Comparison of different NGS approaches to characterize bacterial pathogens

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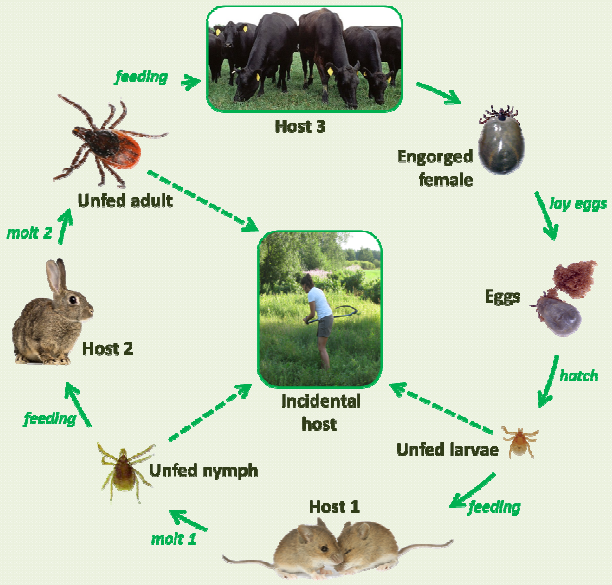
Zoonotic pathogens

Rodents are reservoirs and/or vectors of many human and livestock pathogens.

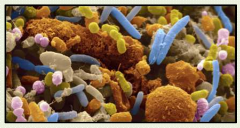
Most of these pathogens are vectored by ticks.



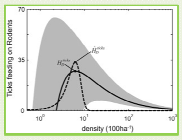
PATHO-ID project

Describe the «pathobiome» (= pathogenic agents in the microbiologic environment) of ticks and rodents:

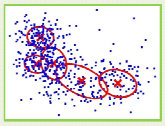
1) To build an **inventory of microorganisms** recovered from ticks and rodents

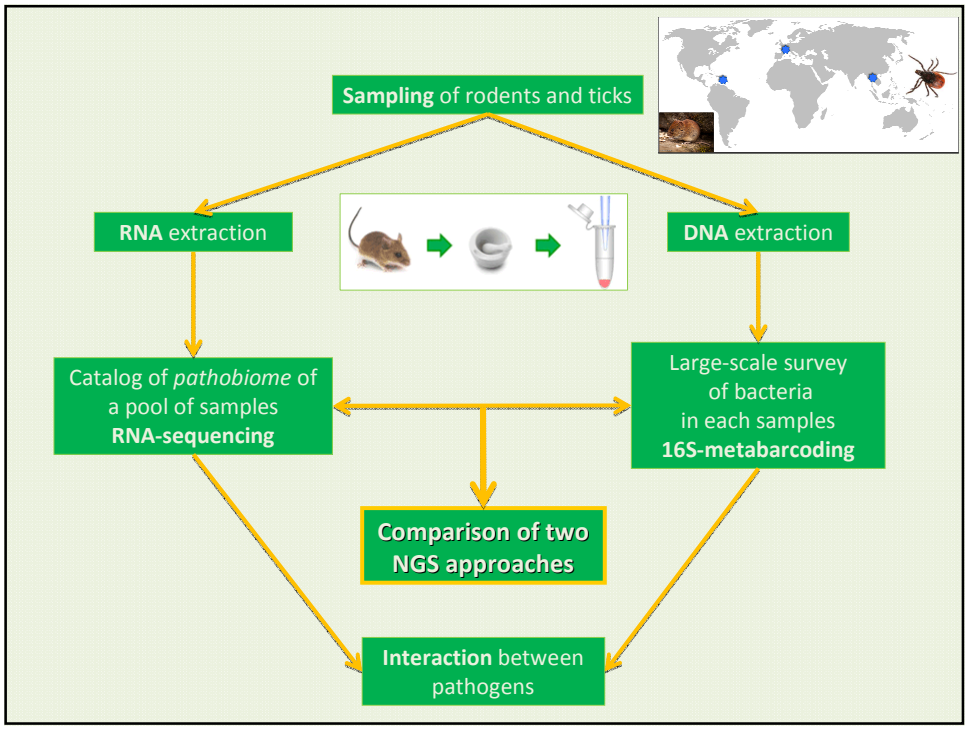


2) To identify pathogens **distribution** and **prevalence** in tick and rodent populations



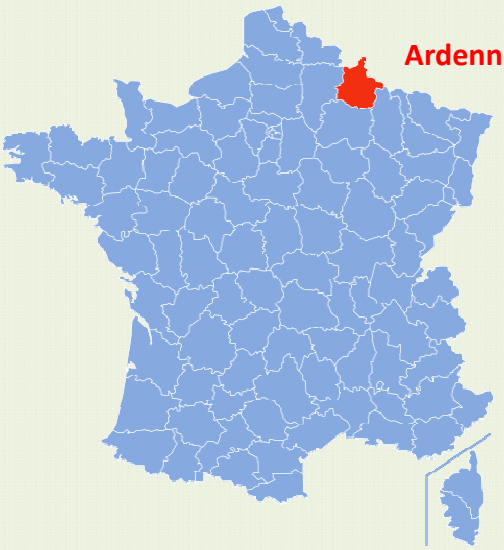
3) To seek for evidence of **interactions** between pathogens



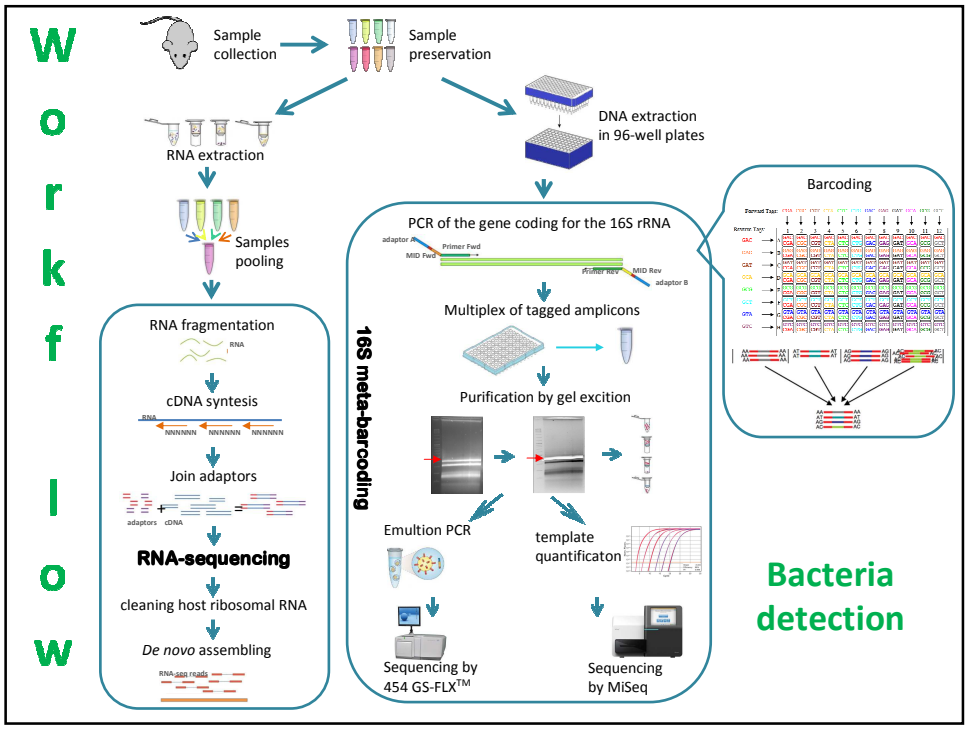


Sampling

192 bank voles studied
(Guivier et al. 2011)

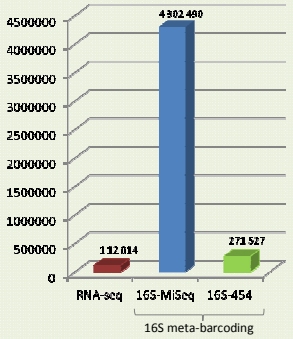


Myodes glareolus

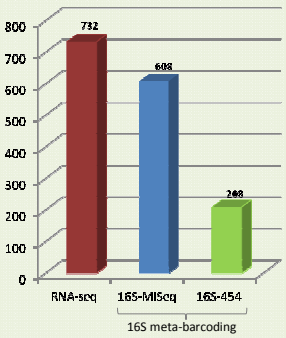


Raw results of the comparison of RNA-Seq and the 16S meta-barcoding

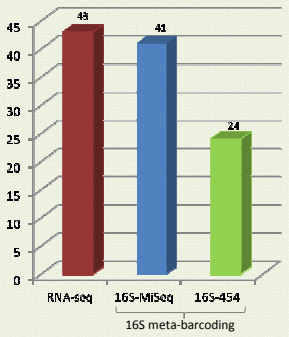
Number of bacterial reads



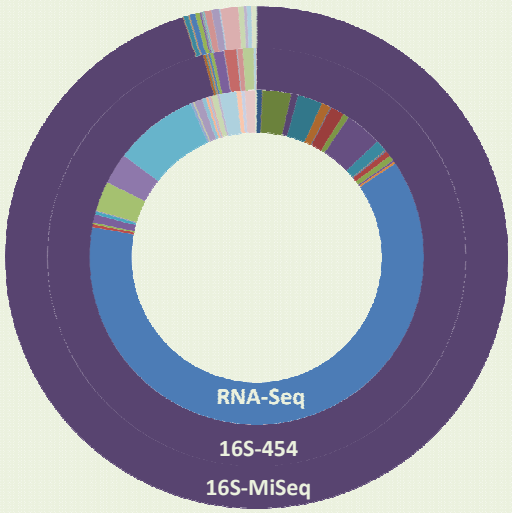
Number of bacterial genera



Number of pathogenic bacteria



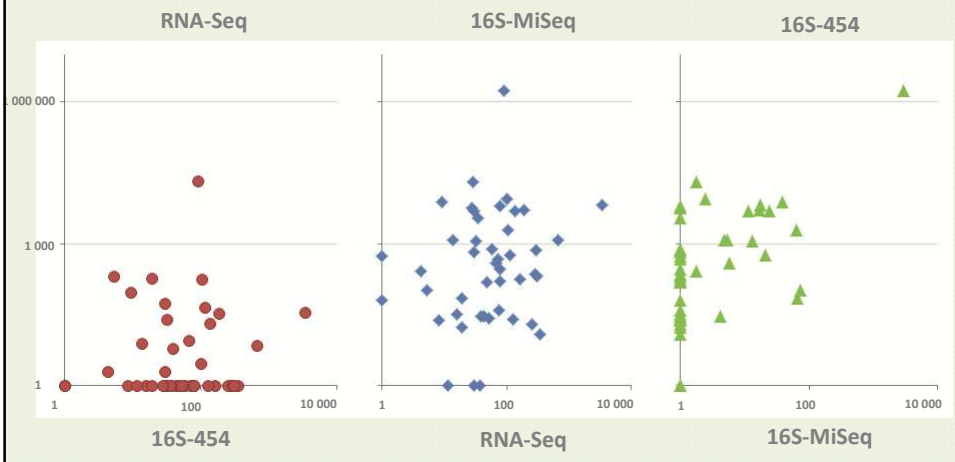
Number of reads detected for each pathogenic bacteria



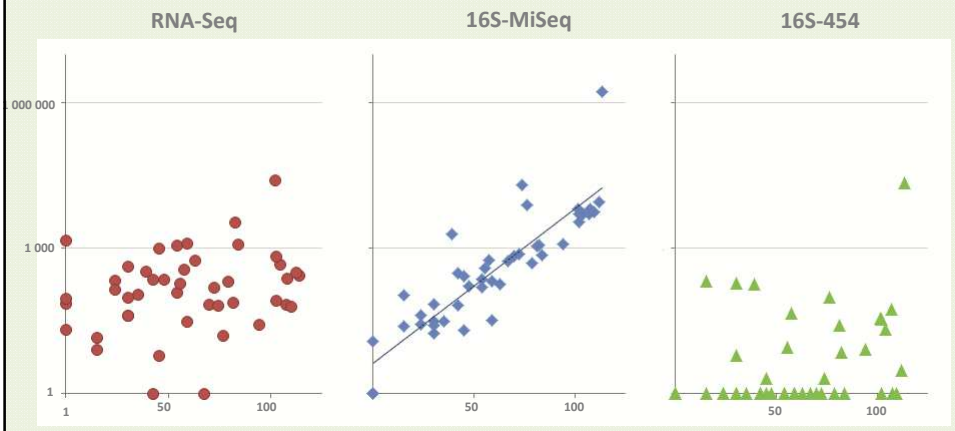
45 pathogenic bacteria detected

Aeromonas sp. | Anaplasma sp. | Bacillus sp. | Bartonella sp. | Bordetella sp.
Borrelia sp. | Brucella sp. | Burkholderia sp. | Campylobacter sp. | Clostridium sp.
Corynebacterium sp. | Coxiella sp. | Ehrlichia sp. | Enterococcus sp. | Eubacterium sp.
Francisella sp. | Granulicatella sp. | Haemophilus sp. | Helicobacter sp. | Klebsiella sp.
Legionella sp. | Leptospira sp. | Listeriasp. | Mannheimia sp. | Micrococcus sp.
Moraxella sp. | Mycobacterium sp. | Mycoplasma sp. | Neisseria sp. | Neochlamydia sp.
Nocardia sp. | Orientia sp. | Pasteurella sp. | Rhodococcus sp. | Rickettsia sp.
Salmonella sp. | Shigella sp. | Spiroplasma sp. | Staphylococcus sp. | Stenotrophomonas sp.
Streptococcus sp. | Treponema sp. | Ureaplasma sp. | Vibrio sp. | Yersinia sp.

Correlation between number of reads (Log-scale) generated for each bacteria genera



Correlation between number of reads (Log-scale) and the prevalence for each bacteria genera



pros and **cons** of both approaches

	RNA-seq	16S meta-barcoding
No. of bacteria detected	higher	lower
A priori documentation	none	universal primers
Taxonomical level	species	genera
Resolution of sample sequencing	pool of samples	individual sample
Prevalence estimations	null	allowed
Bacteria interaction studies	null	allowed

pros and **cons** of the sequencers

Sequencers	HiSeq 2000 (Illumina)	MiSeq (Illumina)	454 GS-FLX (Roche)
Price / run	≈ 5 000 €	≈ 1 200 €	≈ 3 400 €
Price / Mb	≈ 0.008 €	≈ 0.2 €	≈ 5 €
Output data / run	600 Gb	6 Gb	0.7 Gb
Reads / run	3000 millions	12 millions	1 millions
Error with homopolymers	low	low	high
Read length	2x100 bp paired end (short reads assembly)	2x250 bp paired end	400 bp
Time/run	8 day	26 hours	10 hours

Conclusions

❖ RNA-Sequencing:

- more sensitive to bacteria detection (**higher sequencing depth**)
- deeper **level of taxonomy**
- only allows information for **pool** of samples
- **arduous** data analysis

❖ 16S meta-barcoding:

- may be influenced by the **performance of the universal primers**
- might be influenced by **the number of copies of the gene** coding for the 16S ribosomal RNA
- allows **multiplexing** of samples, and hence further analysis on community or a particular sample
- **accessible** data analysis

Collaborators:

- The Centre for Biology and Management of Populations (CBGP-INRA), Montpellier
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