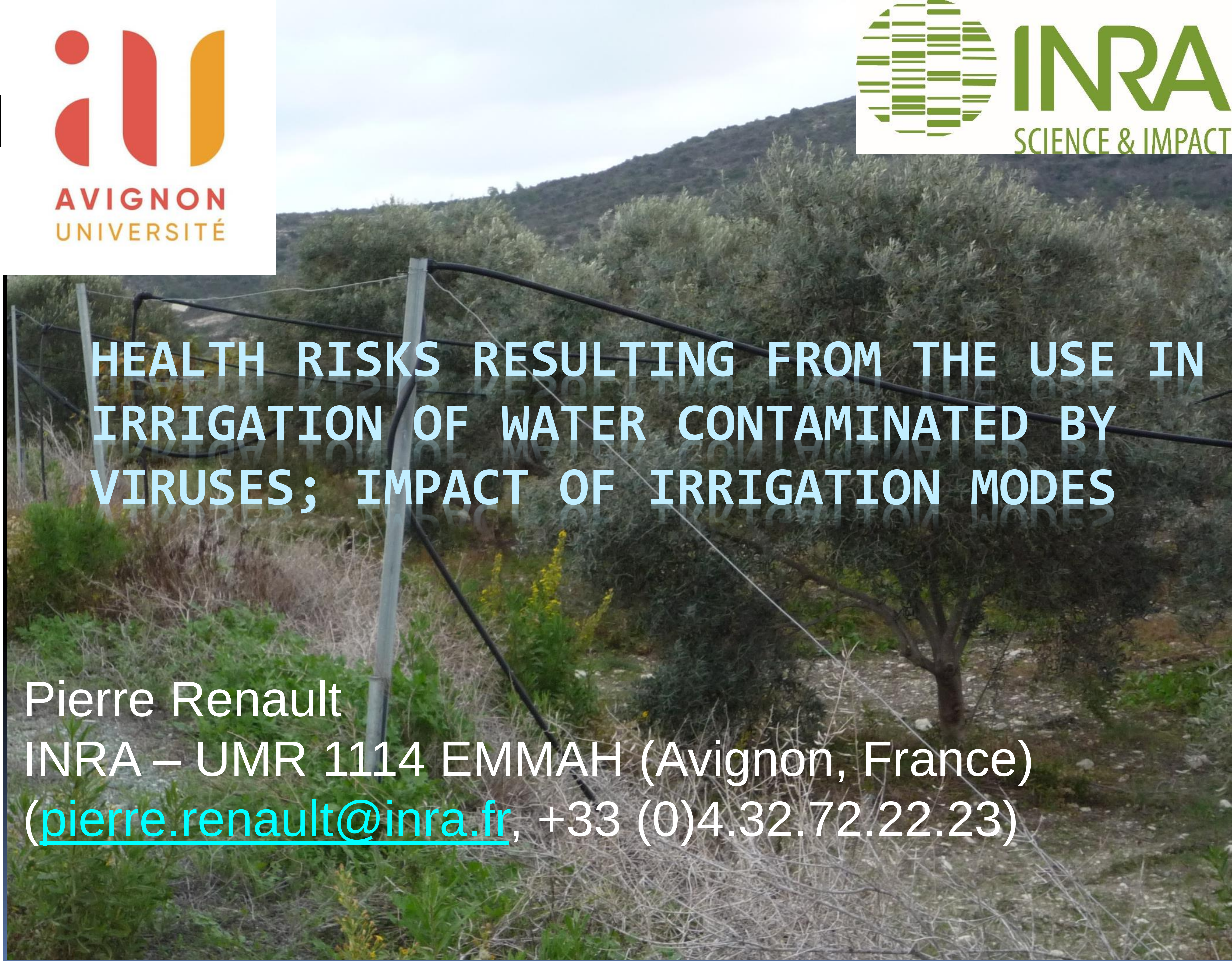
Health risks resulting from the use in irrigation of water contaminated by viruses; impact of irrigation modes

Pierre Renault



HEALTH RISKS RESULTING FROM THE USE IN IRRIGATION OF WATER CONTAMINATED BY VIRUSES; IMPACT OF IRRIGATION MODES

Pierre Renault
INRA – UMR 1114 EMMAH (Avignon, France)
(pierre.renault@inra.fr, +33 (0)4.32.72.22.23)

The importance of foodborne transmission of viral Acute Gastro-Enteritis (AGE) diseases

Acute gastroenteritis: aetiology, frequency, cost

AGEs affect one third of the French population each year

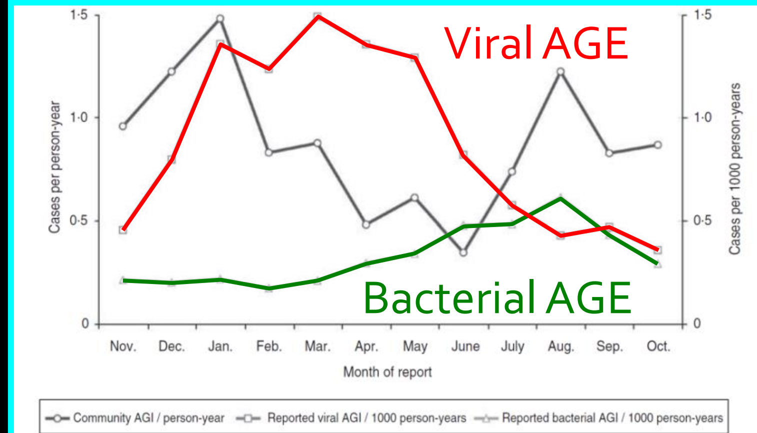
Chikhi-Brachet et al. (2002)

Various AGE aetiologies:

- Viruses; - - - - - - - - - - -> 59%
- Bacteria; - - - - - - - - - - -> 39%
- Unicellular parasites; - -> 2%
- Undefined

in the USA

Esseili et al. (2012)

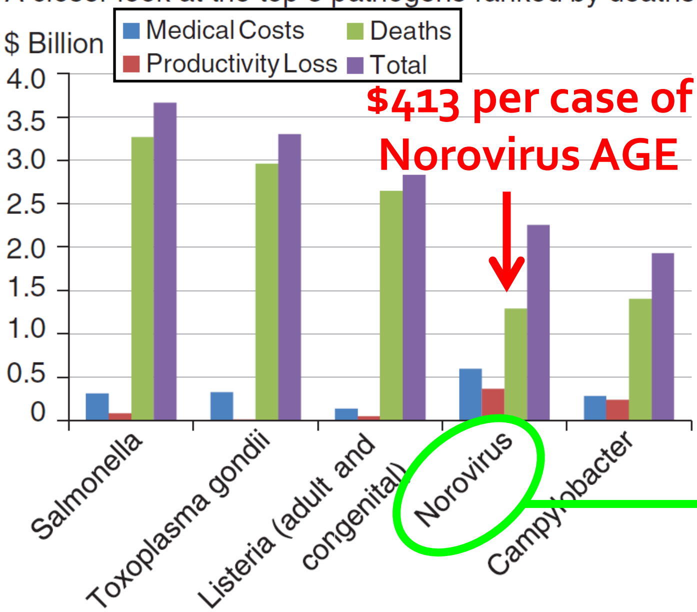


Incidence of self-reported acute gastrointestinal infection (AGI) in the population; calculated for bacterial and viral intestinal infections reported to surveillance, by month, Poland, November 2008 to October 2009.

In Poland *Baumann et al. (2012)*

Total mean cost of foodborne illnesses in the United States (2013 $)

A closer look at the top 5 pathogens ranked by deaths



The cost of AGE and other foodborne illnesses depends on the aetiology, the subject age and the geographical area

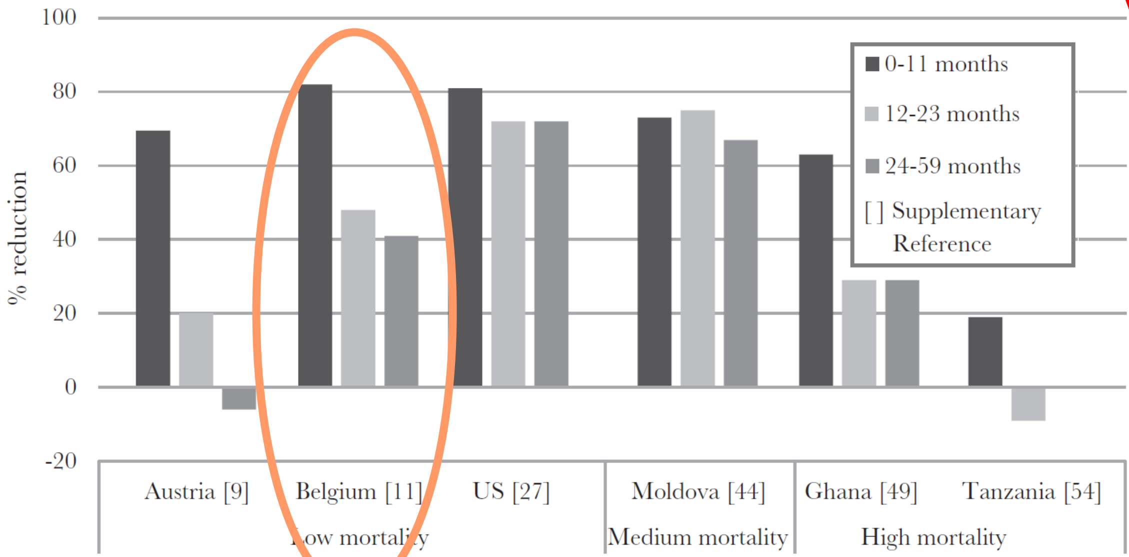
Hoffmann et al. (2015)

Norovirus AGEs represent 58.2% of foodborne illness cases in the USA

Viral AGEs: their importance and specificities

Various viruses: rotavirus, **norovirus**, sapovirus, enterovirus (echovirus, coxakievirus), adenovirus …

Before vaccination, the most important for young children



Reduction in rotavirus hospitalizations by age group in the first full year after vaccine introduction.

Burnett et al. (2017)

Possible clinical symptoms:

- diarrhoea,
- vomiting,
- nausea,
- abdominal pain,
- (fever) …

Causative agents of most of viral AGE (40-80%) … whatever the subject ages.

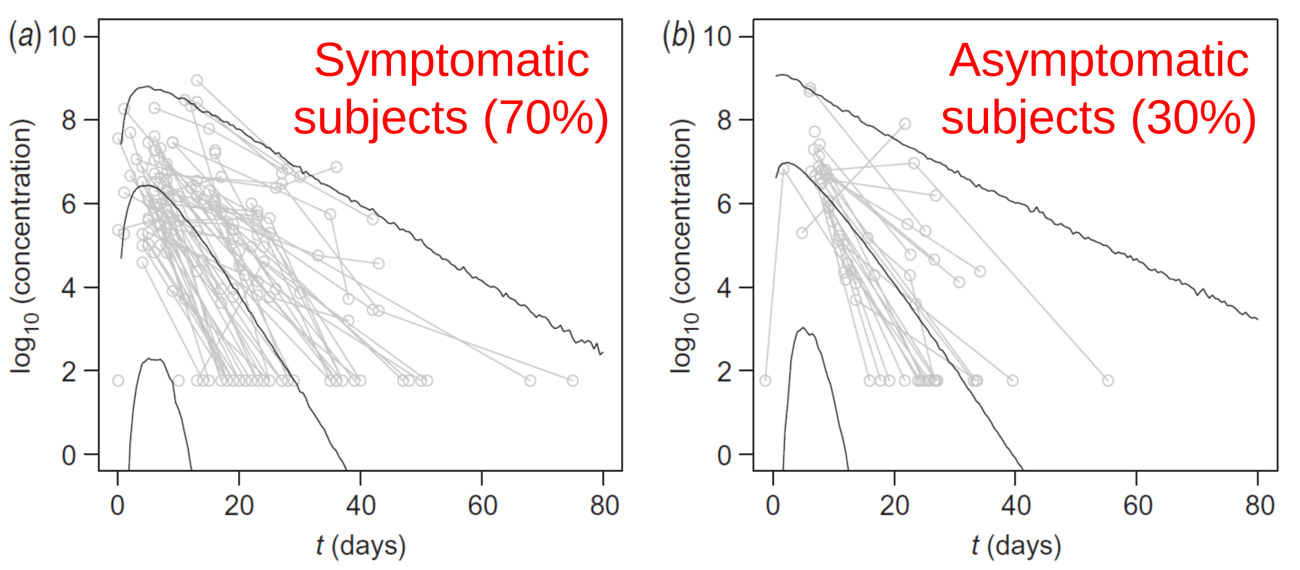
Noroviruses: **200 000 deaths per year worldwide** due to dehydration and malnutrition complications, especially for the young, elderly and immunocompromised

Mallory et al. (2019)

Noroviruses: shedding and infectious dose

Norovirus shedding with faecal excretion:

- Up to 10^{+12} viruses per gram of faeces;
- Shedding starts before the onset of clinical symptoms, and stops well after the end of these symptoms;



Time-course of virus shedding. Median and 95% interval of the predicted virus concentration ($\log_{10}$ numbers of viruses/g stool) are shown.

Teunis et al. (2015)

Low infectious dose: ~18 to 1000 viral particles

Teunis et al. (2008)

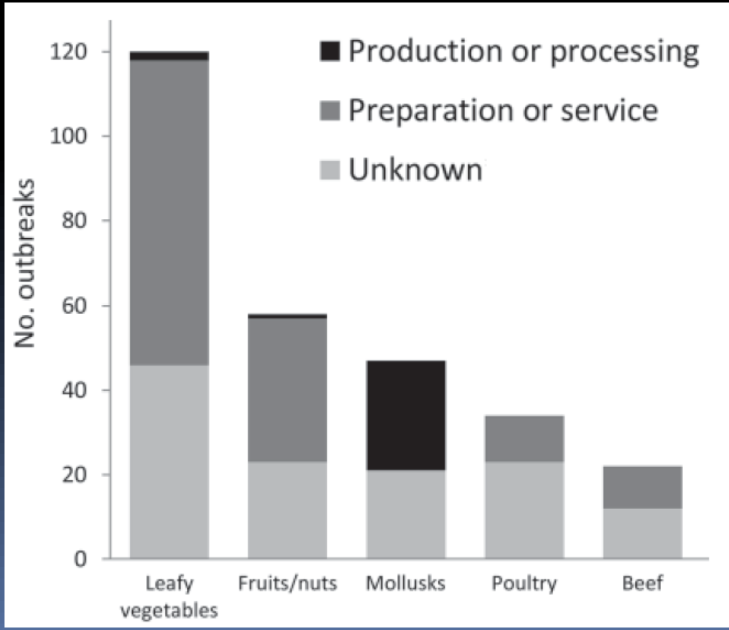
Direct / indirect transmission of NV in the USA

Primary mode of transmission of NV infections in the USA:

Primary mode of transmission among outbreaks reported through the National Outbreak Reporting System (NORS), 2009-2012

Primary Mode of Transmission	Confirmed Norovirus	Suspected Norovirus	Confirmed Non-Viral	Unknown
Animal Contact	0 (0%)	0 (0%)	105 (7%)	4 (0.1%)
Environmental	12 (0.4%)	3 (0.2%)	7 (0.4%)	5 (0.1%)
Food	644 (22%)	355 (27%)	926 (59%)	1006 (27%)
Indeterminate/Other	220 (7%)	86 (7%)	262 (17%)	426 (12%)
Person-to-person	2063 (70%)	877 (66%)	273 (17%)	2253 (61%)

Lively et al. (2018)



Relative contributions of various foods to NV foodborne outbreaks in the USA.

Leafy vegetables > Fruits/nuts > Mollusks

Hall et al. (2012)

Prevalence of NV contamination in France

Frozen marketed mussels supplied by French importers or recovered from different retail outlets.
From Chile, Pacific Ocean, Spain, New Zealand, North West Atlantic Ocean, Vietnam, Ireland.

Fresh lettuces provided by industrial producers.
From Spain, Italy, Belgium, France and Tunisia.

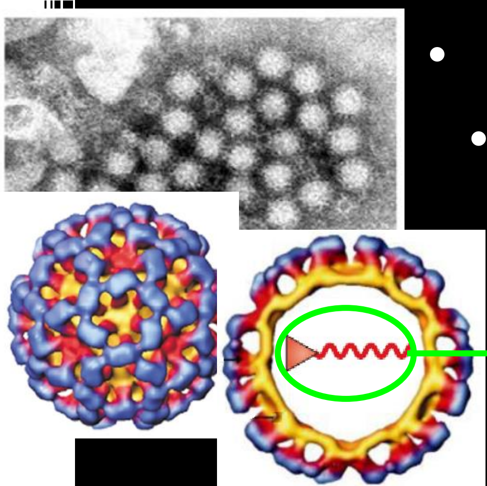
Frozen berries provided by industrial producers.
From Serbia, Chile, Bulgaria, Poland, Spain, Morocco, Turkey and France;

Assessment of the occurrence of NoVs on 493 food matrices using RT-qPCR

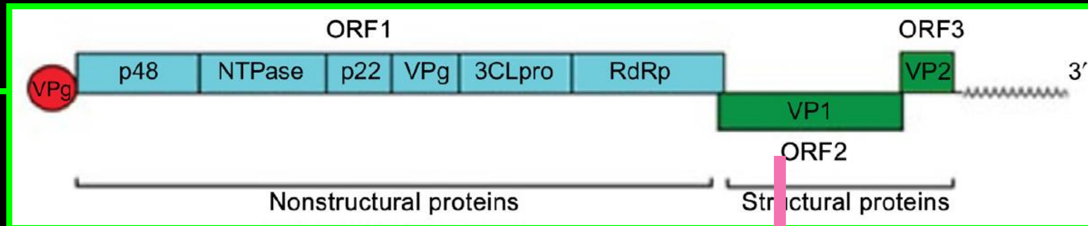
Category of food matrix	Nature	No. of samples tested	% positive for GI NoV	% positive for GII NoV
Bivalve molluscs	Mussel	83	8.4	14.4
Lettuces	*Chicory*	107	11.2	0.9
	Lettuce	77	13.0	0
	Mash	26	11.5	0
	All salads	210	11.9	0.5
Red fruits	*Raspberry*	162	16.8	0
	Strawberry	32	9.4	3.1
	Blackberry	2	50.0	0
	Mix	4	0	0
	All red fruits	200	15.5	0.5
All matrices		493	13.0	2.9

Loutreul et al. (2014)

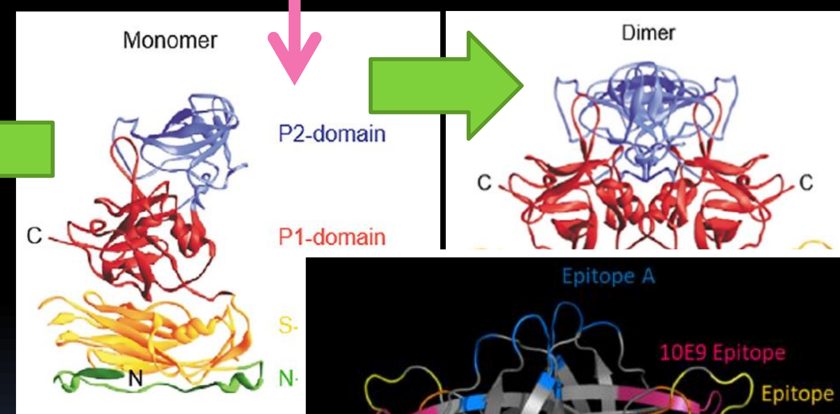
Norovirus: structural/functional properties



- A non-enveloped icosahedral virus, ~40nm in size.
  - isoelectric point (iep) ≈ 4-4.5; surface wettability?
    - a single-stranded positive-sense RNA genome (7.5 kb)



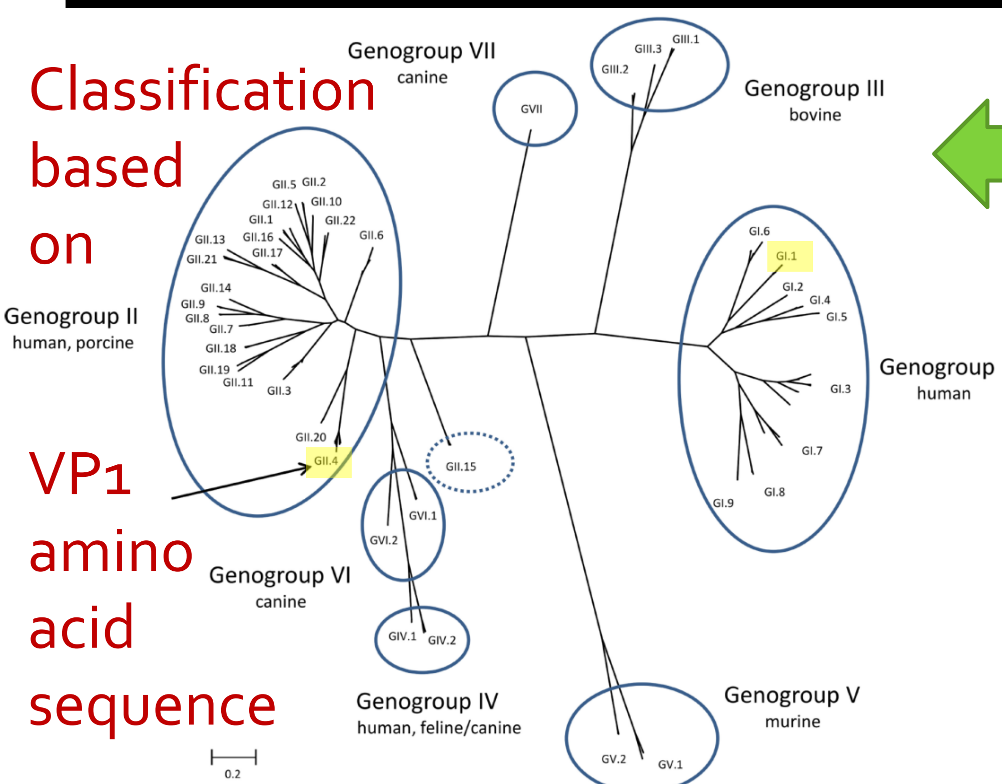
Tarek et al. (2020)



Hutson et al. (2004)

Mallory et al. (2019)

Classification based on VP1 amino acid sequence



Vinjé (2016)

Epitopes are involved in specific adhesions (HBGA affinity ...)

Norovirus: mutations affecting HBGA affinity

Mutations over a few weeks:

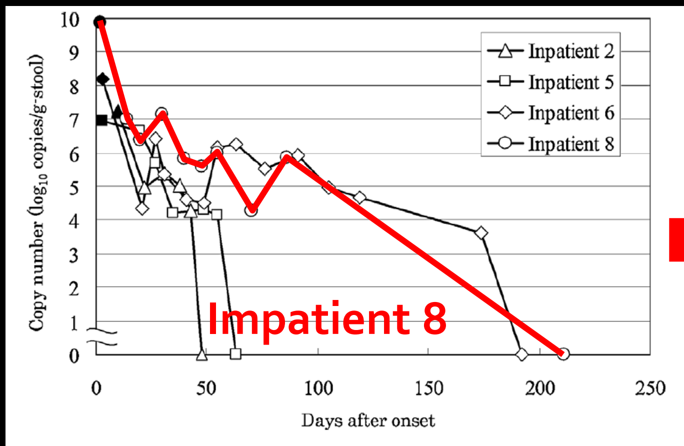


Fig. 3. Viral shedding in four inpatients (infants) in outbreak episode 1. The quantity of NoV genome segments was measured by real-time RT-PCR. Open and closed symbols indicated asymptomatic and symptomatic, respectively.

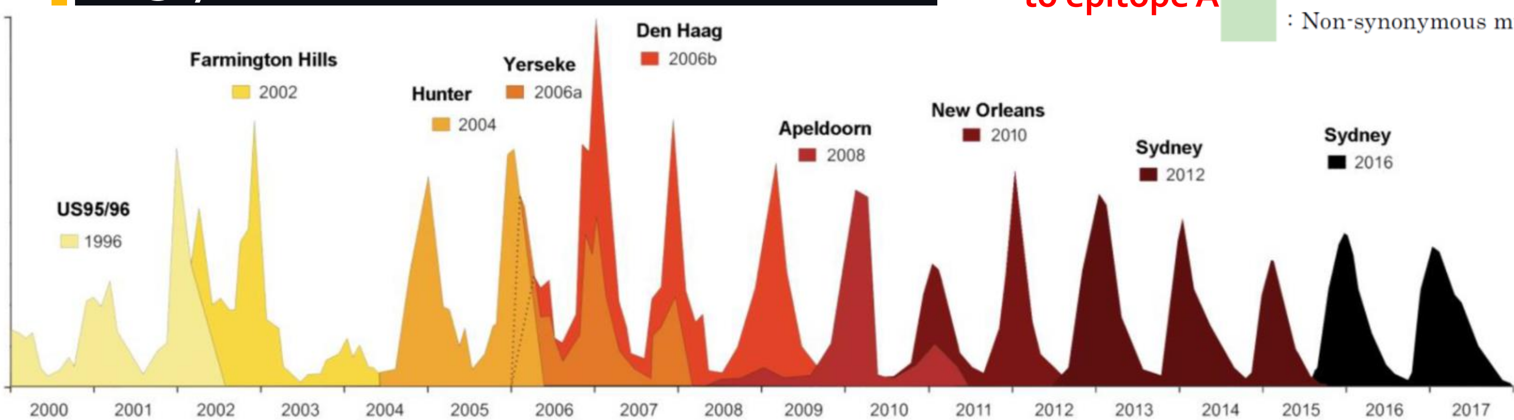
Miyoshi et al. (2015)

Impatient 8

domain	nucleotide position	1d	29d	54d	amino acid position	1d	29d	54d
S	412	G	G or A	G	138	Val	Val or Ile	Val
	429	T	T or C	T				
P	880	C	T or C	C or T	294	Pro	Ser or Pro	Pro or Ser
	883	A	G	A or G	295	Ser	Gly	Asn or Gly
	884	G	G	A G				
	889	C	A or C	C	297	Arg	Ser or Arg	His
	890	G	G	A				
	1,008	A	G or A	A				
	1,102	G	G	A or G	368	Ala	Ala or Asp	Thr or Ala
	1,103	C	C or A	C				
	1,114	G	G or A	G or A	372	Asp	Asp or Asn	Asp G or
	1,123	T	T or C	T	375	Phe	Phe or Leu	Phe
	1,178	G	G	A or G	393	Ser	Ser	Asn or Ser
	1,180	A	G -	G -				
	1,181	G	G or -	G or -	394	Ser	Gly or -	Gly or -
	1,182	T	T -	T -				
	1,231	A	A	G or A	411	Arg	Lys or Arg	Gly or Lys
	1,232	G	A or G	G A				

Contribute to epitope A
: Synonymous mutation
: Non-synonymous mutation
: Mutant amino acid
- : Deletion

New pandemic strains every 2-3 years:



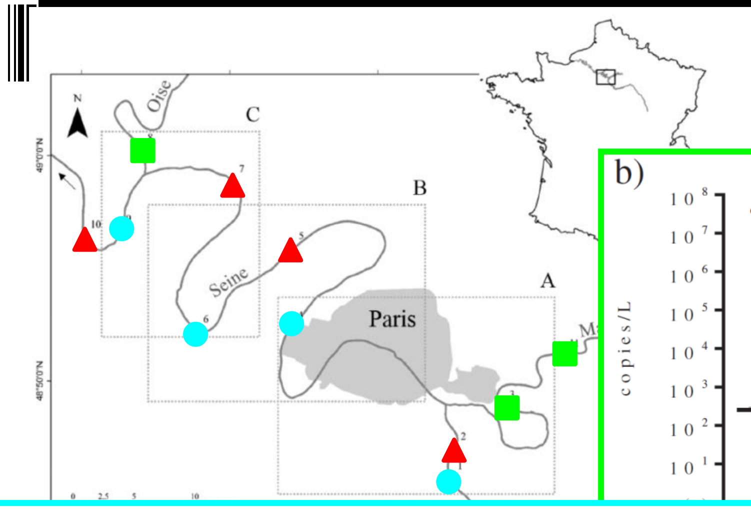
De Rougemont (2018)

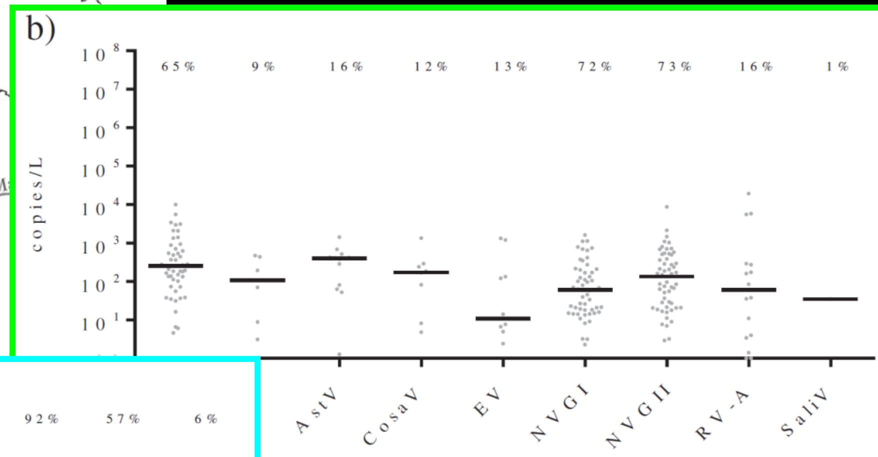
Environmental fate of viruses after wastewater discharge

Contamination of rivers by wastewater discharge

Case of the Seine River near Paris

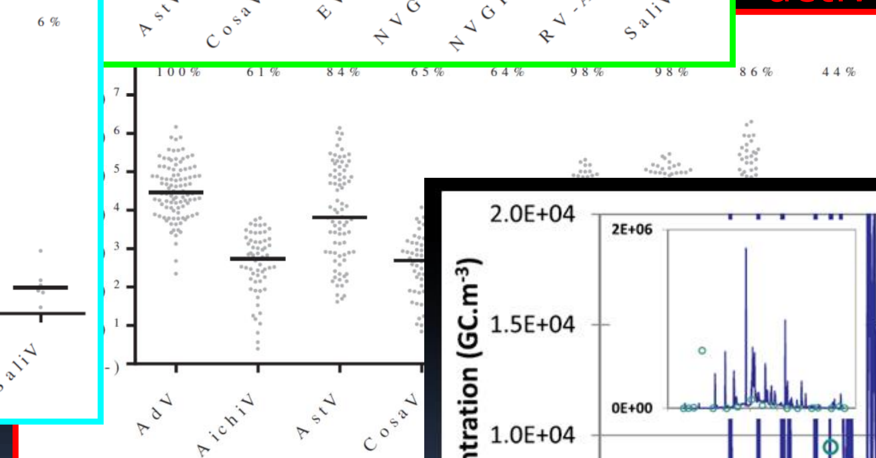
Prevost et al. (2015)

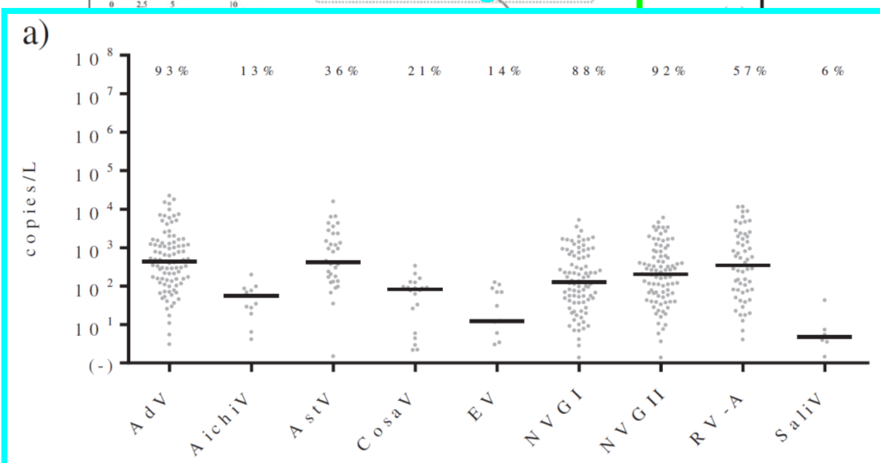




Contamination from the tributaries

Wastewater discharge after activated sludge treatment

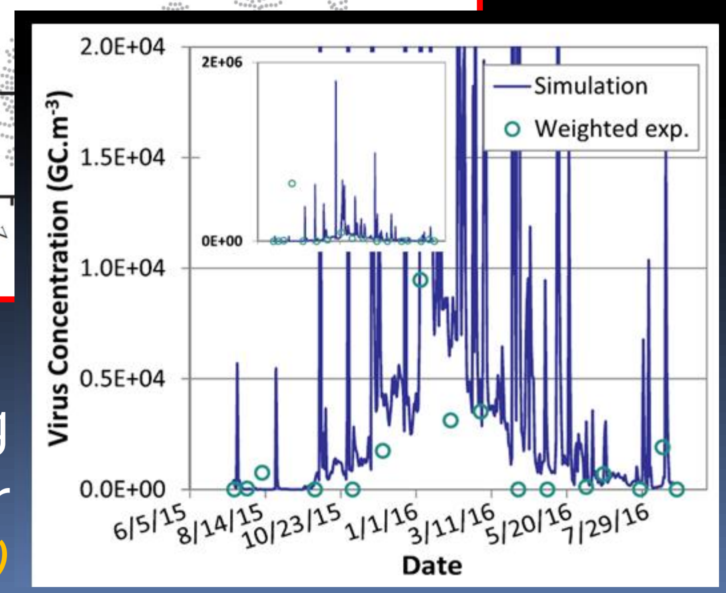




The resulting contamination of the Seine River

Medical prescriptions enable simulating the contamination of the Artière River

Tesson et al. (2019)

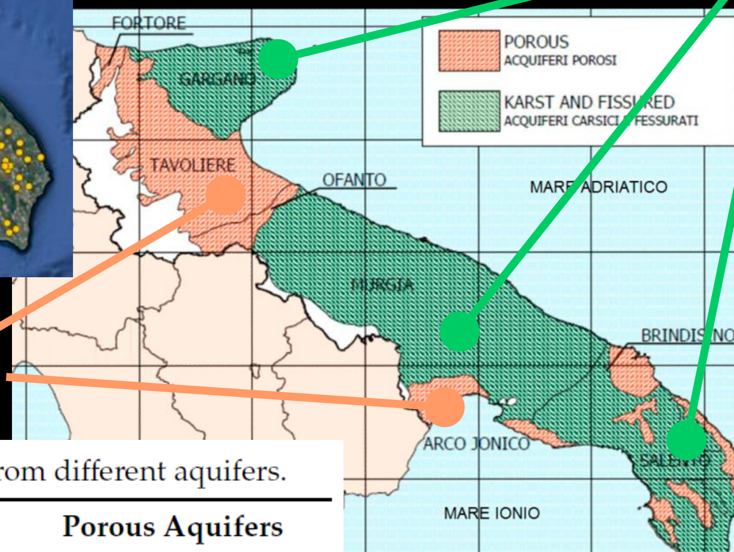


Underground water contaminations

Case of aquifers in Apulia (southern Italy)

De Giglio et al. (2017)

Sampling in 3 karst-fissured aquifers



Sampling in 2 porous aquifers

Isolation frequency of enteric viruses in 364 water samples from different aquifers.

Virus	Karst-Fissured Aquifers			Porous Aquifers	
	GA (28) No. (%)	SA (82) No. (%)	MU (126) No. (%)	JON (40) No. (%)	TAV (88) No. (%)
Enterovirus	0 (-)	2 (2.4)	0 (-)	1 (2.5)	1 (1.1)
Norovirus	1 (3.6)	3 (3.7)	10 (7.9)	3 (7.5)	1 (1.1)
Rotavirus	1 (3.6)	2 (2.4)	0 (-)	0 (-)	2 (2.3)
Hepatitis A virus	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
TOTAL	2 (7.1)	7 (8.5)	10 (7.9)	4 (10.0)	4 (4.5)

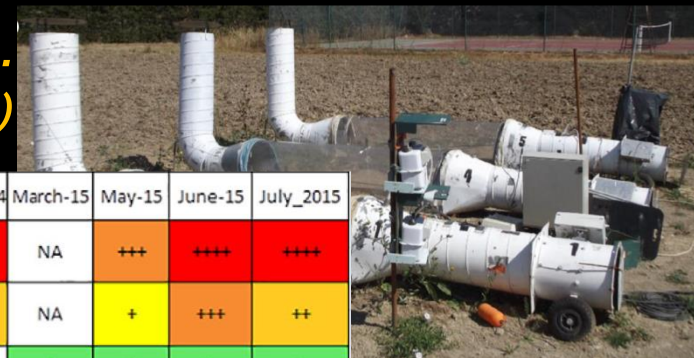
Wells with potential infectious risk owing to the presence of viruses.

Aquifers	Presence of Virus/Suitable Wells * No. (%)
Karst-Fissured	17/98 (17.3)
Porous	6/49 (12.2)
Total	23/147 (15.6)

Environmental virus fate during/after irrigation

Virus as aerosol

Girardin et al. (2016)



NoV GI	3 July-2014	March-15	May-15	June-15	July_2015
Raw water (WWTP input)	++++	NA	+++	++++	++++
Treated water (output WWTP)	++	NA	+	+++	++
WWTP air from filter	NA	-	-	-	-
WWTP air from impinger	NA	-	-	-	-
Pond water	-	NA	NA	NA	NA
Pond air from filter	+	+	NA	NA	-
Pond air from impinger	-	-	NA	-	NA
Air above beetroot field (filter)	-	NA	-	+	-
Air above beetroot field (impinger)	-	NA	-	+	-

Courault et al. (2017)

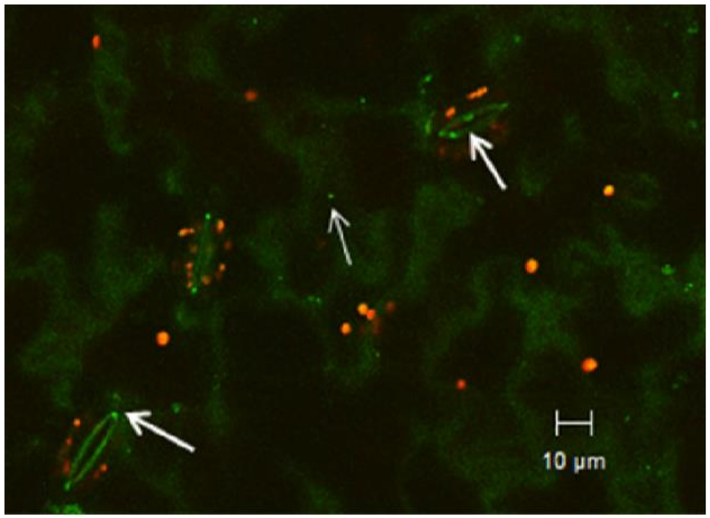
Contamination of external surfaces of crops

Virus flow in the soil with water infiltration

Virus internalization in plants via their roots

Attachment on external surface or plant materials

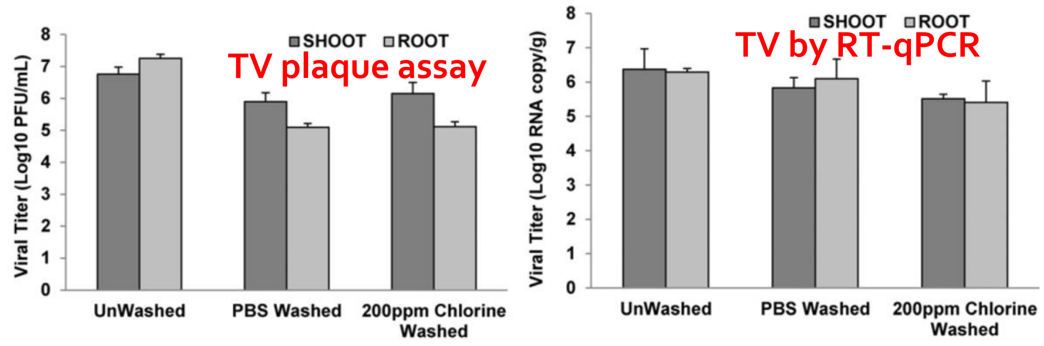
Murine NV (MNV-1) observed on lettuce surface, inside open cuts, and within stomata



Confocal microscopy images of MNV attached to Romaine lettuce leaves. The arrows indicate MNV. Green indicates plant cell walls, and red indicates autofluorescence from plant chlorophyll.

Wei et al. (2010)

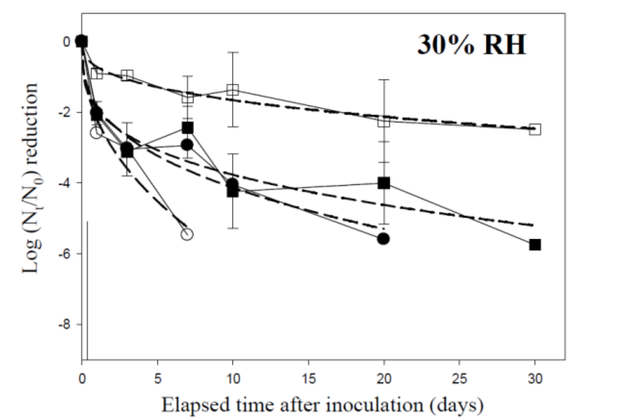
Tulane virus (TV) attachment/washing vary with the virus, the plant organ and the liquid phase



Effect of washing on the removal of TV from Romaine lettuce

DiCaprio et al. (2015)

MNV inactivation on inert surface varies with the surface type, and the air temperature and relative moisture



Inactivation curves of MNVon the wooden surface. 15°C (■), 25°C (□), 32°C (●), and 40°C (○) conditions.

Kim et al. (2012)

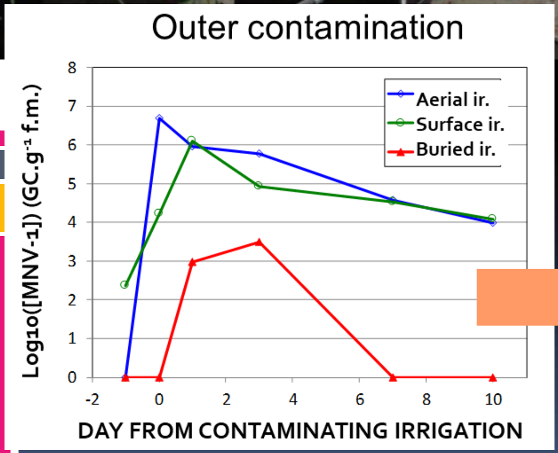
Irrigation/handling impact on outer contamination

Initial contamination + Decay (inactivation …) + Washing (rain …)

Green onion outer contamination varies with irrigation mode (buried, surface or aerial drip irrigation), as well as washing

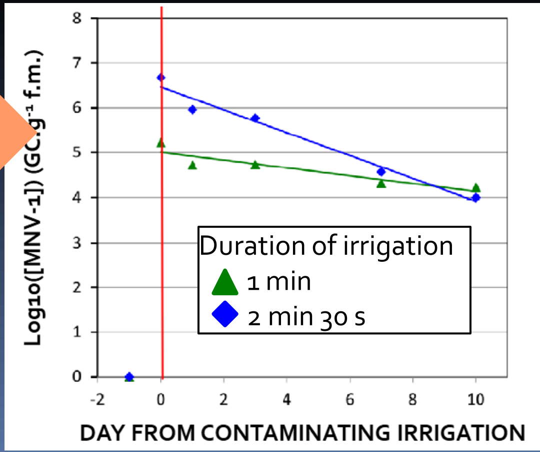
But handling may explain most of vegetable and fruit contamination.

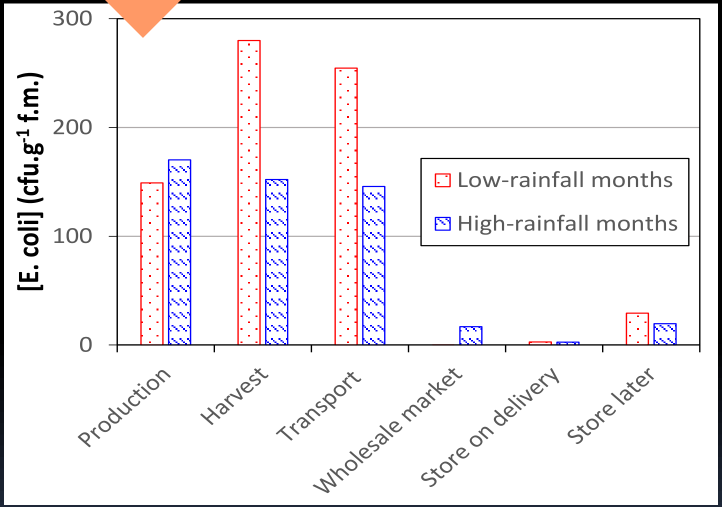
Pérez-Rodríguez (2019)



Renault et al. (2017)

Washing proportional to the irrigation amount



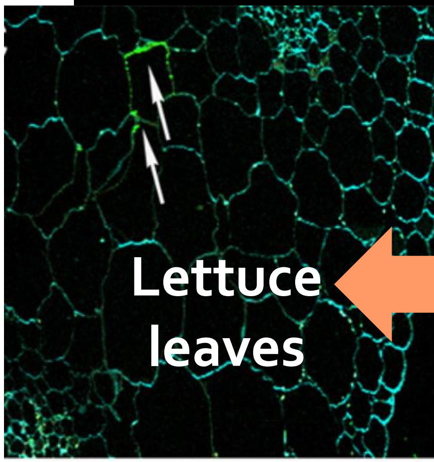


Chards produced in *La Ramada* near *Bogotá* (Colombia) are contaminated by irrigation, “washing” and handling.

Jordan Lozano (2020)

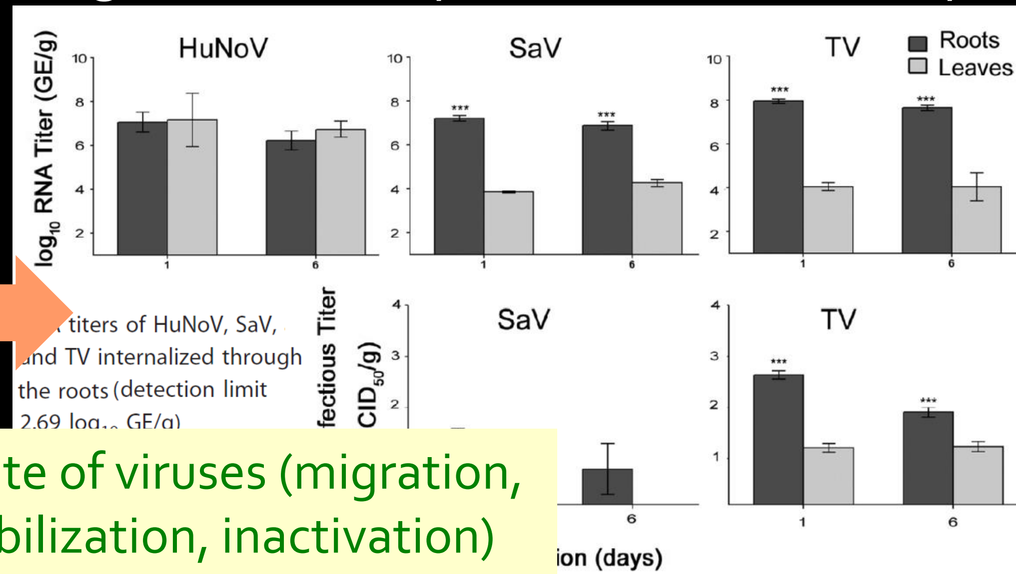
Virus internalization in vegetables and fruits

Internalization *via* the roots followed by migration without inactivation to parts eaten raw observed on lettuce, green onion, spinach and strawberry.



Lettuce leaves

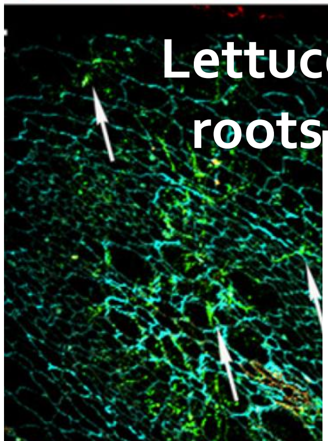
On whole lettuces after washing their root from soil and immersing them in a viral solution



titers of HuNoV, SaV, and TV internalized through the roots (detection limit 2.69 log$_{10}$ GE/g)

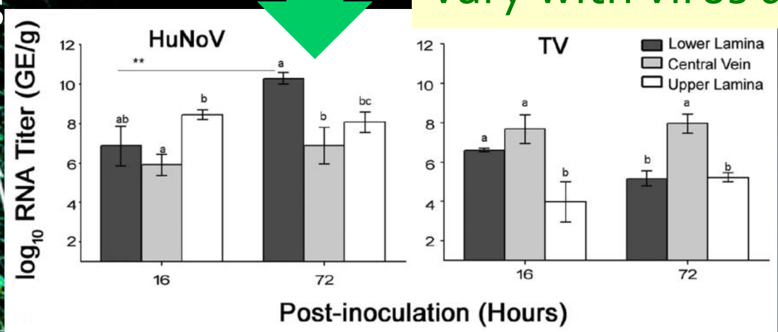
On cut leaves after immersing th[...] in a viral solut[...]

The fate of viruses (migration, immobilization, inactivation) vary with virus and strain



Lettuce roots

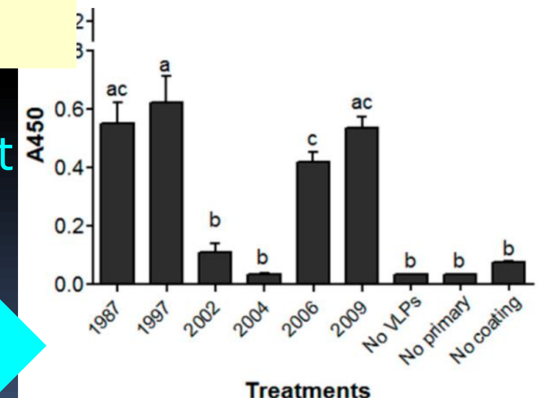
Esseili et al. (2018)



RNA titers (in genomic equivalents per gram) of HuNoV and TV internalized through the petiole of lettuce into their central veins and leaf lamina (lower and upper portions). (detection limit was 2.69 log$_{10}$ GE/g)

Attachment On cell wall materials

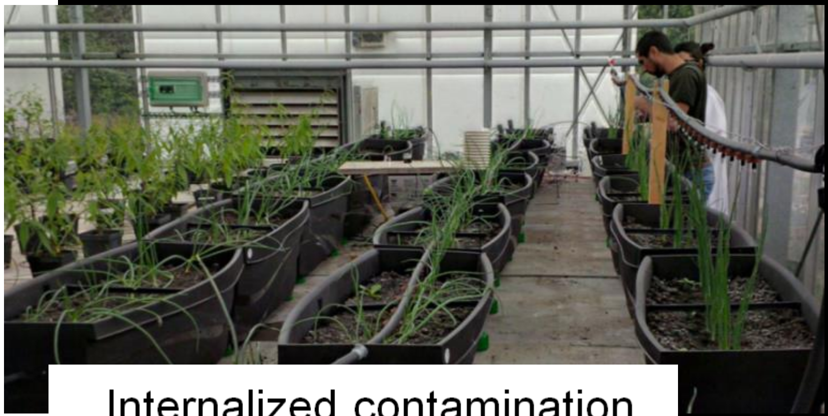
Esseili et al. (2019)

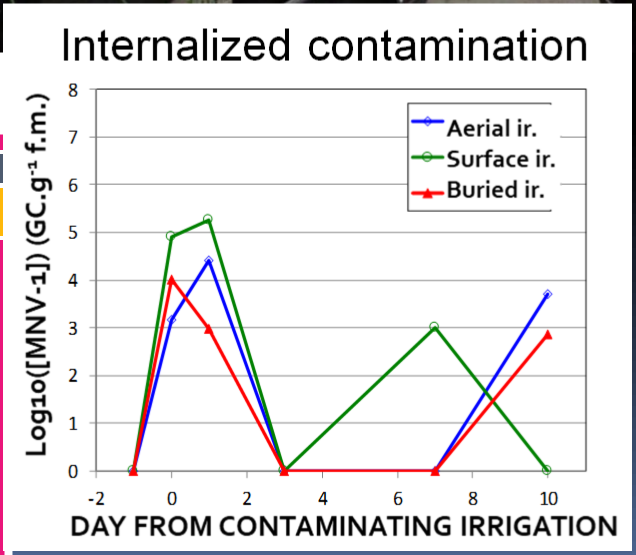


Enzyme linked immunosorbent assay (ELISA) screening of historical HuNoV GII.4 virus-like particles (VLPs) for their binding to lettuce leaves cell wall material (CWM).

Irrigation mode and soil impact on internalization

Internalization of MNV-1 doesn't vary with irrigation mode (aerial, surface, buried) as long as contaminated water reach absorbing roots of green onions

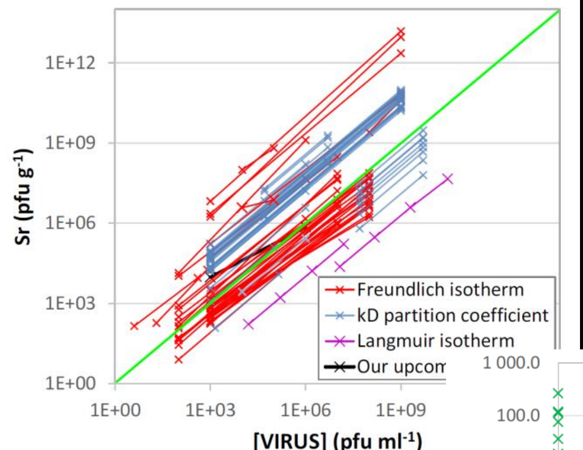




Renault et al. (2017)

But the soil may affect the fate of viruses (immobilization, inactivation)

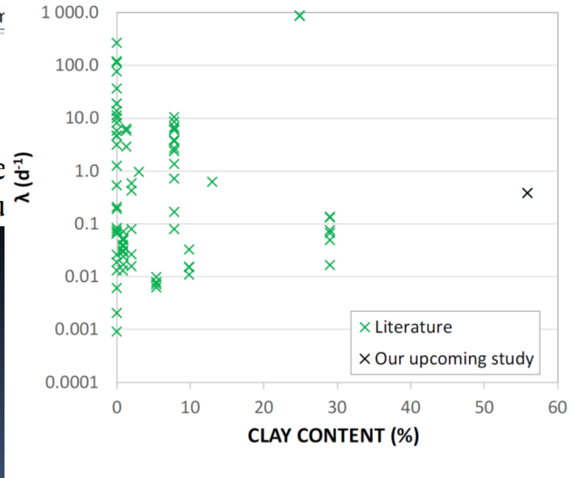
Tesson and Renault (2019)



Published relationships between virus concentrations S_r and fre concentrations C_i in soil solu

Reversible immobilization (from 25 papers)

Irreversible inactivation (from 27 papers)



Daily removal rates as reported in literature

Main topics to explore

They include:

- Additional knowledge on the fate of human enteric viruses;
- The development of systemic models to be combined with Quantitative Microbial Risk Assessment (QMRA)
- The development of new methods and probes for scientific research as well as the monitoring in real times of contaminations.


Thank you for your attention