Prevalence and characterization of *Salmonella spp.* in three shellfish-harvesting areas in France.

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Shellfish farming is an important economic activity in the Brittany and Normandy regions. However, a part of the production sites corresponds to relatively sensitive areas where the presence of faecal microorganisms is a major concern for shellfish and constitutes a possible health risk. Indeed, shellfish bioaccumulates in their tissues pathogenic contaminants present in water and can cause food-borne diseases such as salmonellosis.

During a two-year study, we evaluated the presence of faecal indicators, measured the prevalence of *Salmonella spp.*, isolated and characterized *Salmonella spp.* from three French shellfish-harvesting areas (shellfish and sediment) and their watersheds (from river water samples).

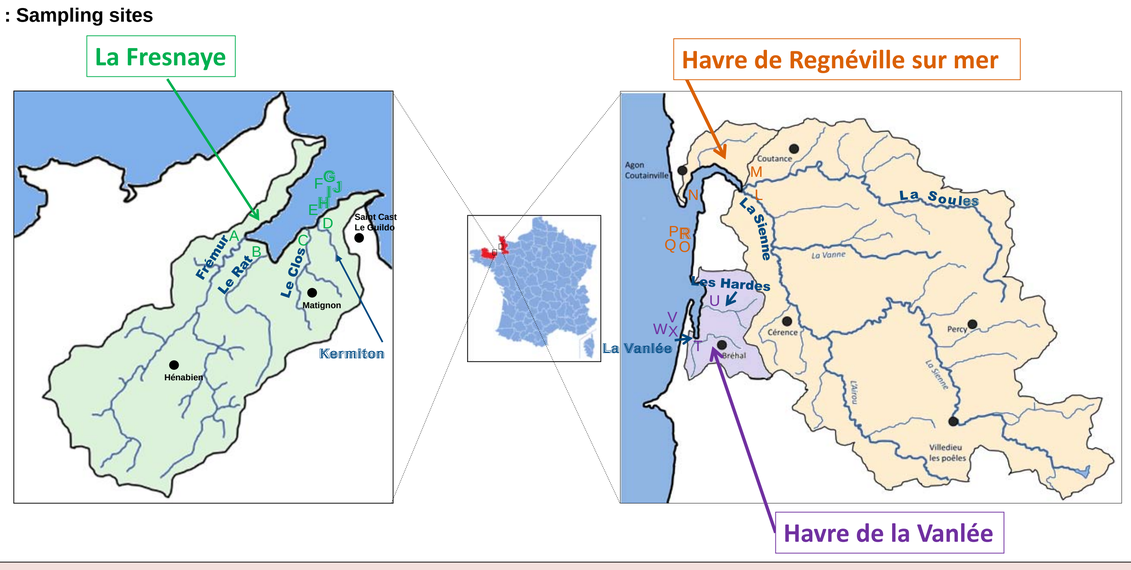
Studied catchment sites

In Brittany, the site "**La Fresnaye**", is a 121 km² watershed, characterized by intensive livestock farming (cattle, pig, and poultry), with a human population of about 9,000 inhabitants. Main rivers are "Frémur", "Le rat", "Le clos", and "Kermiton" (Fig. 1).

The 1,000 Km² catchment "**Havre de Regnéville sur mer**" includes 2 main rivers "La Sienne" and "La Soules". It is characterized by a population of 40,000 inhabitants and a large livestock (cattle, sheep, pigs, poultry).

The catchment "**Havre de La Vanlée**" (50 Km²) on which live about 7,000 people (twice more during the summer) is also important for breeding animals (cattle, sheep, pigs). Main rivers are "La Vanlée", and "Les Hardes".

Fig 1 : Sampling sites



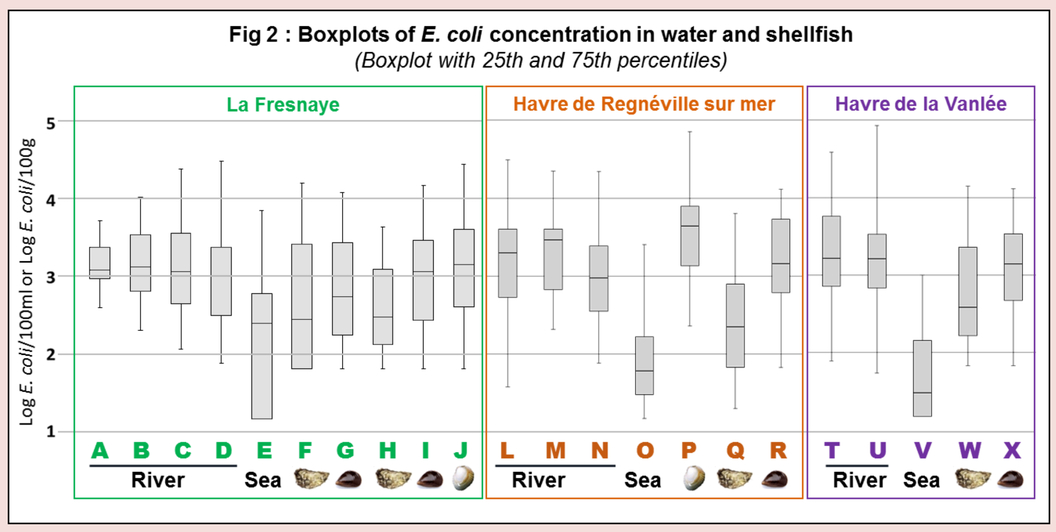
samples

Shellfish (oyster, mussel, and cockle, n=232), upstream water samples (n=225), seawaters (n=39) and sediment samples (n=39) were collected monthly from February 2013 to January 2015. In the same time, the height of 2-days cumulative rainfall before sampling date was measured.

Enumeration of the faecal indicator *E. coli*

Concentrations of *E. coli* were measured in shellfish samples, river waters but also in seawaters collected next to shellfish. They were usually relatively low in seawaters while they were generally high for water samples from rivers and for shellfish (Fig. 2). Oysters were found to be the less contaminated shellfish.

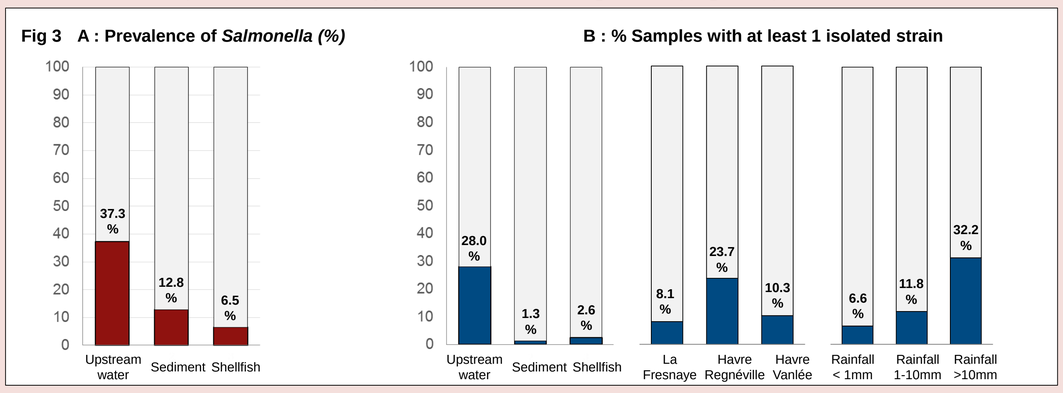
Fig 2 : Boxplots of *E. coli* concentration in water and shellfish
(Boxplot with 25th and 75th percentiles)



Detection and isolation of *Salmonella spp.*

Salmonella spp. was detected according to the protocol based on NF EN ISO 6579 and detection of *invA* and *ttrBCA* genes was done by rt-PCR using primers and probes described in Gonzales et al., 2012 [1] after an enrichment step. 37.3% of water samples, 12.8% of sediment samples and only 6.5% of shellfish enrichments (15/232) were positive for both genes (Fig. 3A).

Fig 3 A : Prevalence of *Salmonella (%)* **B : % Samples with at least 1 isolated strain**



In total, 462 clones from 67 samples were isolated. Of these, 74 isolates were retained for our analysis. The remaining 388 isolates were considered replicates for the purpose of the present study (same serotype from the same sample).

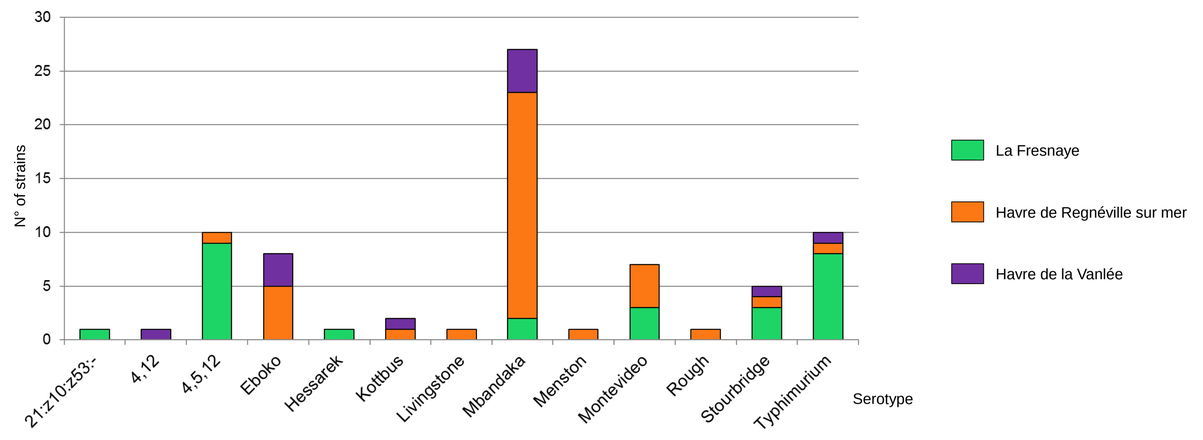
Most of the samples with at least one *Salmonella* strain isolated were waters (28.0%) and it is at the site Régneville sur mer and when the height of rainfall exceeded 10 mm/48 h that *Salmonella spp.* were most frequently isolated (Fig. 3B).

[1] : González-Escalona et al., 2012, Food Research International 48 : 202–208

Characterization of *Salmonella spp.*

From the 74 *Salmonella* strains retained for further analyses, 70 came from water, 1 from sediment and 3 from shellfish (1 from each type of shellfish and from each site). All of them were serotyped and the 74 strains were assigned to 13 different serotypes (Fig. 4).

Fig 4 : Serotyping of *Salmonella strains*



Mbandaka was the most frequently found serotype and was the only one serotype found for the three shellfish positive samples. Ten strains from the Typhimurium serotype were isolated and 10 other carrying O antigens 4, 5, and 12 were immobile or monophasic with H antigen i.

CRISPOL typing was performed on 18 strains, 9 *Salmonella* Typhimurium strains belonged to CRISPOL types (CT) 1, 18, 21, 30 or 76 and 9 immobile or monophasic strains carrying O antigens 4, 5, and 12 belonged to CT 1 or 18.

Although E. coli enumerations have revealed relatively high faecal contamination of the shellfish taken from the three sites (shellfish are classified as B or C category according to European Directive 91/492/EEC of the shellfish), and while Salmonella spp. were frequently detected and isolated from river waters, they were rarely detected in shellfish. The risk of a human infection by Salmonella spp. caused by shellfish consumption from these sites seems limited due to the low prevalence of Salmonella spp. in the shellfish batches analysed in this study and due to the depuration step or relaying step which have to be performed before shellfish from these categories reach market.
Seventy-four Salmonella strains were isolated and characterized. Twenty-seven belonged to the serotype Mbandaka and these mainly came from river water samples of the site "Havre de Regnéville sur mer" while strains from Typhimurium serotype or non-motile or monophasic variants of Salmonella Typhimurium carrying O antigens 4, 5, and 12 mainly came from river waters collected at "La Fresnaye" site.

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