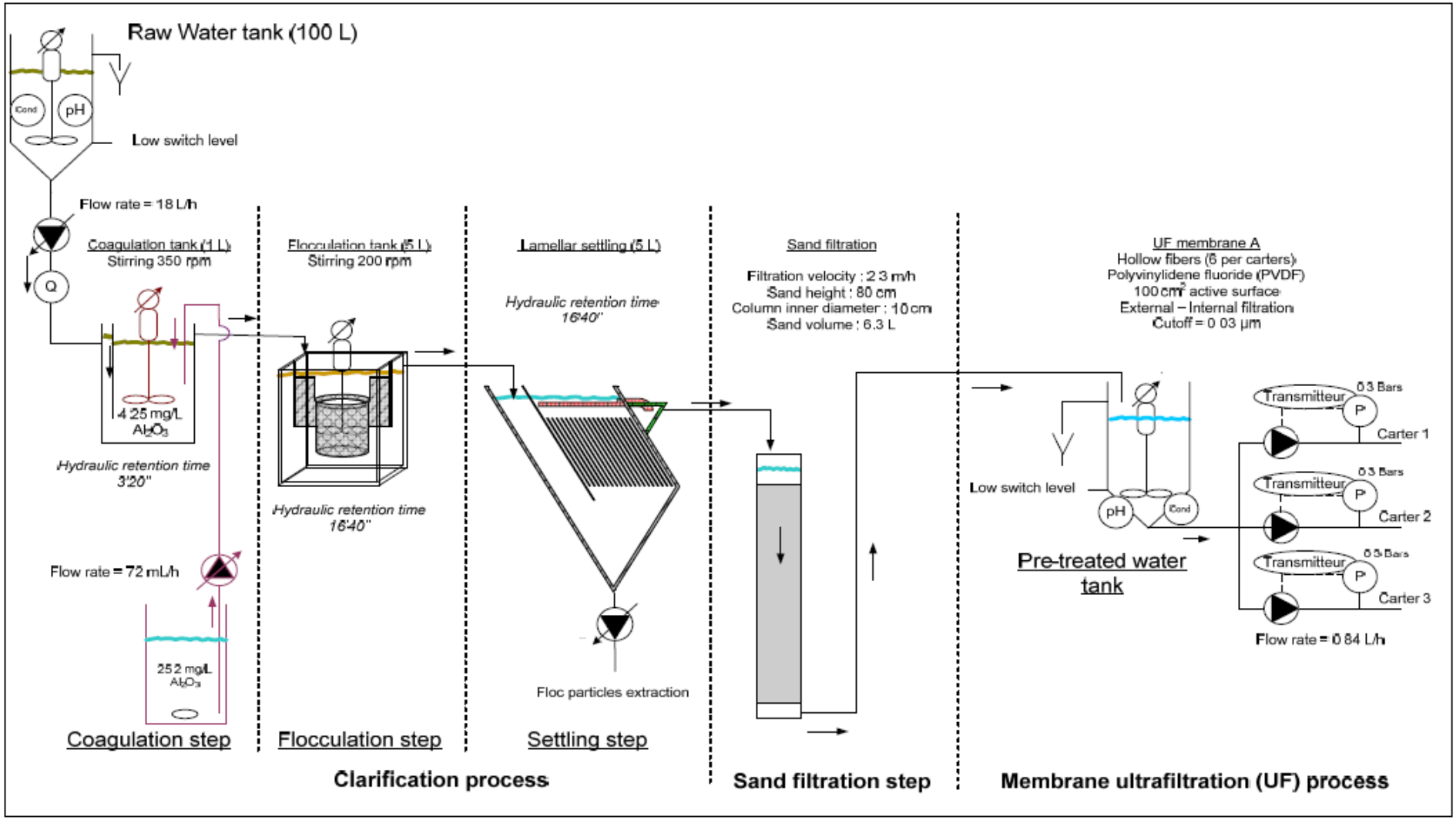
	MS2	Qβ	GA
Diameter	20-30 nm	20-30 nm	20-30 nm
Genome (RNAss)	3569 nts	4217 nts	3577 nts
IEP	3.9	1.9 to 5.3	2.3
Amino acid sequences of capsid protein	• 20 % similarity between MS2 and Qβ • 62 % similarity between MS2 and GA		

Similar fundamental structures

Amino acid exposed at the capsid surface are different → Different interfacial properties ?

Behavior of three bacteriophages during (physical) drinking water treatment

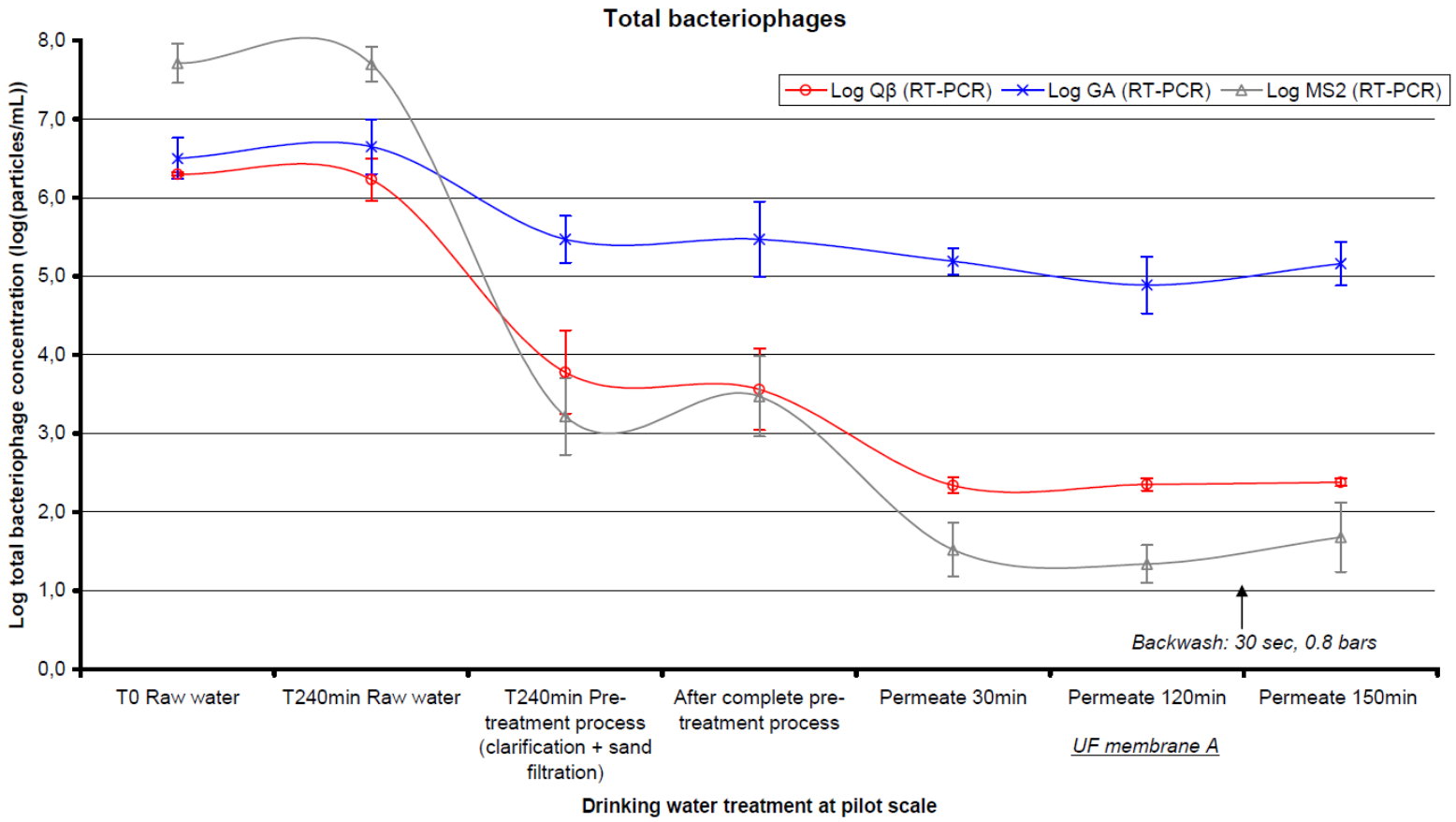
Schematic drinking water treatment at pilot scale



Q: Flow meter
Cond.: Conductivity

(Boudaud *et al.* 2012)

Behavior of three bacteriophages during drinking water treatment



Separate experiments for infectivity : log reduction

Treatments	MS2	Qβ	GA
Coagulation/floculation + sand filtration	4.5	3	1
Membrane ultrafiltration	6	4	1.5

The elimination efficiency in these treatments follows the phage hydrophobicity sequence

(Boudaud *et al.* 2012)

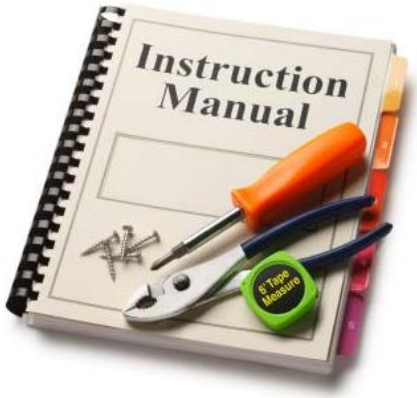
Choose the right tool to do the right thing….

1) Estimation of fecal/viral pollution in water

2) Estimation of virus removal by treatment

3) Tracking the origin of fecal pollution

http://www.copywriters-toolkit.com/images/perfect_tool.gif



http://therecordingrevolution.com/wp-content/uploads/2011/06/instruction-manual.jpg

Universal indicator does not exist :

Viral pollution

1) Define objectives and situations
2) Use the tool box

Fecal phages

Old fecal pollution
Viral behavior
Treatment efficiency
Discrimination of fecal pollution ?

Detection of viral pathogens by molecular tools

Epidemiologic studies
Diversity of viral pollution
Absence of virus

Bacterial fecal indicators

Recent fecal pollution

Cost ?

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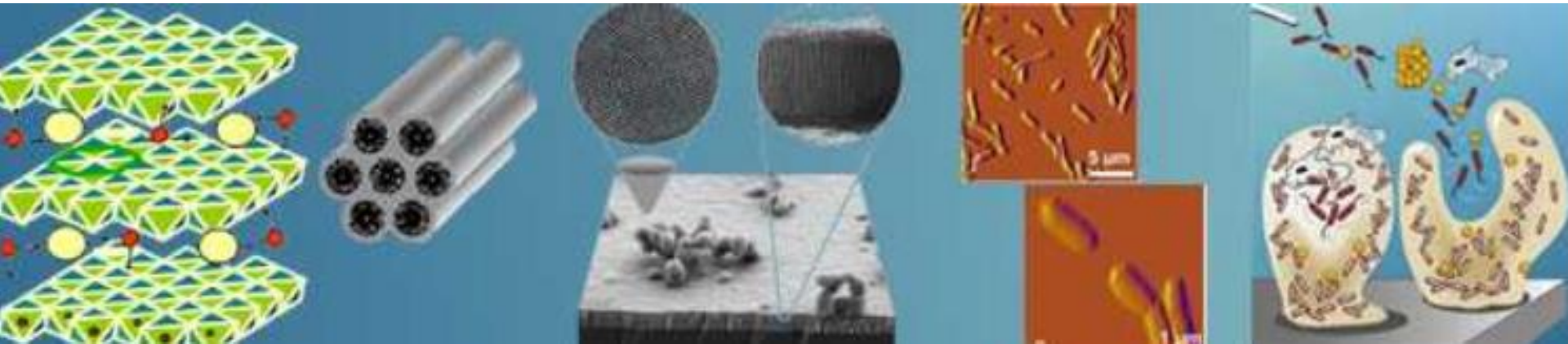
Thank you for your attention

LCPME



AFSSA (ANSES) 2007

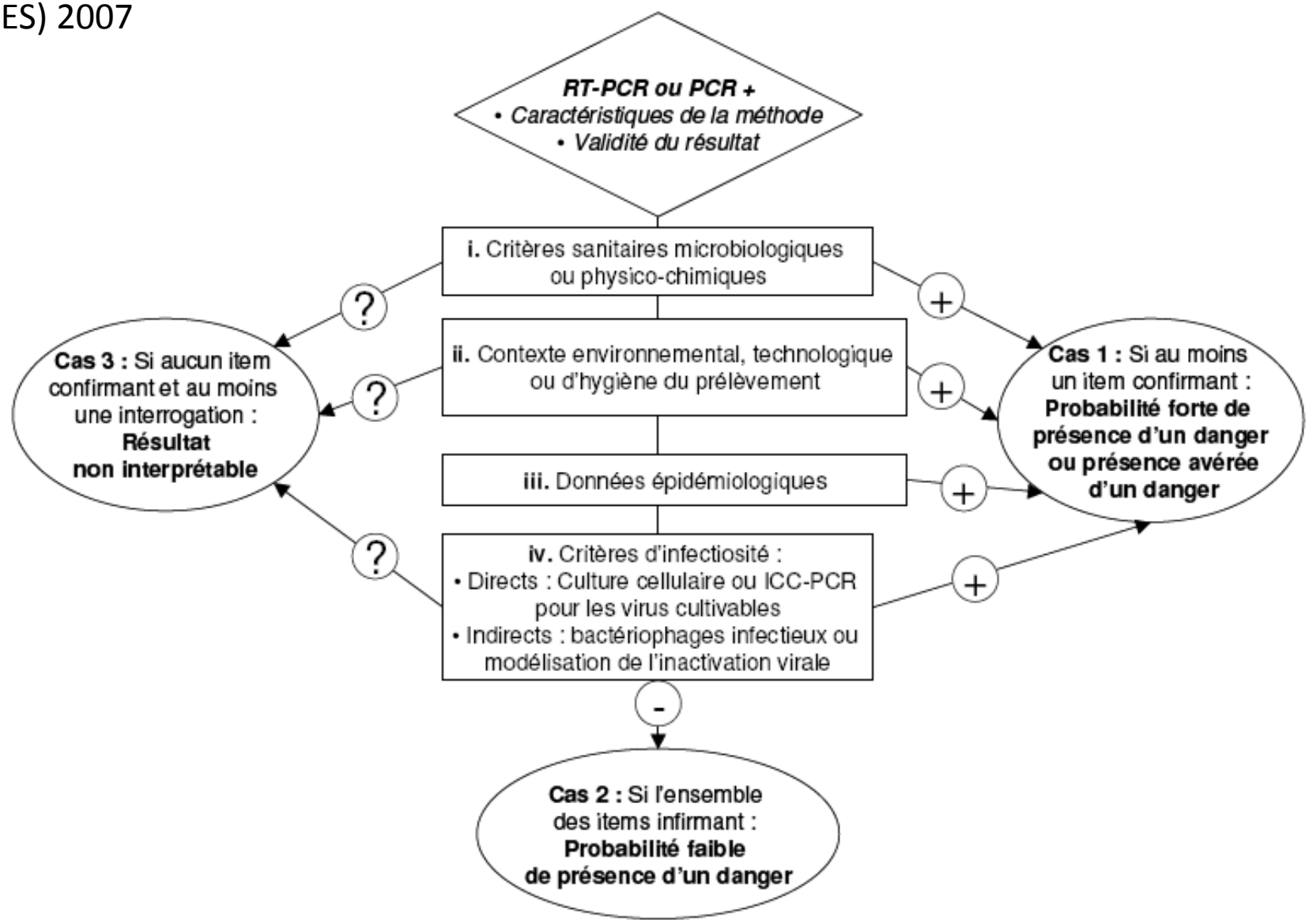


Figure 2 : Logigramme d'interprétation d'un résultat positif par les techniques de biologie moléculaire