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Does environmental water support the survival of *Francisella tularensis*, the etiological agent of tularemia?

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BACKGROUND

- Tularemia : zoonosis caused by the bacterium *Francisella tularensis*
- Human contamination mainly occurs through contact with wildlife animals or arthropod bites
- Tularemia can also be a water-borne disease
- ➔ *F. tularensis* mechanisms of survival in aquatic environments are poorly characterized

AIM

Characterize relationships between the aquatic environment and *F. tularensis*

METHODS

Experimental study :

in vitro models for investigation of interactions of *F. tularensis* subsp. *holarctica* (the tularemia agent found in the northern hemisphere), and the aquatic species *F. novicida* and *F. philomiragia* with amoebae of the *Acanthamoeba* species

	Amoeba Plate Test model	Infection model	Co-culture model
Aim	Screening model : study interactions of <i>Francisella</i> sp. with amoebae in agar plates	Evaluate if <i>Francisella</i> sp. are able to survive or even multiply <u>inside</u> amoebae	Evaluate if <i>Francisella</i> sp. are able to survive or even multiply <u>in the presence</u> of amoebae
Method	<ul style="list-style-type: none"> • Amoebae monolayer on an agar plate • Spots of bacteria on the amoebae • Monitoring of bacterial growth over 10 days 	<ul style="list-style-type: none"> • Infection MOI 10 • In Starvation Medium (SM) : allows <i>Acanthamoeba</i> sp. survival in trophozoite form for 16 days without amoebae multiplication • Removal of extracellular bacteria by washing and gentamicin • At day 0, D1, D2, D5, and D7 post-infection: removal of supernatant, lysis of amoebae, and numeration of intracellular bacteria 	<ul style="list-style-type: none"> • Infection MOI 10 • In Starvation Medium • No removal of extracellular bacteria • At day 0, D2, D7, D12, D16: no removal of supernatant, lysis of amoebae, and numeration of total intracellular and extracellular bacteria

Environmental study:

- Two sampling campaigns in West of France, in July 2019 and January 2020
- Collection of 87 water samples
- Analysis by a set of three qPCRs

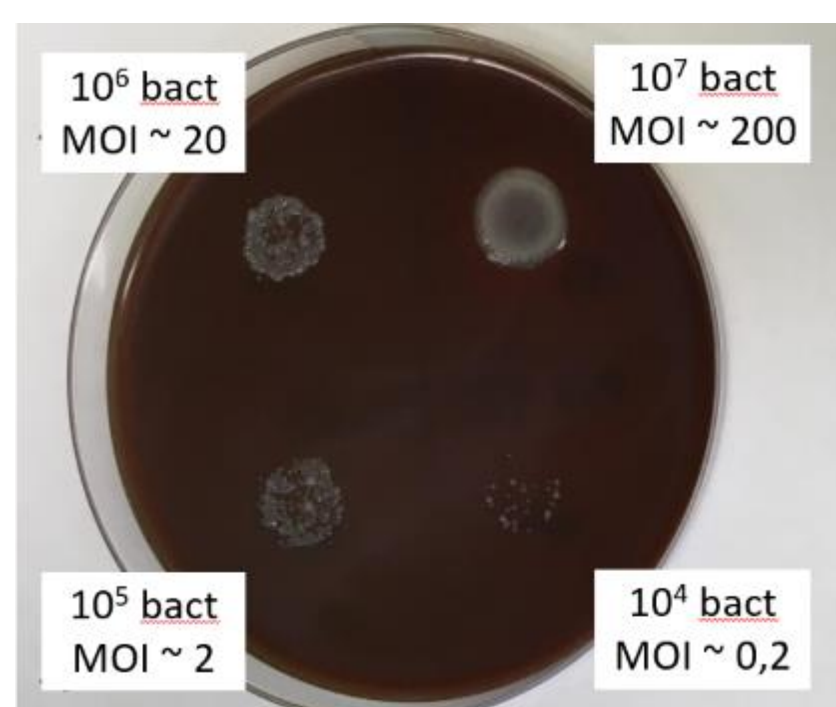
	ISFtu2-qPCR	Tul4-qPCR	Type B-qPCR
Target	insertion element-like sequence ISFtu2	<i>tul4</i> gene	specific junction between ISFtu2 and a flanking 3' region
<i>Francisella</i> species detected	<i>Francisella</i> sp.	<i>F. tularensis</i> (i.e., <i>F. tularensis</i> subsp. <i>tularensis</i> , subsp. <i>holarctica</i> and subsp. <i>mediasiatica</i>) and <i>F. novicida</i>	<i>F. tularensis</i> subsp. <i>holarctica</i>

RESULTS

Experimental study

Amoeba Plate Test model:

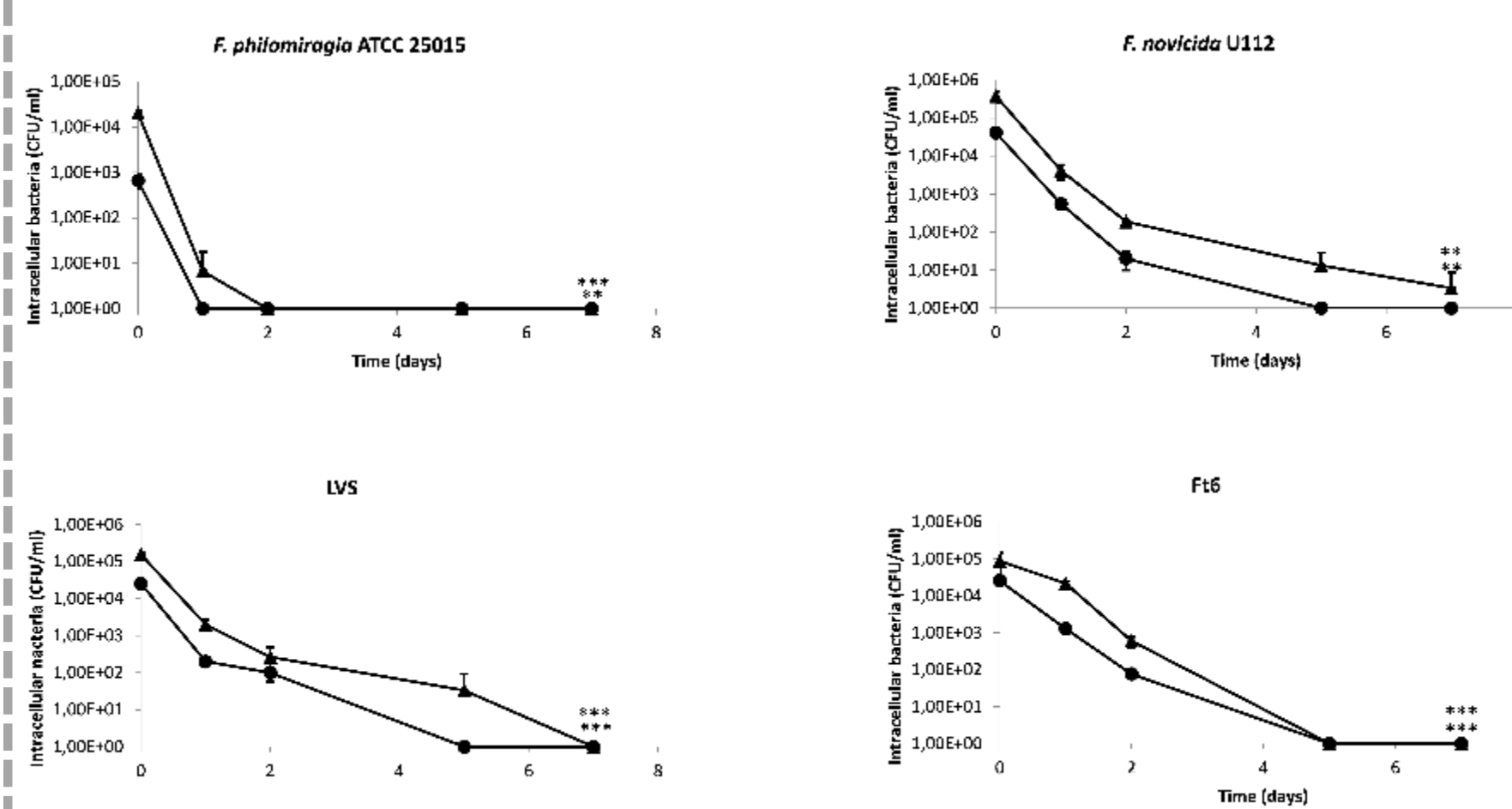
➔ *F. tularensis*, *F. novicida* and *F. philomiragia* are resistant to predation by amoebae



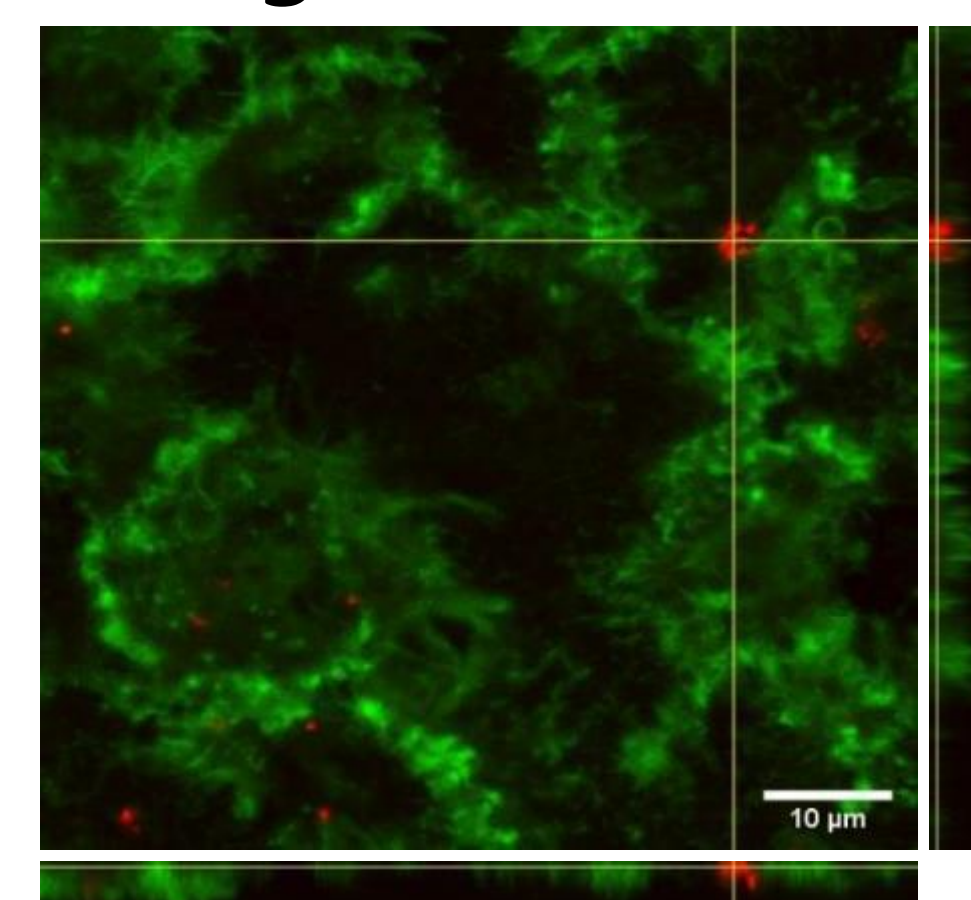
Amoeba Plate Test results of *A. polyphaga* and *F. tularensis* Ft6 at D9

Infection model:

➔ *F. tularensis*, *F. novicida* and *F. philomiragia* do not multiply inside amoebae



Infection of *A. castellanii* and *A. polyphaga* by *Francisella* sp. in SM. The error bars represent standard deviations of a triplicate. Comparison of T0 versus D7. NS: not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.



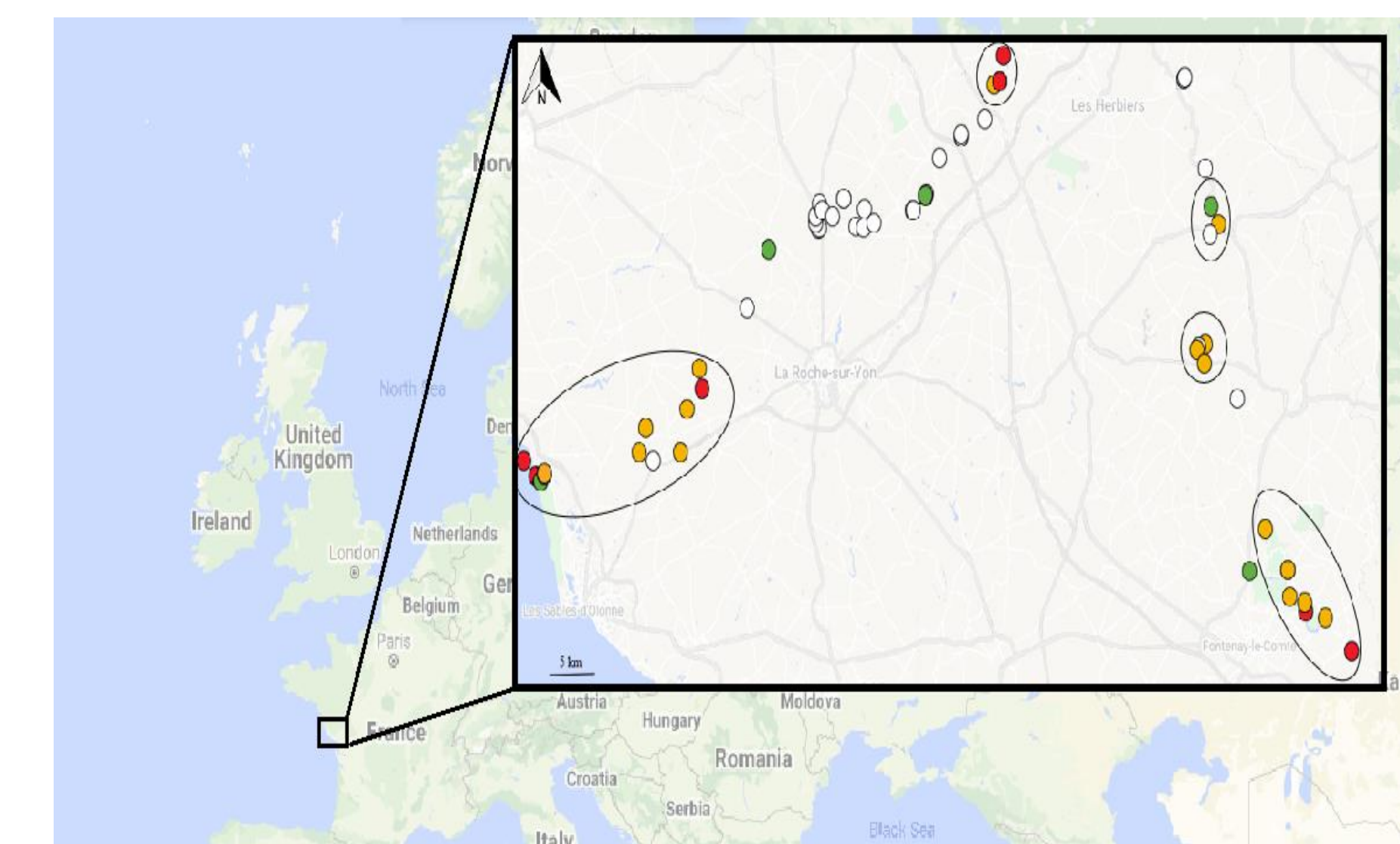
Confocal microscopy of *A. polyphaga* infected by *F. tularensis* Ft6 at T0. Green: actin (phalloidin Alexa Fluor 488). Red: *Francisella* sp. (rabbit primary antibody against *Francisella*, goat secondary antibody against rabbit Alexa Fluor 594).

Environmental study

➔ Contamination of the aquatic environment with *Francisella* sp. DNA, including *F. tularensis* subsp. *holarctica*, is relatively common.

	First campaign (july 2019)	Second campaign (january 2020)
<i>Francisella</i> sp.	26.3% (15/57)	83.3% (25/30)
<i>F. tularensis</i> / <i>F. novicida</i>	15.8% (9/57)	80.0% (24/30)
<i>F. tularensis</i> subsp. <i>holarctica</i>	7.0% (4/57)	13.3% (4/30)

9/30 sites sampled during the two campaigns were positive both times.



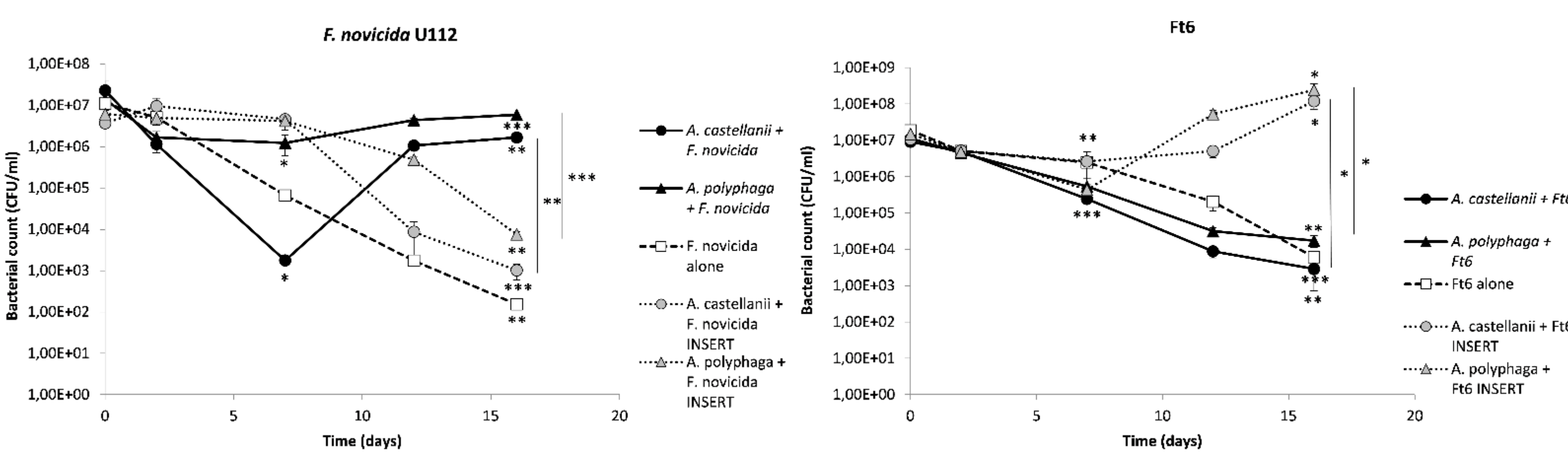
Francisella sp. detection in surface water samples collected during both campaigns.

Disk: water sampling site. White disk: sample with no positive qPCR. Green disk: sample with positive ISFtu2-qPCR in the first campaign or the second campaign or both, and negative Tul4 and Type B-qPCR. Orange disk: sample with positive ISFtu2 and Tul4-qPCR in the first campaign or the second campaign or both and negative Type B-qPCR. Red disk: sample with positive ISFtu2, Tul4, and Type B-qPCR in the first campaign or the second campaign or both. Circles indicate sites where water samples were collected during the two campaigns.

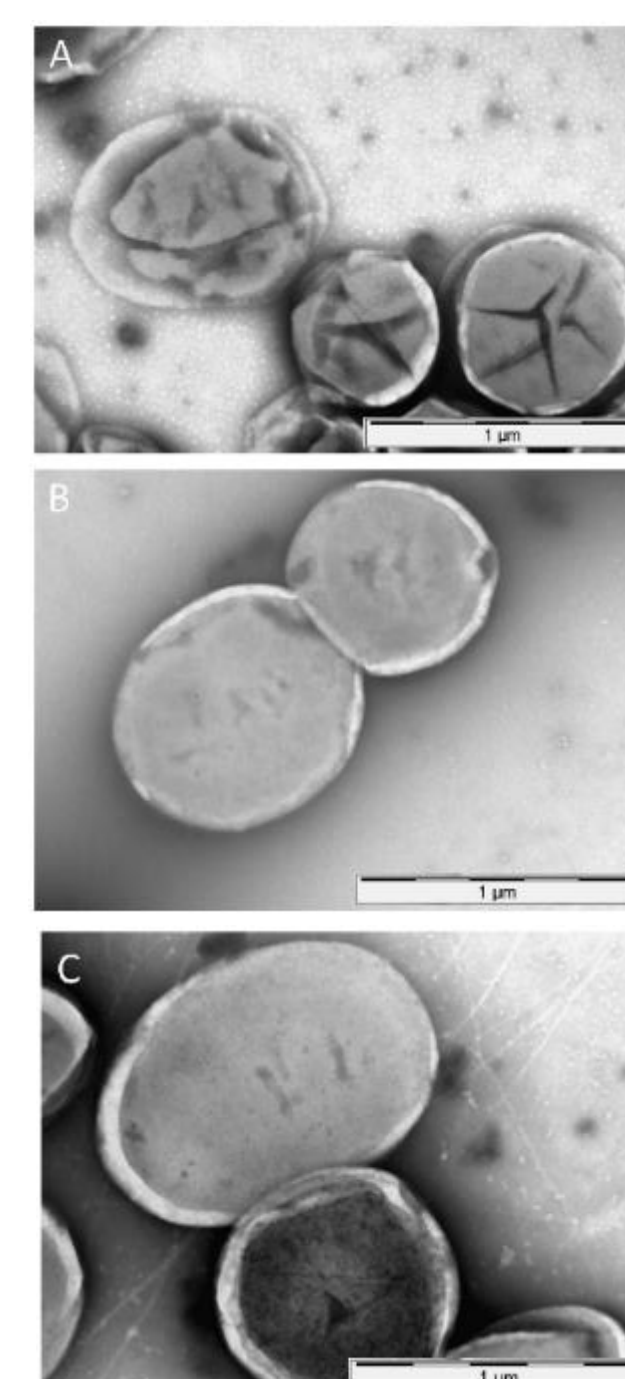
Co-culture model:

➔ *F. tularensis*, *F. novicida* and *F. philomiragia* survive and multiply in the presence of amoebae over a 16 days period

➔ Amoebae preserved bacterial morphology in co-culture model



Co-culture of *A. castellanii* and *A. polyphaga* with *Francisella* sp. in SM. The error bars represent standard deviations of a triplicate. Comparison of T0 versus D7 or D16; or D7 vs D16. Comparison of bacteria with amoebae separated or not by an insert at D16. NS: not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.



Morphology and structure of *F. novicida*, after 15 days incubation in spring water (A), co-incubation with *A. castellanii* in spring water (B), and as a control after growth on BCYE agar (C) by TEM.

CONCLUSION - DISCUSSION

- Survival of *F. tularensis* in the aquatic environment favored by amoebae
- High prevalence of *F. tularensis* in the aquatic environment
- ➔ Aquatic environment can be a natural reservoir of *F. tularensis*, and thus could participate in the maintenance of this pathogen in tularemia endemic areas.

Still to be explored :

- Other ways of *F. tularensis* survival in water: planktonic form, viable but non-culturable form (VBNC), biofilms, ...
- Infectivity of the forms recovered in water