# 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

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# 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

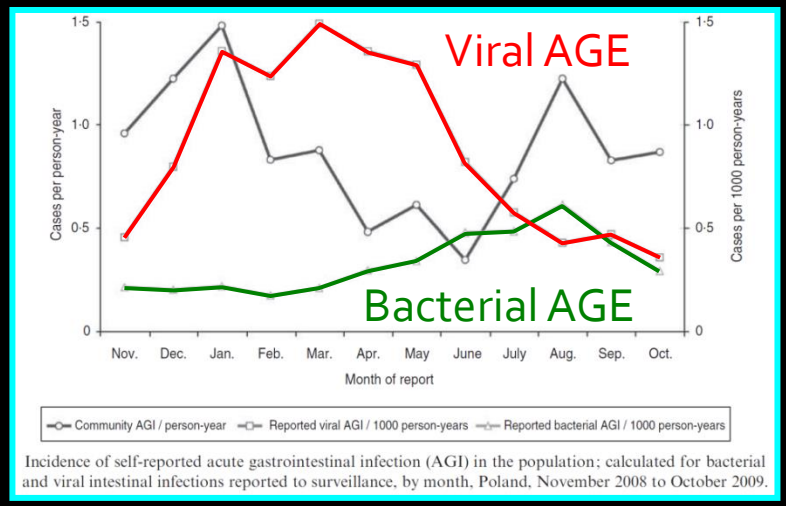
*Chikhi-Brachet et al. (2002)*

Various AGE aetiologies:

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

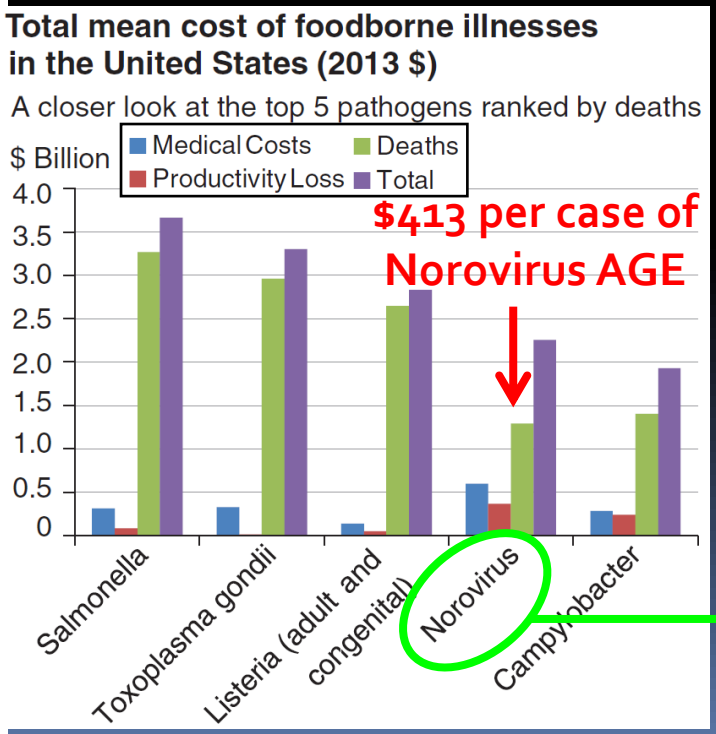
in the USA

*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)*




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

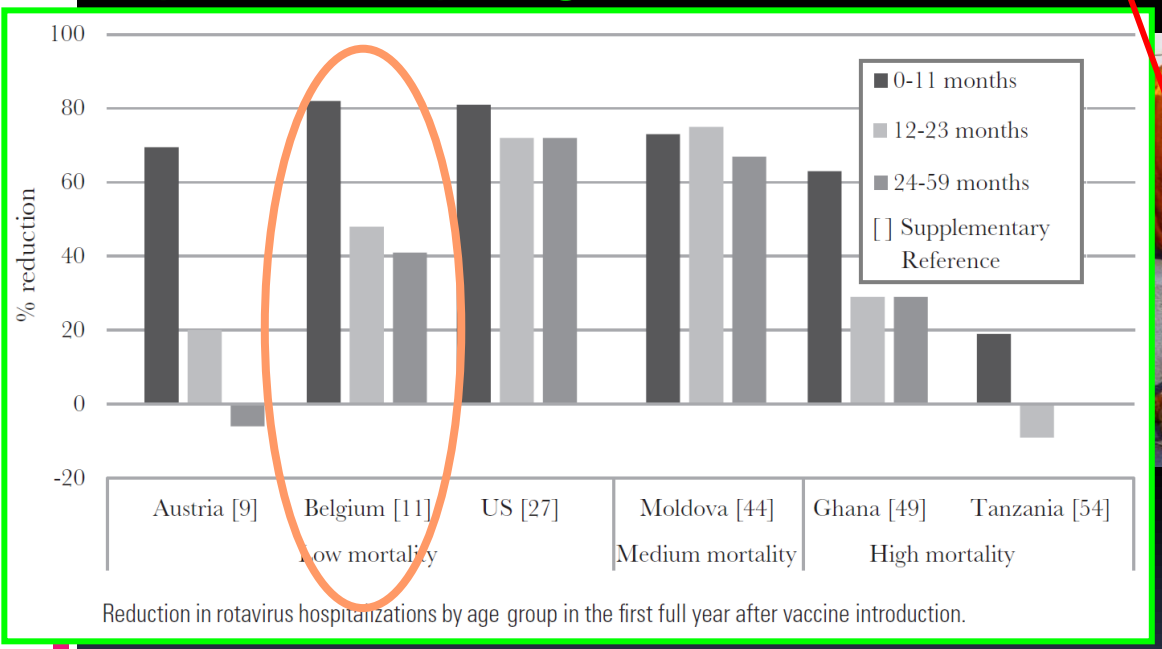
*Hoffmann et al. (2015)*

**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.

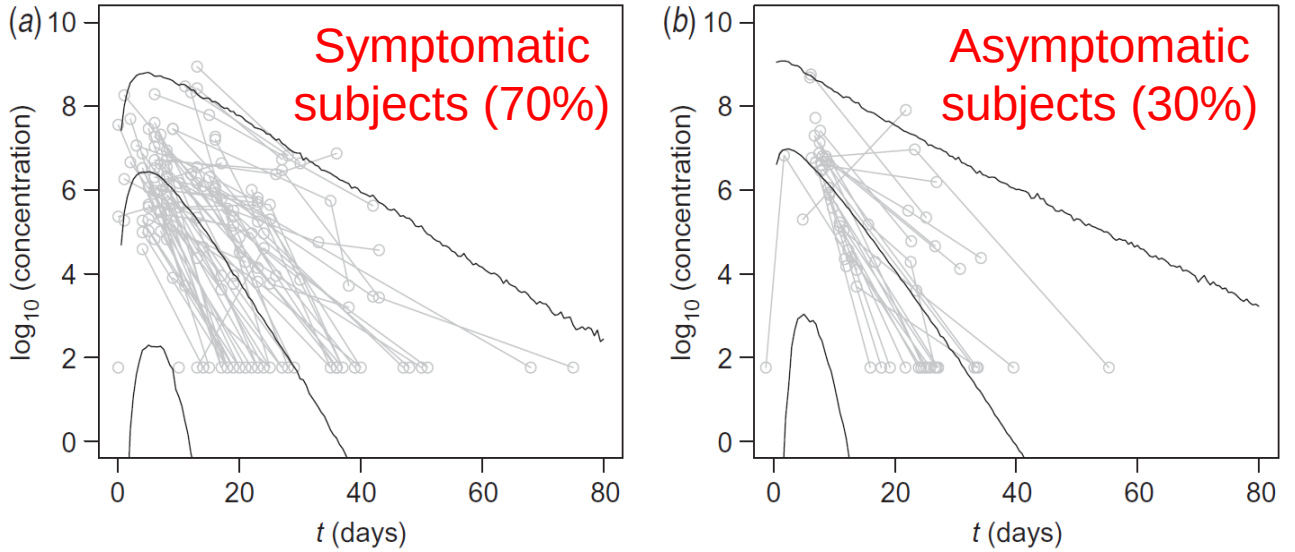
Noroviruses: **200 000 deaths per year worldwide** due to dehydration and malnutrition complications, especially for the young, elderly and immunocompromised

***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)*

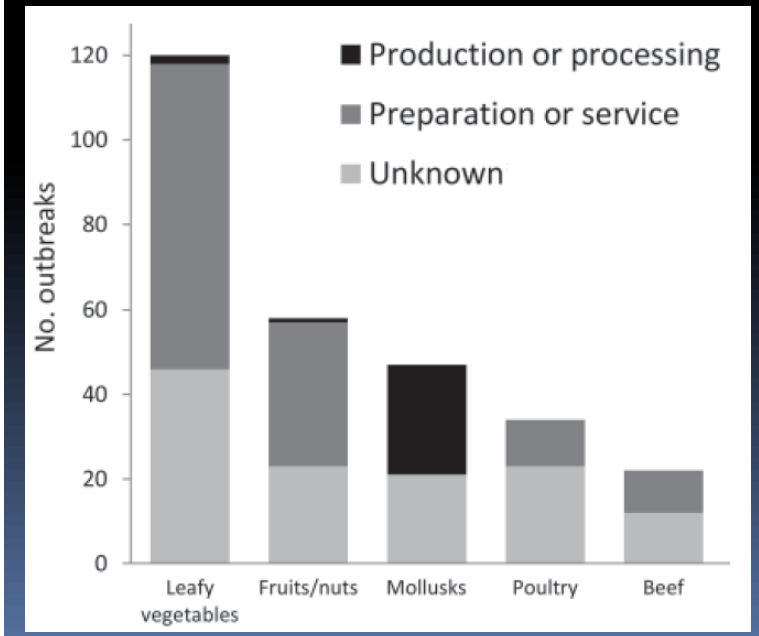
# Direct / indirect transmission of NV in the USA

## 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.

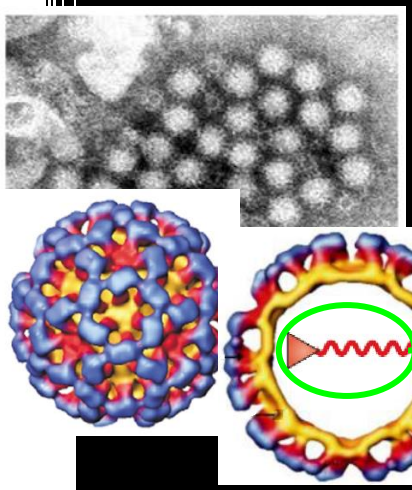
Frozen berries provided by industrial producers.
From Serbia, Chile, Bulgaria, Poland, Spain, Morocco, Turkey and France;

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

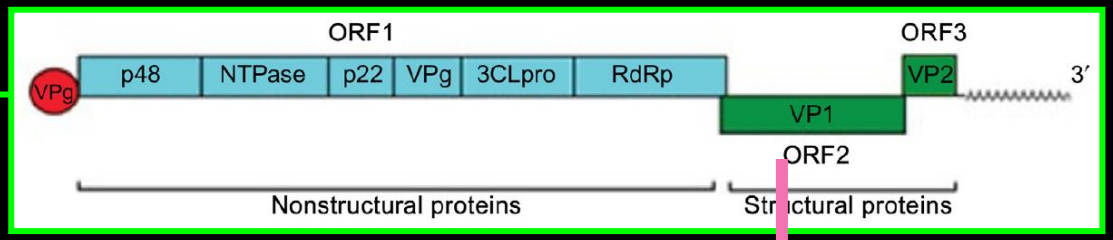
| Category of food matrix | Nature | No. of samples tested | % positive for GI NoV | % positive for GII NoV |
|---|---|---|---|---|
| Bivalve molluscs | Mussel | 83 | 8.4 | 14.4 |
| Lettuces | *Chicory* | 107 | 11.2 | 0.9 |
| | *Lettuce* | 77 | 13.0 | 0 |
| | *Mash* | 26 | 11.5 | 0 |
| | All salads | 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 )

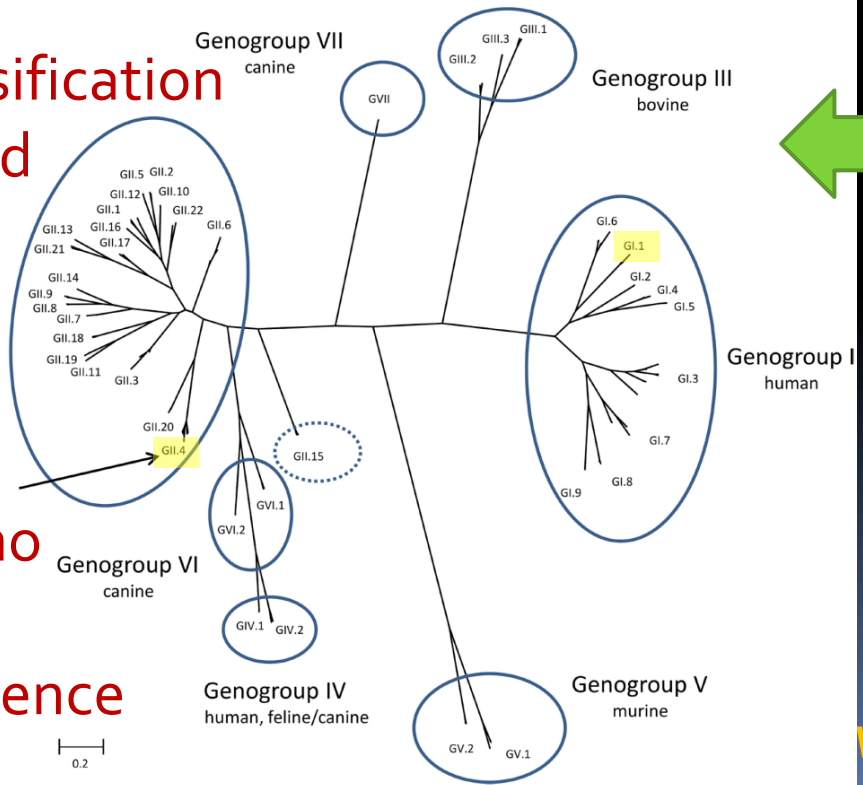


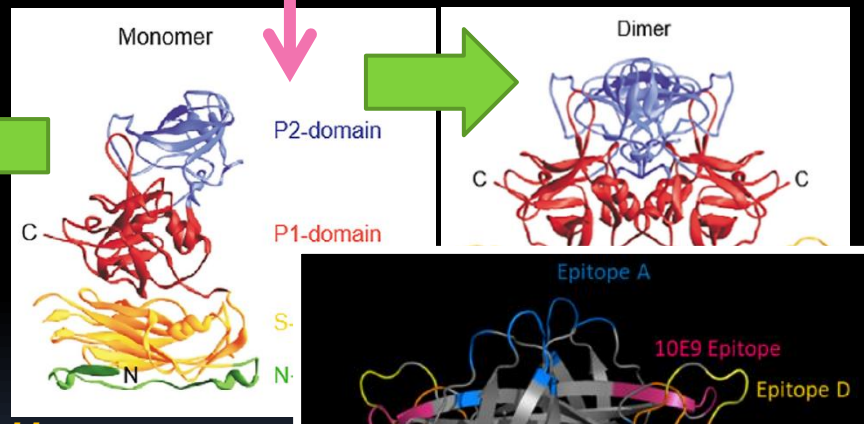

*Tarek et al. (2020)*

Classification based on

Genogroup II
human, porcine

VP1 amino acid sequence

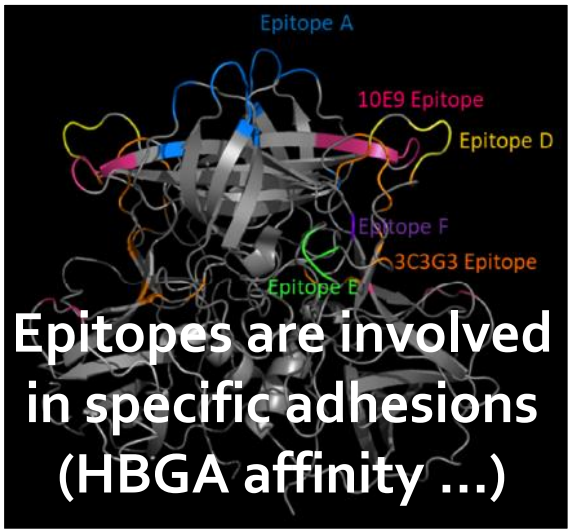



*Hutson et al. (2004)*

*Mallory et al. (2019)*

*Vinjé (2016)*




**Epitopes are involved in specific adhesions (HBGA affinity …)**

# Norovirus: mutations affecting HBGA affinity

## Mutations over a few weeks:

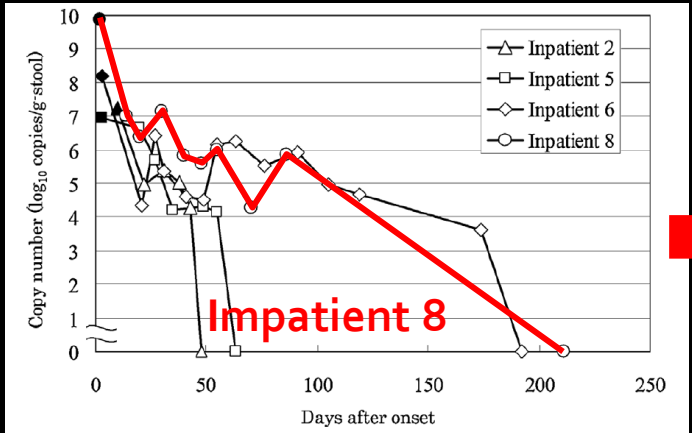



Fig. 3. Viral shedding in four inpatients (infants) in outbreak episode 1. The quantity of NoV genome segments was measured by real-time RT-PCR. Open and closed symbols indicated asymptomatic and symptomatic, respectively.

*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 G | | | | |
| | 884 | G | G | A or G | 295 | Ser | Gly | Asn or Gly |
| | 889 | C | A or C | C | | | | |
| | 890 | G | G | A | 297 | Arg | Ser or Arg | His |
| | 1,008 | A | G or A | A | | | | |
| | 1,102 | G | G | A or G | | | | |
| | 1,103 | C | C or A | C | 368 | Ala | Ala or Asp | Thr or Ala |
| | 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 A | 411 | Arg | Lys or Arg | Gly or Lys |
| | 1,232 | G | A or G | G A | | | | |

Contribute to epitope A

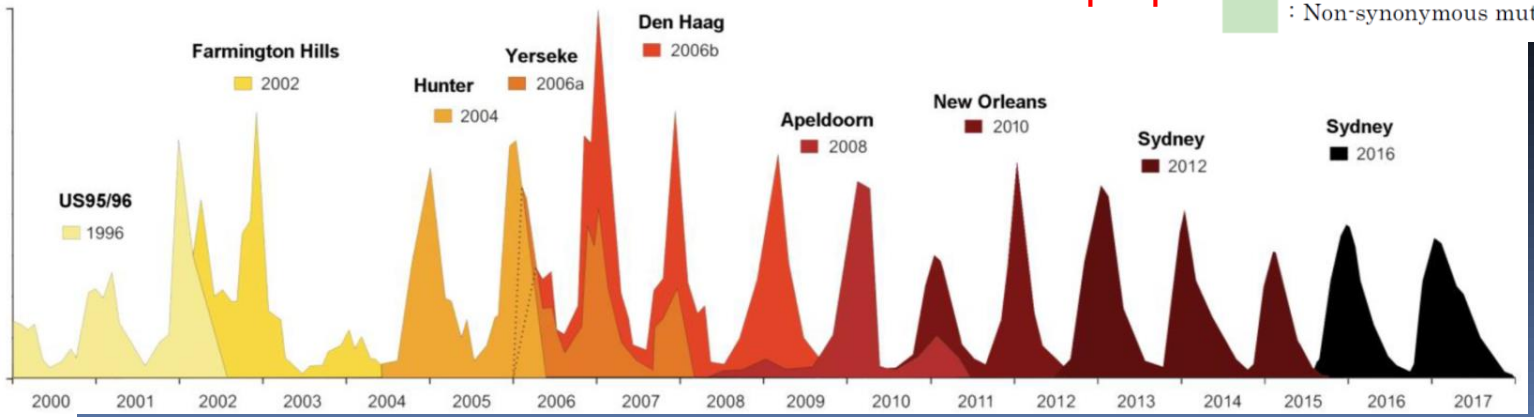
: Synonymous mutation

: Non-synonymous mutation

: Mutant amino acid

- : Deletion

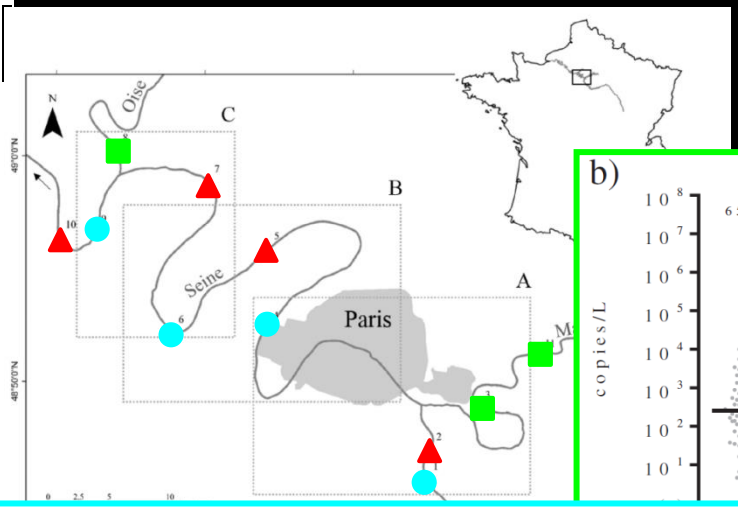
## New pandemic strains every 2-3 years:




*De Rougemont (2018)*

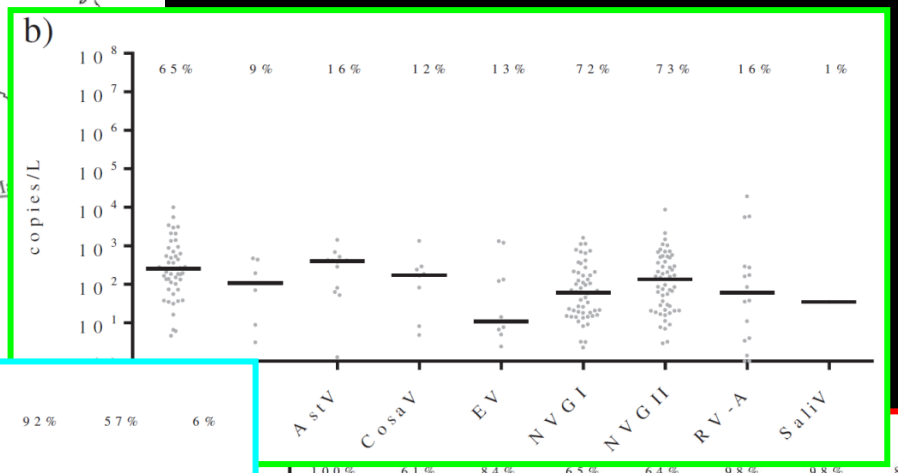
# Environmental fate of viruses after wastewater discharge

# Contamination of rivers by wastewater discharge



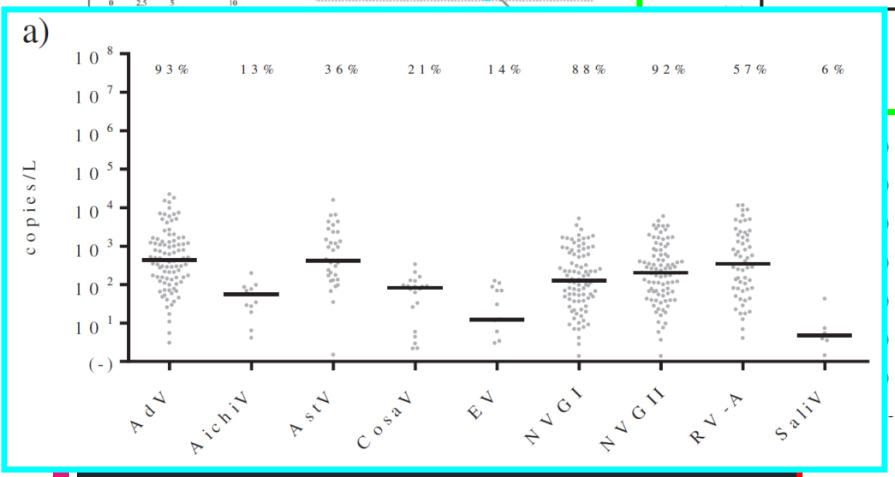

Case of the Seine River near Paris

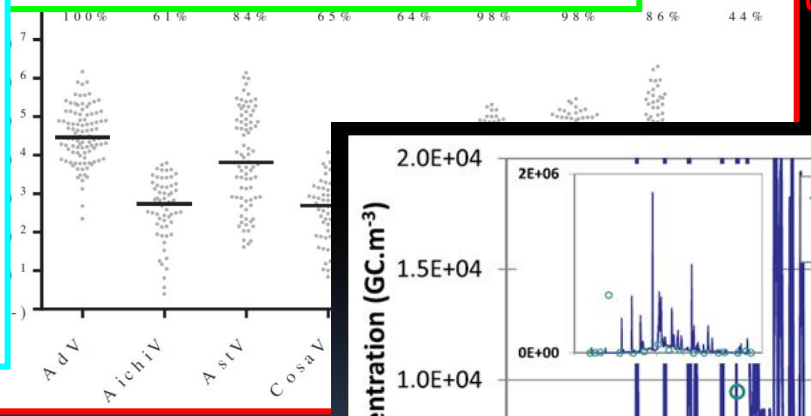
***Prevost et al. (2015)***




Contamination from the tributaries

Wastewater discharge after activated sludge treatment

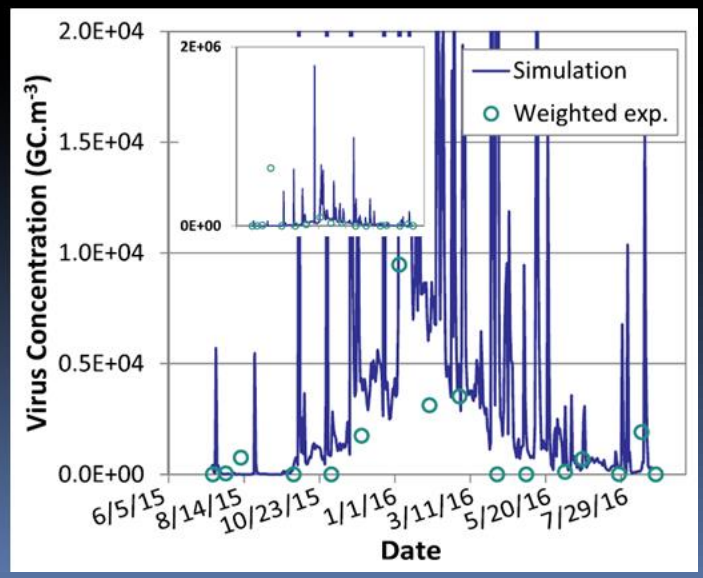



The resulting contamination of the Seine River

Medical prescriptions enable simulating the contamination of the Artière River

***Tesson et al. (2019)***



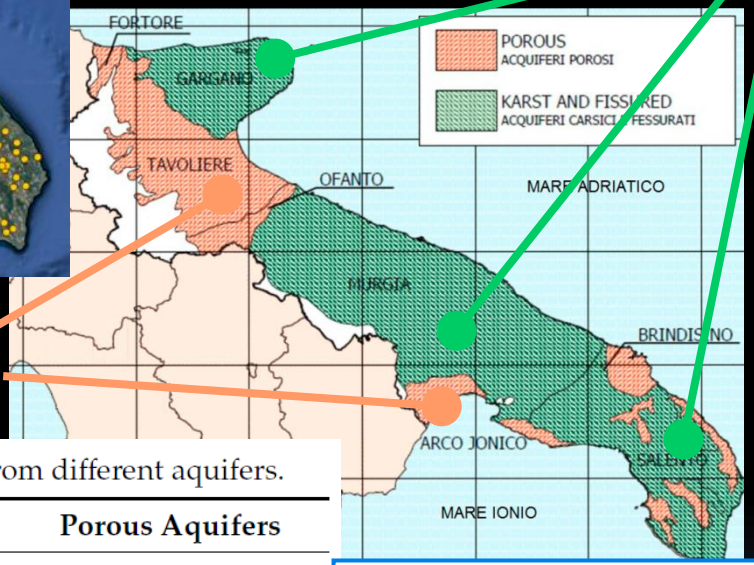
# Underground water contaminations


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) |

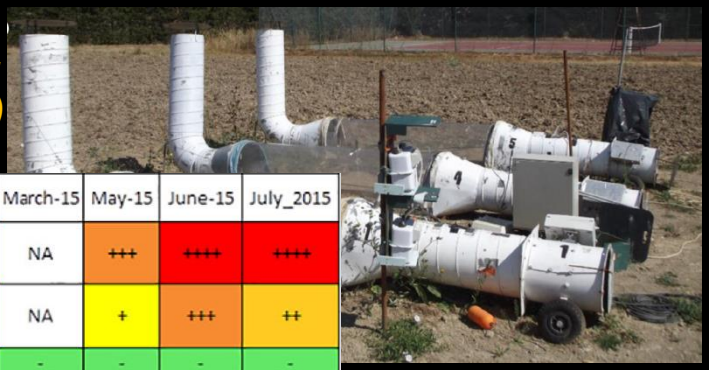
Wells with potential infectious risk owing to the presence of viruses.

| 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)*

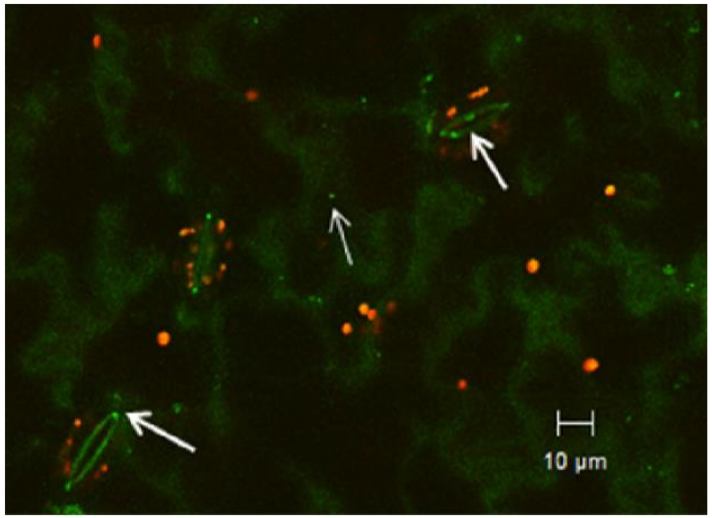
Contamination of external surfaces of crops

Virus flow in the soil with water infiltration

Virus internalization in plants via their roots

# Attachment on external surface or plant materials

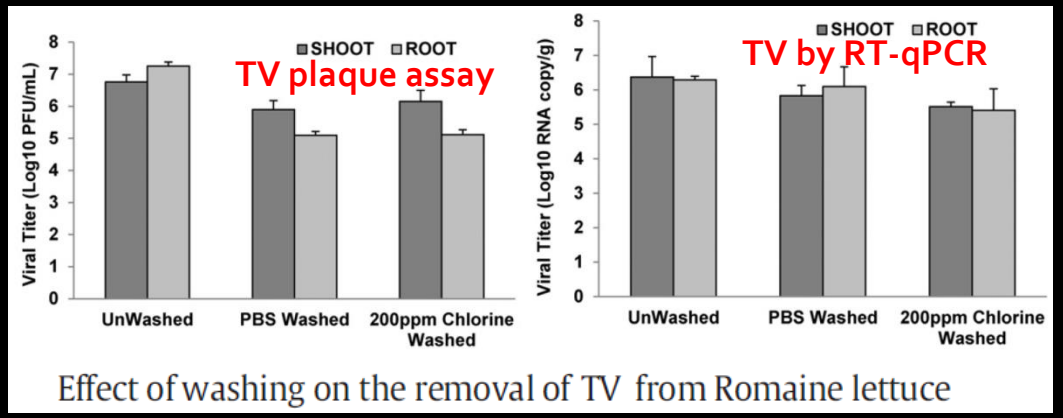
Murine NV (MNV-1) observed on lettuce surface, inside open cuts, and within stomata




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)***

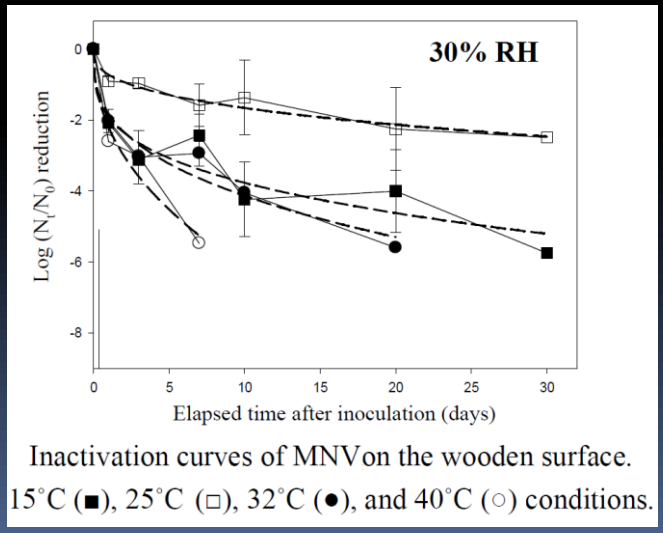
Tulane virus (TV) attachment/washing vary with the virus, the plant organ and the liquid phase




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



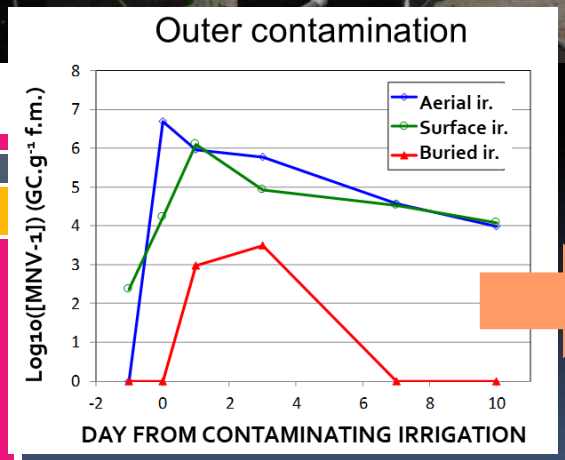

Inactivation curves of MNVon the wooden surface. 15˚C (■), 25˚C (□), 32˚C (●), and 40˚C (○) conditions.

***Kim et al. (2012)***

# Irrigation/handling impact on outer contamination

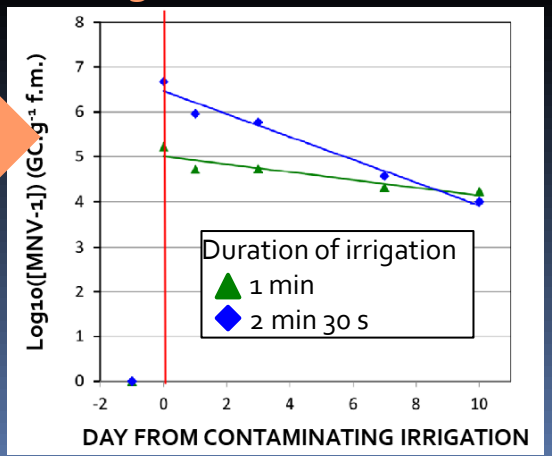
Initial contamination + Decay (inactivation …) + Washing (rain …)

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



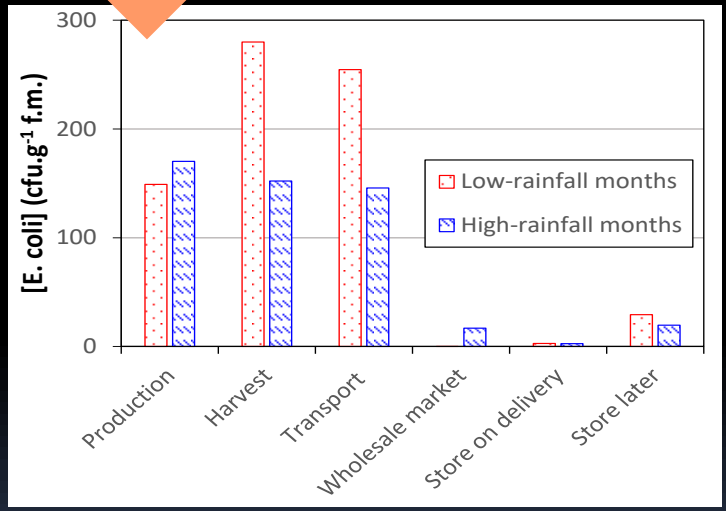

*Renault et al. (2017)*

Washing proportional to the irrigation amount




But handling may explain most of vegetable and fruit contamination.

*Pérez-Rodríguez (2019)*

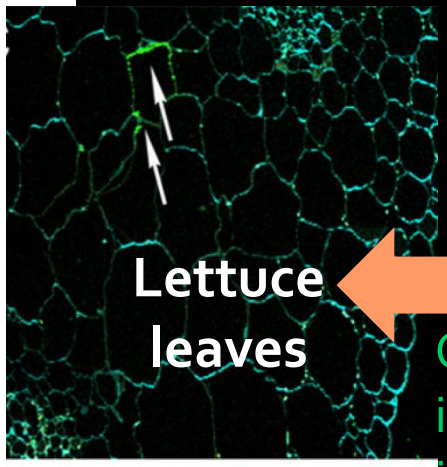



Chards produced in *La Ramada* near *Bogotá* (Colombia) are contaminated by irrigation, "washing" and handling.

*Jordan Lozano (2020)*

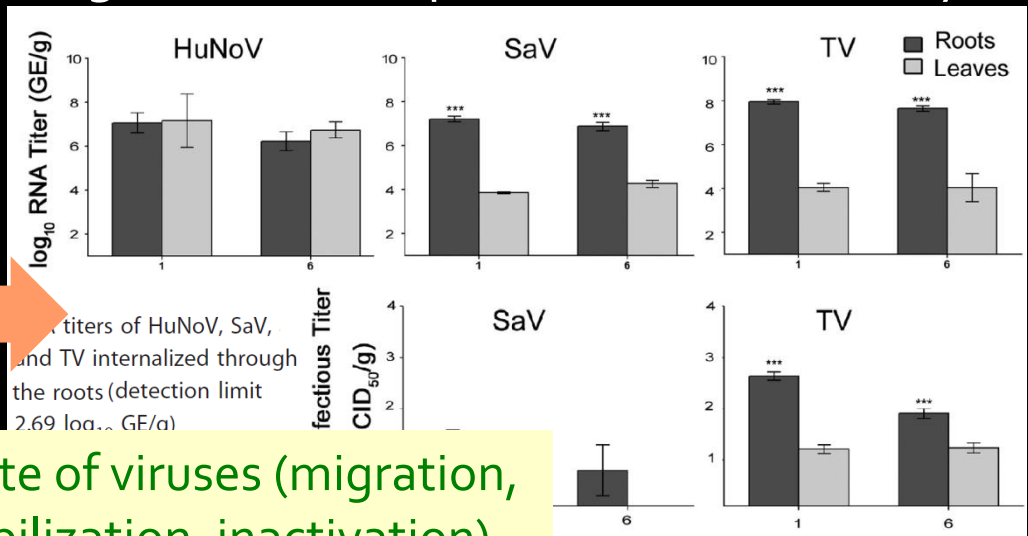
# Virus internalization in vegetables and fruits

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

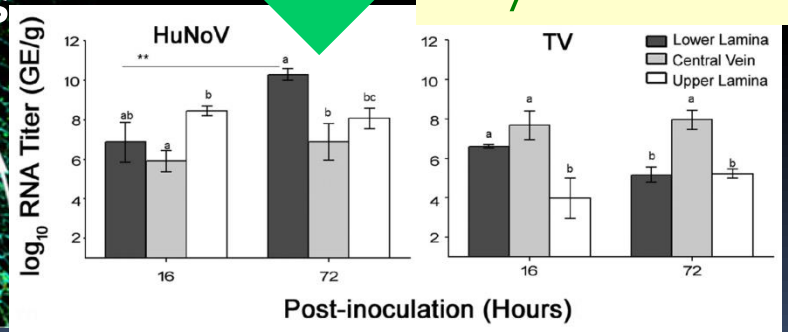



On whole lettuces after washing their root from soil and immersing them in a viral solution

On cut leaves after immersing th… in a viral solut…

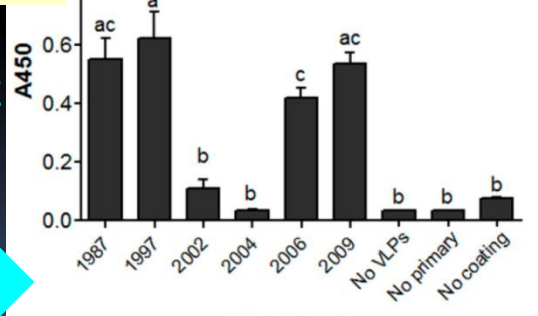

The fate of viruses (migration, immobilization, inactivation) vary with virus and strain




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)

*Esseili et al. (2018)*

Attachment On cell wall materials

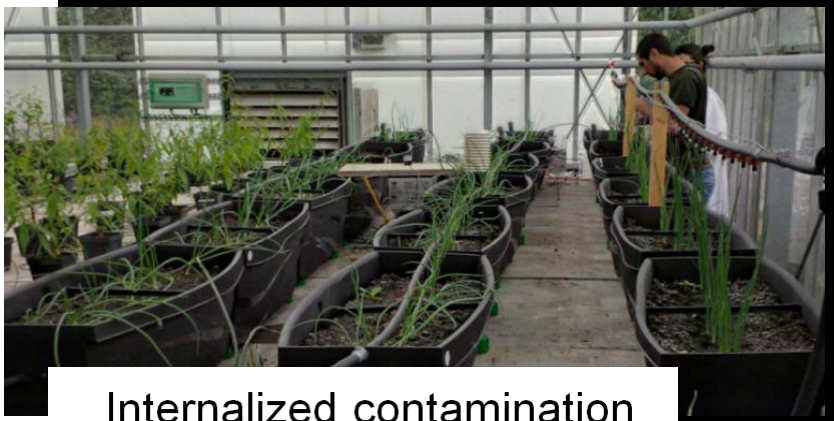


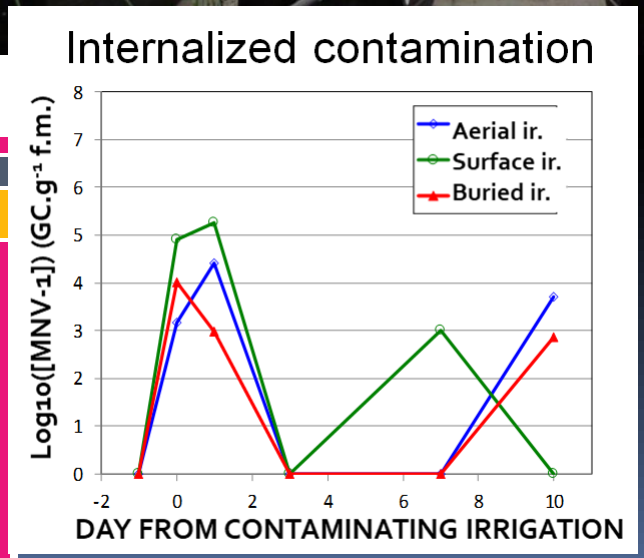

Enzyme linked immunosorbent assay (ELISA) screening of historical HuNoV GII.4 virus-like particles (VLPs) for their binding to lettuce leaves cell wall material (CWM).

*Esseili et al. (2019)*

# 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

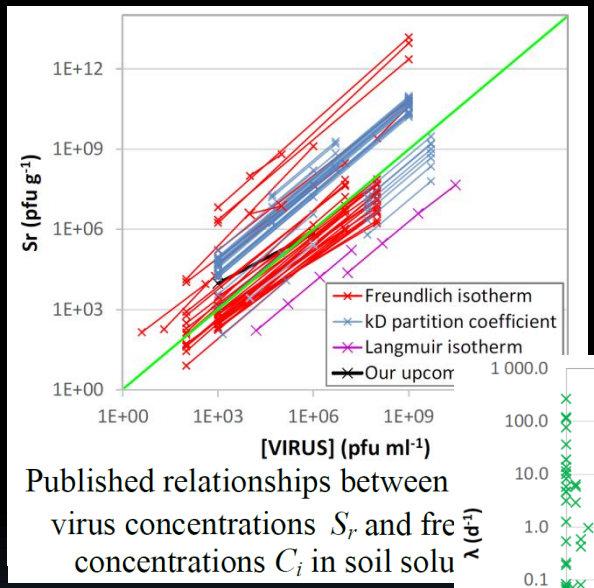





*Renault et al. (2017)*

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

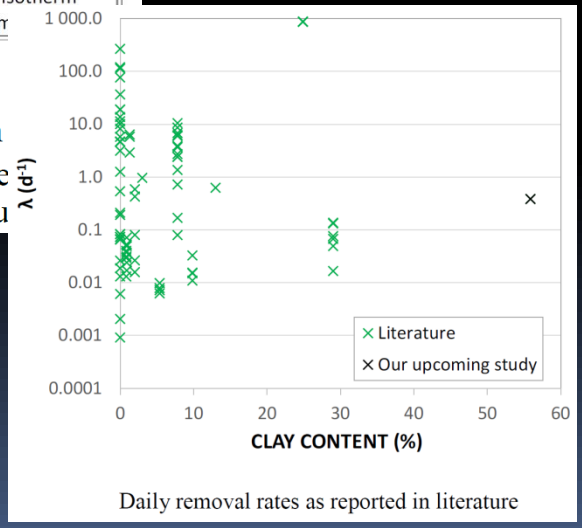
***Tesson and Renault (2019)***




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