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Food safety analysis of Moroccan camel milk samples

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Introduction

Camel milk is considered as the staple food in most of the Saharan pastoral areas. It possesses all the advantages of milk and several therapeutic virtues are attributed to it (1). However, camel breeding still plays a relatively weak role at the global level. **Food safety concerns** remain one of the **major constraints** limiting the widespread consumption of this milk. In addition to the danger associated with the potential presence of **human pathogenic bacteria** in camel milk, **mycotoxins**, in particular **aflatoxin M1**, must be the subject of special monitoring.

The main objective of the **EU ARIMNet 2 CA.RA.VA.N project "Toward a CAmel tRAnsnational VAlue chaiN"** is to promote a set of interdisciplinary measures capable of generating **knowledge and practices contributing to the socio-economic development of the camel sector in North Africa** (Algeria, Morocco and Tunisia) and in particular to **promote the safety of foods from camel origin**.

This poster presents the safety evaluation of Moroccan camel milk samples by bacterial communities and aflatoxin M1 analysis.

Camel milk sampling

Sixty-five samples of camel milk collected in 2018 by IAV (Rabat)
- originated from different areas of **Morocco**, in particular from **Laâyoune, Essmara, Jrifila , Msayed , Dakhlah , Rabat** and **Fes**
- during **milking** but also at different **points of sale**

Mycotoxins analysis

Aflatoxin M1 (AFM1) was determined using Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS)

- Preparation of camel milk samples

- LC/ESI-MS/MS analysis (2)

Ultra-High-Performance Liquid Chromatography (UHPLC, Shimadzu, Tokyo, Japan) coupled with a mass spectrometer (8040, Shimadzu, Tokyo, Japan) with an electrospray Ionisation (ESI)
Flow : 0.4 mL/min
- Injection volume : 50 µl
- Kinetex 2.6 µm C18 100A 50 × 2.1 mm ID column (Phenomenex)
- Column temperature was maintained
- Mobile phases
  phase A : 99.5 % ultra pure water with 0.5 % acetic acid
  phase B : 99.5 % isopropanol ; 0.5 % acetic acid
- Retention time for AFM1 = 1.32 min

- Optimization and validation of the **analytical method** on camel milk samples according to the French Standardization Association (AFNOR) FD CEN / TR 16059, ISO 5725 standard and COMMISSION REGULATION (EC) No 401/2006
- Analysis of the **65 samples of camel milk** for the presence of **Aflatoxin M1**
- Multi-mycotoxins analysis

RESULTS

Performance criteria for Aflatoxins M1

- **Calibration Curve** : y = 1,60303x +0,0477802
- **R2** = 0,9971
- **Linearity** : AFM1 Range 0,0625 to 5 ng/mL
- **LOD** : 0.00625 µg/kg
- **LOQ** : 0.01875 µg/kg
- **Recovery** : 92.4 %
- **RSD** : 92 %

An analysis of multi-mycotoxins (aflatoxins B1, B2, G2, G1, ochratoxins, fumonisins, trichothecens) was carried out on all the samples by the same analytical method and the concentrations of the multi-mycotoxins analysed were below their detection threshold. The results showed that aflatoxin M1 was not detected in any of the samples analysed.

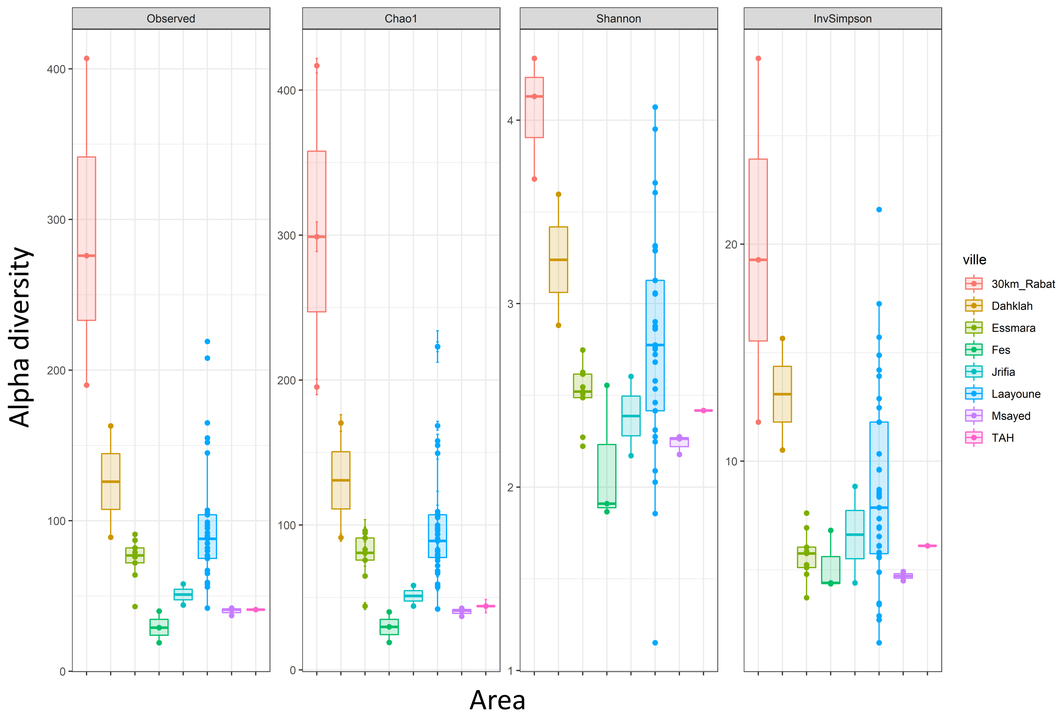
Bacterial community analysis

Bacterial communities were characterized using metabarcoding approach targeting V3-V4 16S rRNA gene amplified from phenol-chloroform extracted DNA

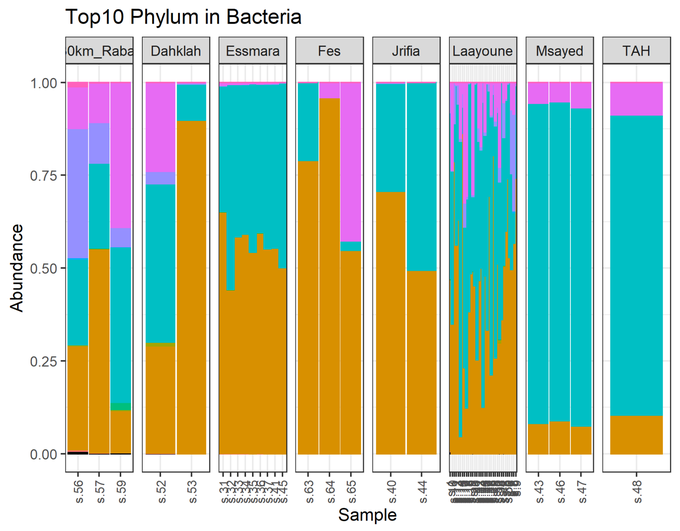
A 444 bp fragment of 16S bacterial DNA was amplified using primers 341F and 785R and sequenced on an Illumina Miseq platform (University of Montpellier)
Sequence data were analysed using dada2 and phyloseq R packages

RESULTS

Alpha diversity distribution by area



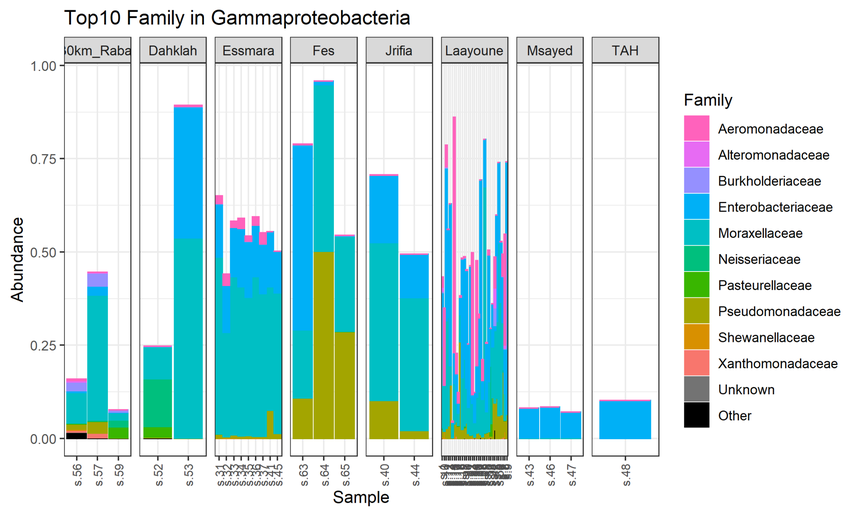
Bacterial alpha diversity was greater in samples from Rabat compared to others



***Firmicutes*, *Actinobacteria* and *Proteobacteria* were the main phyla**

Among *Proteobacteria*, *Gammaproteobacteria* was the most represented class (not shown)

Potential presence of human pathogenic bacteria (*Enterobacteriaceae* but also *Pseudomonadaceae*, etc.) in most samples



Bacterial diversity seemed more linked to geographic origin than to sampling methods. Bacterial genera should be investigated in order to conclude on the safety of the milk samples.

Conclusion

In term of mycotoxin content, all camel milk samples could be considerer as safe. The metabarcoding approach showed complex bacterial communities. Bacterial diversity seemed more linked to geographic origin than to sampling methods. Additional sampling from different North-African countries should be performed in order to confirm these preliminary results.

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