Development of various quantitative detection methods for a better risk assessment of enteric viruses in germinated seeds

Audrey FRAISSE[1] ; Prunelle WALDMAN[1], Fabien VORIMORE[2], Sylvie PERELLE[1]

[1] ***ANSES, Enteric Viruses Unit, Laboratory for Food Safety, Maisons-Alfort, France.*** ***audrey.fraisse@anses.fr***

[2] ***ANSES, IdentyPath platform, Laboratory for Food Safety, Maisons-Alfort, France.***

INTRODUCTION

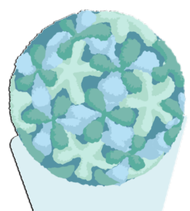
Consumption of germinated seeds has grown in popularity in recent years, mainly due to their ease of cultivation and their **positive impact on health**. However, as unprocessed foods, raw sprouts can be contaminated with viruses, bacteria or fungi and represent a **food poisoning hazard**.

AIM — **To develop wide-ranging quantitative detection methods for a better risk assessment of enteric viruses in germinated seeds**

METHODOLOGY

❶ germination — ❷ sprouts inoculation — ❸ virus recovery — ❹ virus detection & quantification

❷ sprouts inoculation:
- hepatitis A virus (HAV)
- or human norovirus (NoV GII)
- or raw sewage
- process control : murine norovirus (MNV)

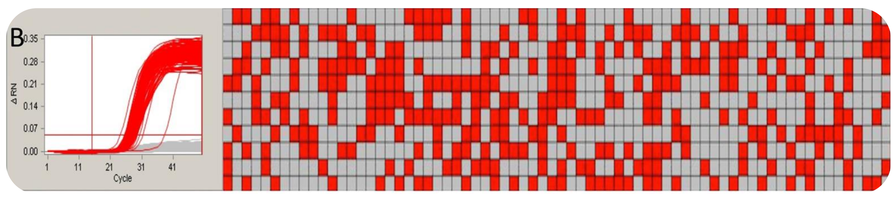


❸ virus recovery:
- elution/concentration ISO 15216
- direct elution *(vortex & repetitive pipetting)*

❹ virus detection & quantification:
- elution/concentration ISO 15216 → RT-qPCR; capsid integrity - RT-qPCR; metagenomic sequencing
- direct elution *(vortex & repetitive pipetting)* → infectious virus titration

FIRST RESULTS

RT-qPCR & DIGITAL-RT-qPCR

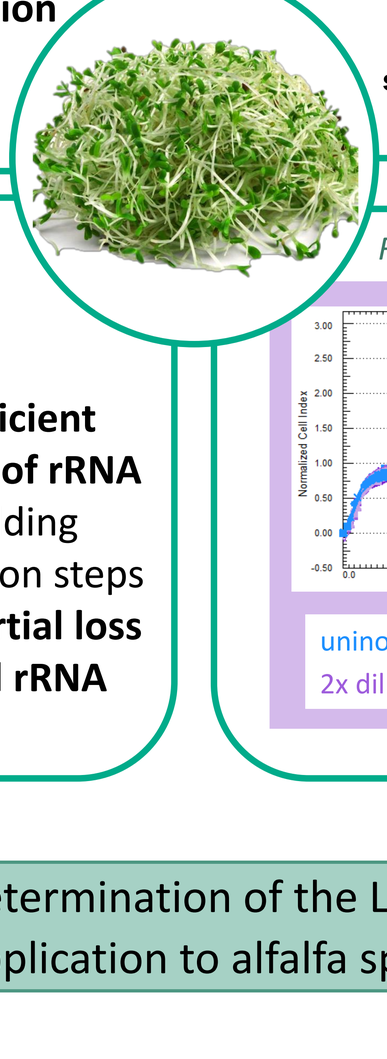


Recovery rates (mean ± SEM)

	digital RT-qPCR	RT-qPCR
HAV	34.8% ± 10.9% (n=2)	30.0% (n=1)
NoV GII	36.2% ± 4.9% (n=2)	*not tested yet*
MNV	*not tested yet*	37.3% (n=1)

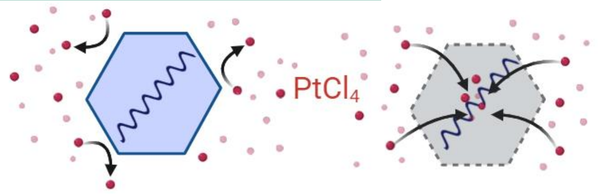
→ **high virus recovery rates**

→ **low PCR inhibition rate** : < 10% *(RT-qPCR ; n=2)*



INTEGRITY-RT-qPCR

30 min incubation with $PtCl_4$ 1.5mM

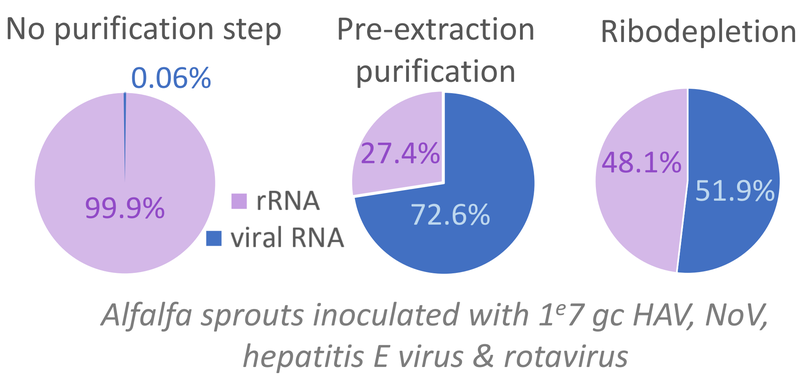


HAV genomic titer log_{10} reduction (mean ± SEM ; n=2)		**RT-qPCR**	**integrity RT-qPCR**
PBS suspension	5°C	-	-0.18 ± 0.00
	80°C 10min	-0.77 ± 0.11	-2.44 ± 0.35
Alfalfa sprouts eluate	5°C	-0.30 ± 0.12	-0.21 ± 0.06
	80°C 10min	-1.18 ± 0.62	-3.65 ± 0.60

→ **discrimination between infectious and heat-inactivated HAV**

METAGENOMICS

Evaluation of a viral RNA purification step prior to MinION sequencing *(digital RT-qPCR; n=2)*

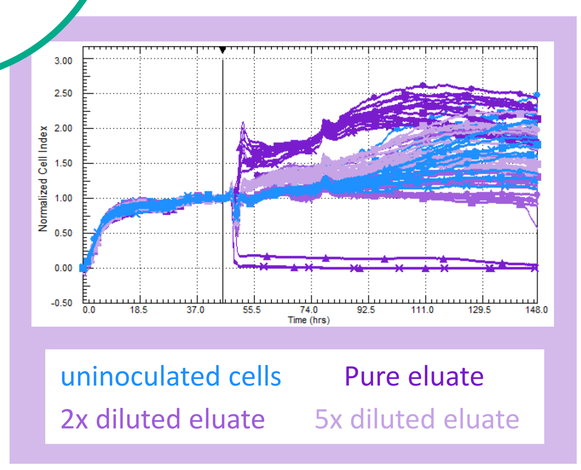


Alfalfa sprouts inoculated with 1e7 gc HAV, NoV, hepatitis E virus & rotavirus

→ **efficient removal of rRNA** by adding purification steps **but a partial loss of viral rRNA**

INFECTIOUS VIRUS TITRATION

FRhK-4 cells treated with uninoculated alfalfa sprouts eluates (n=1)



Xcelligence MP system (Agilent)

→ **Cytotoxicity of pure eluates**

→ **No cytotoxicity of diluted eluates**

⇒ **Evaluation of $TCID_{50}$ titration of HAV and MNV inoculated on alfalfa sprouts**

FUTURE PERSPECTIVES
- determination of the LOQ of each method
- application to alfalfa sprouts inoculated with raw sewage

CONCLUSION

Developing methods for identifying and characterising enteric viruses in germinated legume seeds could provide a **better assessment for viral risk management**.

Funding: *Holistic approach for tackling food systems risks in a changing global environment (HOLIFOOD) project – HORIZON Europe.*