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LABERCA

[a] Oniris, INRAE, LABERCA, 44300 Nantes, France
Fax : +33 (0)2 40 68 78 78 - Tél : +33 (0)2 40 68 78 80
laberca@oniris-nantes.fr
[b] INRAE, Oniris, BIOEPAR, 44300 Nantes, France
[c] INRAE, VetAgro Sup, Univ Lyon, EPIA, 69280 Marcy-L'Etoile, France

MELISA — METabolomics LIpidomics Steroidomics Analysis
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Introduction

The tick *Ixodes ricinus* (Arthropoda; Chelicerata; Arachnida; Acari) is an obligate hematophagous ectoparasite of vertebrates, and the main vector of tick-borne diseases for humans and animals in Europe. The time a tick spends searching for a suitable host can vary from days to years (from 2 to 6 years). **Knowing the age of ticks is thus an important data** for understanding the epidemiology of tick-borne diseases. Unfortunately, there is currently no appropriate method for determining tick age that has been experimentally validated.

Because between each blood meal, the tick uses its lipid reserves while waiting for a new host, in this study we investigated potential triglycerides, a major constituent of tick lipids and a non-renewable energy source, as a marker of aging (*i.e.*, time since last blood meal).

Monday morning at BioEpar — How old are you ? — Easy ! look at my lipids profile
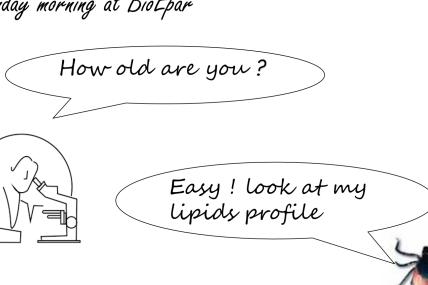
Sample preparation

Ixodes ricinus female adult (A0:year 2014, A1:year 2015, host: roe deer) and nymph of known age from 0 to 3 month after moulting (N0, N1, N2, N3 year 2015, host: Gerbil) were obtained after their metamorphosis in the laboratory and individualized.

Female Adult — Male Adult — Larvae — Nymph — 0 — 1.5 cm
Developments stage *Ixodes ricinus* (InVS, 2015, from Parola *et al*)

✓ Several protocols were evaluated and the one below was selected.
✓ Due to the small weight of samples (80 µg to 300 µg) the drying step have been optimized
✓ Grinding can be done with a Precellys

Fresh weight with **microbalance** (Sartorius MSA2.7S-000-DM) → Drying step 1 h 60°C for nymphs & 2 h for adults with **Speed vac** → Dry weight with **microbalance** → Grinding with **Tissu Lyzer** → Extraction **MTBE** MTBE:MeOH:H2O (Matyash et al., 2008)
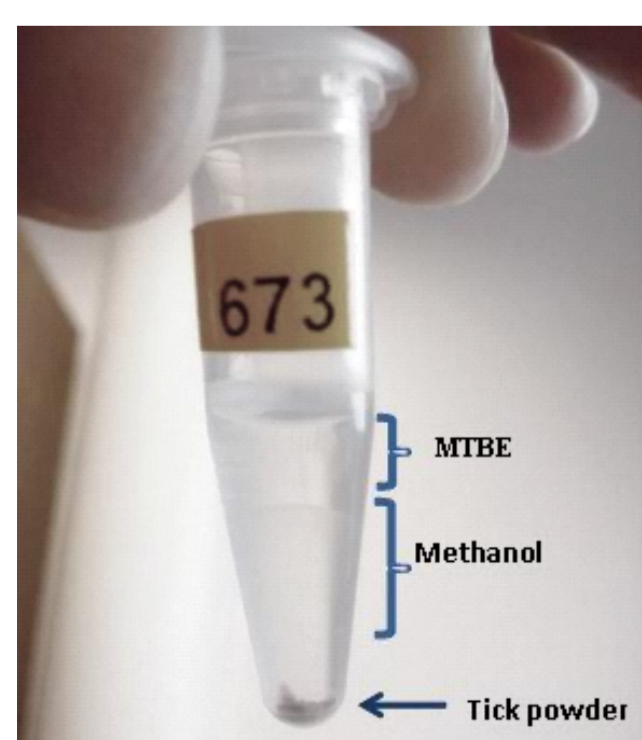
In brief
+ 220 µl MeOH
+ 675 µl MTBE
+ 188 µl water
+ Vortex
Centrifugation
Transfert 300 µl supernatant to an HPLC vial
ISTD addition & evaporation
Resuspention in ACN:IPA:water (65:30:5)

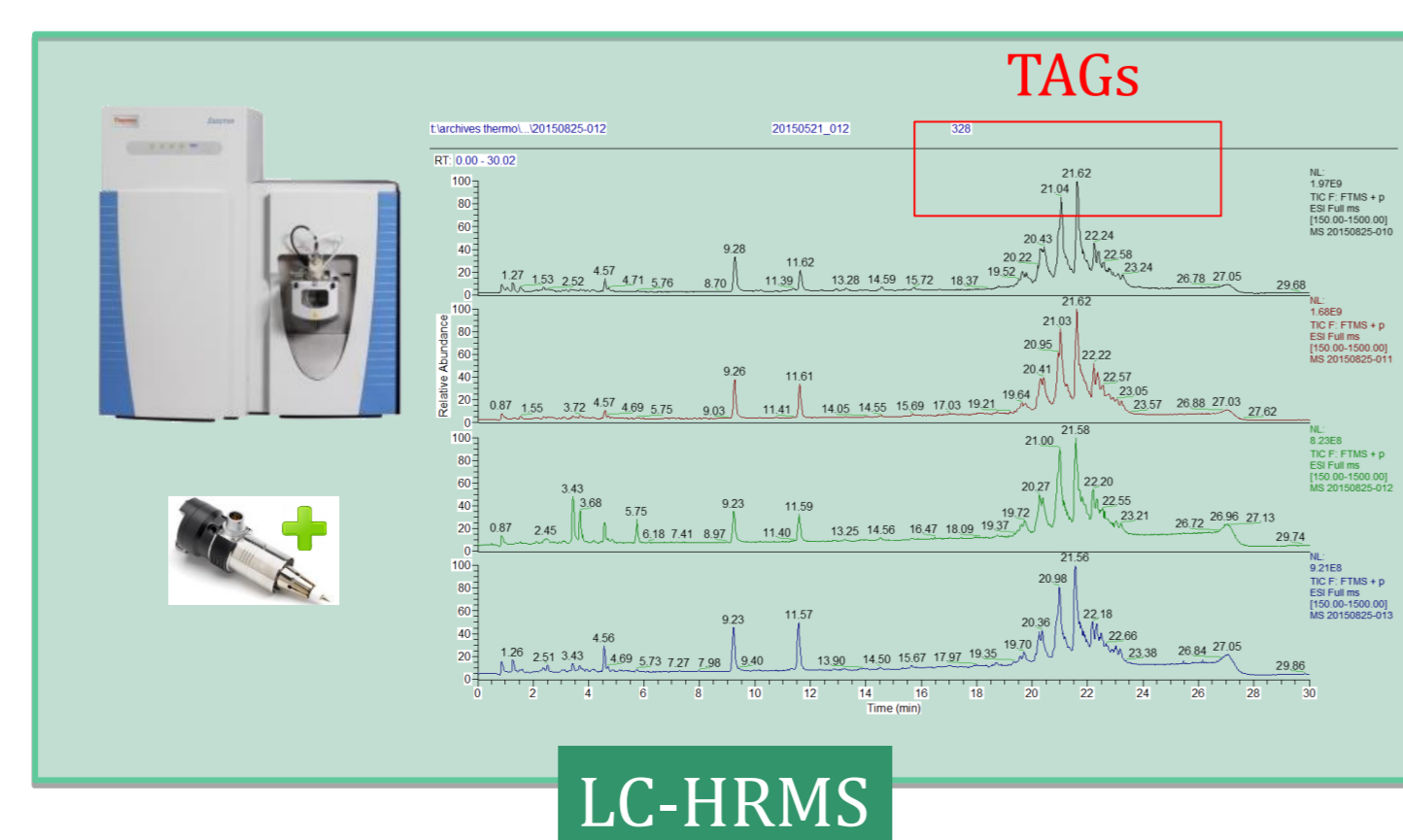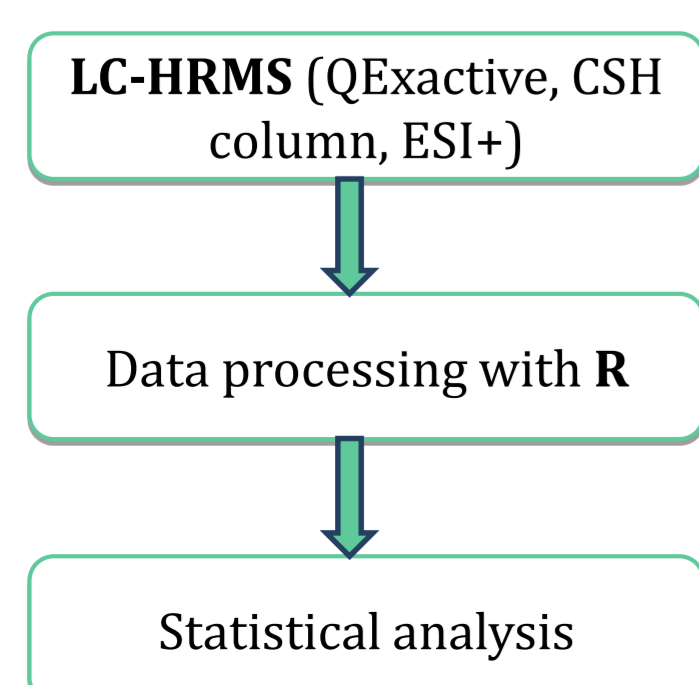MTBE — Methanol — Tick powder

LC-HRMS (QExactive, CSH column, ESI+) → Data processing with **R** → Statistical analysis

TAGs
LC-HRMS

Results

Principal Component Analysis (PCA) were applied to describe the patterns observed in the data. For all multivariate statistics, the data wee log10 transformed and Pareto scaled. A total of 52 nymphs (13 for each the 4 groups N0, N1, N2, N3) and 10 adults (5 for each two groups A2 and A14) were analyzed. The PCA was performed on the data acquired in ESI+ mode with R considering the totality of the extracted MS signals in the TAG portion of the chromatogram. With our data processing workflow 1510 variables (features) were extracted for the nymphs and 1595 for the adults. The number of features was finally reduced to 457 and 526 ions using quality filters (reproducibility of the signal in quality controls, chromatographic peak shape...).

A — Scores - PCs 1x2 PC1 55.827 % PC2 24.806 %
B — Scores - PCs 1x2 PC1 42.977 % PC2 13.324 %
Principal Component Analysis of TAG portion of LC-HRMS lipids profiles for Adults (A) and Nymphs (B)

A — M1140T1424
B — M1140T1425
Boxplot for feature M1140T1424 (Intensity=area under the curve) found statistically significant between profiles for Adults (A) and Nymphs (B)

Method — The methodology is now in place and can be applied to new studies

The formal identification of the statistically significant ions is not done yet. — Biomarker identification

Conclusion

Total lipids profiles of individual ticks (especially nymphs) were analyzed by LC-HRMS (QExactive Orbitrap). By Principal Component Analysis based on potential triglycerides signals, we were able to reveal a gradual change in lipid composition between nymphs aged from 0 to 3 months since the last blood meal, validating the proof of concept of this method for estimating tick age. Potential applications of this method will be investigated, as well as its utility for tick-borne disease epidemiology and vector control.