Do amoeba contribute to the environmental survival of *Francisella tularensis*?

Hennebique A[1, 2], Peyroux J[2], Boisset S[1, 2], Maurin M[1, 2]
[1]Institut de Biologie et de Pathologie, Centre Hospitalier Universitaire Grenoble Alpes, Grenoble, France.
[2]Université Grenoble Alpes, TheREx, TIMC-IMAG, CNRS, UMR5525, Grenoble, France.

CONTEXT - *Francisella tularensis*

- Bacteria responsible for **tularemia**, a potentially lethal disease
- Potential agent of bioterrorism

→Need to control the bacterial reservoirs

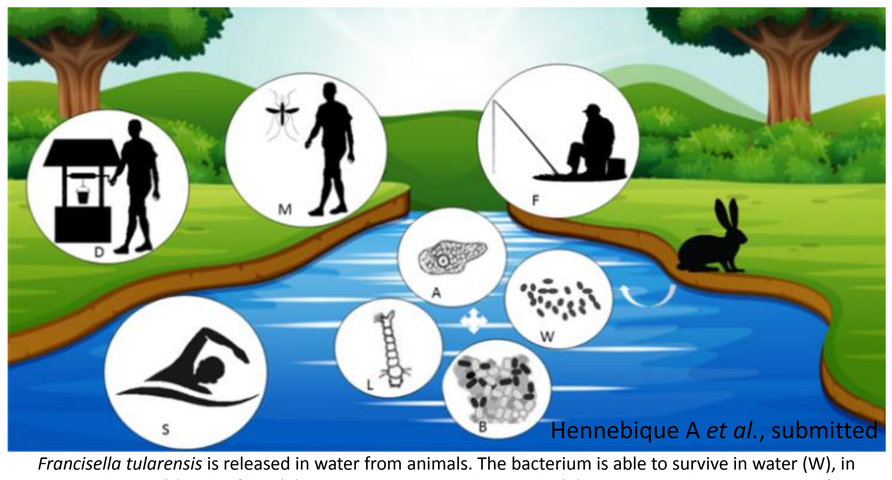
- Main modes of human contamination: contact with animals (hares ++) or arthropod bites (ticks)

- However, **contamination from aquatic environments** also exist[1]:
  - Outbreaks linked to ingestion of contaminated water: Turkey, Balkans, Norway
  - Outbreaks linked to mosquito bites: Sweden, Finland
  - Sporadic cases related to aquatic activities (fishing, swimming): multiple countries

→ Suspicion of an aquatic reservoir of *F. tularensis*

→ Potential mechanisms of *F. tularensis* survival in aquatic environment

- Long-term survival in water[2,3]?
- Biofilms[4]?
- Mosquito larvae[5]?
- **Amoebae[6-8]?**



Francisella tularensis is released in water from animals. The bacterium is able to survive in water (W), in mosquito larvae (L), in biofilms (B), or in cooperation with amoeba (A). Human can be contaminated from the aquatic reservoir either by drinking contaminated water (D), after a mosquito bite (M), or during swimming (S) and fishing (F) activities.

AIM

Evaluate if *F. tularensis* is able to survive in the aquatic environment inside amoebae and/or through interaction with amoebae

1st model: AMOEBA PLATE TEST

Aim: study interactions between amoebae and *F. tularensis* in agar plates

Method:

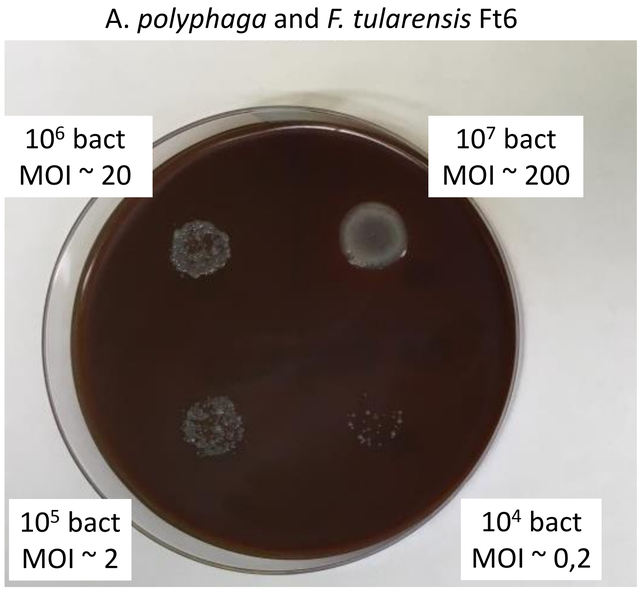
- amoebae (*Acanthamoeba polyphaga*) monolayer on an agar plate
- Spots of serial dilutions of the tested *Francisella* strain on the same plate
- 10 days follow-up of bacterial growth on the plate

→Bacterial growth on the plate = interaction with amoeba

→No bacterial growth on the plate = no interaction with amoeba

Results:

A. *polyphaga* and *F. tularensis* Ft6



Conclusion:
Interaction between *F. tularensis* and the amoeba *A. polyphaga*

2nd model: INFECTION

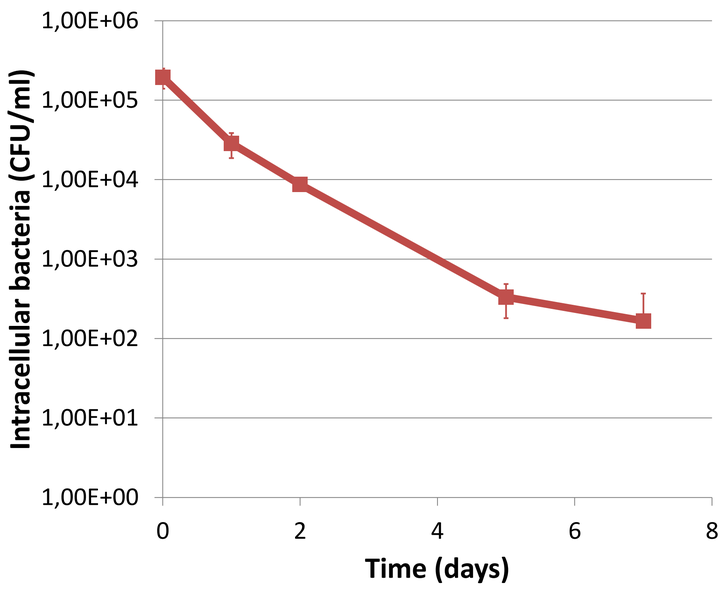
Aim: evaluate if *F. tularensis* is able to survive or even multiply **inside** amoebae

Method:

- Infection of amoebae with *F. tularensis,* MOI 10, 1h
- Removal of extracellular bacteria by washing and gentamicin
- At day 0 (D0), D1, D2, D5, and D7 post-infection: removal of supernatant, lysis of amoebae, and numeration of intracellular bacteria by CFU counting

Results:

Infection of *A. polyphaga* by *F. tularensis* Ft6



Conclusion:
No replication of *F. tularensis* inside amoebae and very short intracellular survival time

3rd model: CO-CULTURE

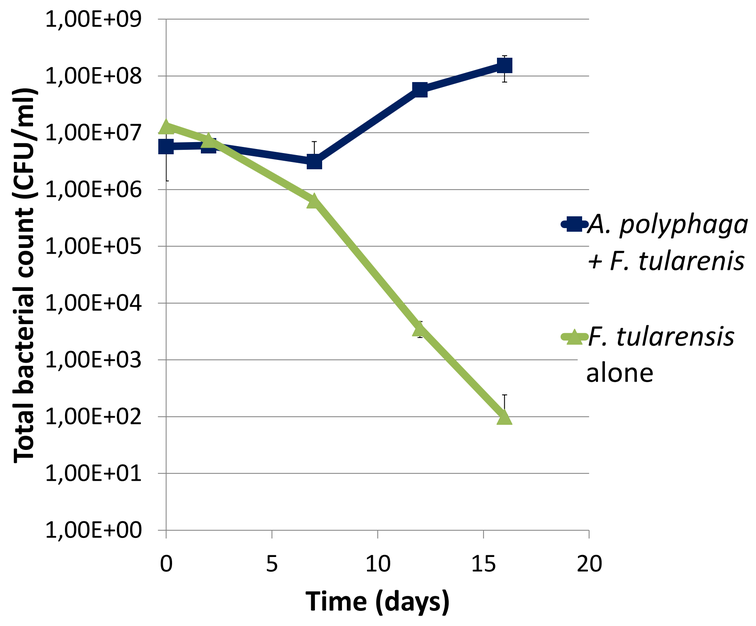
Aim: evaluate if *F. tularensis* is able to survive or even multiply **in the presence** of amoebae

Method:

- Infection of amoebae with *F. tularensis,* MOI 10, 1h
- No removal of extracellular bacteria
- At day 0 (D0), D2, D7, D12, D16: no removal of supernatant, lysis of amoebae, and numeration of total bacteria (i.e. intracellular and extracellular bacteria) by CFU counting

Results:

Co-culture of *A. polyphaga* and *F. tularensis* Ft6



Conclusion:
Replication of *F. tularensis* in the presence of amoebae in contrast to death in the absence of amoebae

CONCLUSION and PROSPECTS

Amoebae likely enhance survival of *F. tularensis* in the aquatic environment

→Survival of *F. tularensis* in the aquatic environment is favored by interactions with amoebae, but not related to intra-amoebal replication of this bacterium

→Mechanisms of enhanced survival of *F. tularensis* in the presence of amoebae are being investigated…

This work was supported by the Direction Générale de l'Armement (DGA, ANR-17-ASTR-0024)

REFERENCES

1. Maurin M and Gyuranecz M. Lancet Infect Dis. 2016
2. Gilbert SE and Rose LJ. Lett Appl Microbiol. 2012
3. Forsman M *et al.* FEMS Microbiol Ecol. 2000
4. Van Hoek ML. Virulence. 2013
5. Lundström Jo *et al.* Emerg Infect Dis. 2011
6. El-Etr SH *et al.* Appl Environ Microbiol. 2009
7. Buse HY *et al.* Acta Microbiol Immunol Hung. 2017
8. Abd H *et al.* Appl Environ Microbiol. 2003