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Molecular typing of Belgian *Echinococcus multilocularis* specimens from alveolar echinococcosis human lesions using EmsB microsattelites analysis

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INTRODUCTION

The genetic diversity of *Echinococcus multilocularis* (Em) is a major field of investigations to correlate with sources of infection or variable clinical manifestations of the alveolar echinococcosis (AE). Molecular markers able to distinguish strains are already used such as the EmsB microsatellite (Knapp J. *et al.*, 2007). This marker is present in about 40 copies in the Em genome.

AIM

The molecular characterization of Em has never been done before in Belgium. Here, we report the use of EmsB microsatellite polymorphism for the typing of Belgian specimens isolated from patients with AE between 2015 and 2020 and their introduction into the European EWET data.

METHOD

Total DNA was isolated from liver, pleural fluid and lung biopsy samples using a DNA extraction kit for tissue (Promega). The PCR was performed according to Knapp et al, 2020. The EmsB A primer was 5'-labeled with FAM-fluorochrome. Fragment size analysis was performed on an ABI3500 automatic sequencer (ThermoFisher). The fluorescence signal was detected by colorimetric reading. Correspondences were established to assess the size of the amplified fragments using Gene mapper (ThermoFisher). "R studio" was used to generate a distance matrix, calculate the Euclidian distance and obtain a UPGMA method dendrogram in order to assess the genetic similarity among samples. The profiles obtained were compared with those included in the EWET data collection (Knapp *et al*, 2017).

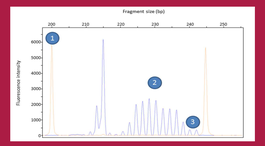

Figure 1: Electrophoregram of EmsB-PCR products (1): size standard in orange, (2): the EmsB fragments classified by size in blue (in base pair) and (3) peaks under 5% of the highest peak to be removed from the analysis. (From « EmsB analysis guidelines V.7 » Knapp J. et al, 2017)

Sample results obtained after migration on a ABI3500 were treated as followed:

- The **peak size was adjusted** compared to **the calibrator** values (our own values) and the reference values
- The peaks that showed intensities **lower than 5%** of the higher fluorescence intensity, **were removed** and putted to zero.
- Then the fluorescence values were **normalized**: for each peak the fluorescence is divided by the entire fluorescence values sum for a given sample.

Adjusted peak size	215	217	219	221	223	225	227	229	231	233	235	237	239	241	243	
Original fluorescence intensity (FI)	6185	0	0	300	831	1985	2210	2368	2252	1741	1716	1642	867	455	450	Sum
FI without peak 5%	6185	0	0	0	831	1985	2210	2368	2252	1741	1716	1642	867	0	0	21797
Normalized FI values	0.28	0	0	0	0.038	0.091	0.101	0.108	0.103	0.079	0.078	0.075	0.039	0	0	1

Table 1: Table showing the different steps of the treatment of the data obtained after EmsB amplification by PCR. (From EmsB analysis guidelines V.7 Knapp J., 2017)

RESULTS

- Twenty six specimens have been successfully analyzed. **In figure 2**, only the Belgian strains are represented on the dendrogram of similarity. We can distinguish five different groups (G) among our strains. One comprising 9/26 strains is the biggest group, a second group contained 4/26 strains and the Fe792 French reference strain, then three small groups of 2 strains each are also visible in the dendrogram. "Groups" were defined when the Euclidian distance was lower than 0.1 between samples.
- According to a comparison with European samples previously characterized from the **EWET** collection (Knapp J. *et al.*, 2017), 9 Belgian specimens shared the same **P1 genomic profile** (P) while two other strains shared a profile near to P1 that we renamed **"like P1".** This P1 profile has already been characterized in Switzerland, Germany and France. Three strains are included into the **P8 profile** (together with the reference strain Fe792). This P8 profile has essentially been found in France since now. Two strains shared the **P4 profile** (already found in France and Germany) while one strain was **assimilated to P9** profile. P9 profile was present in France and Switzerland. Nine out of 26 strains, were out of known profiles (**Figure 3**).

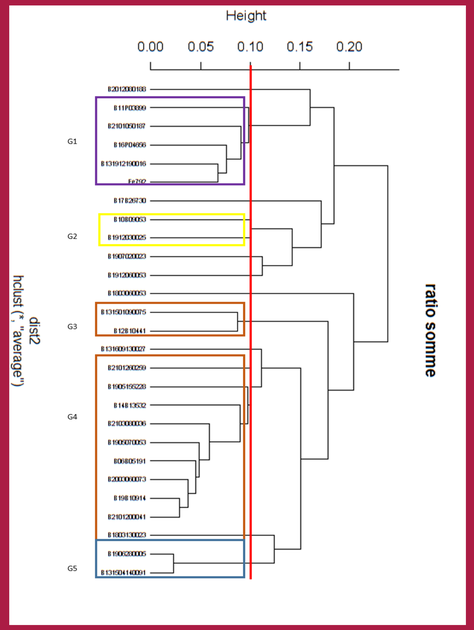

***Figure 2**: Dendrogram representing the twenty six Belgian strains and 1 French reference strain (Fe792) (Based on Euclidian distances and UPGMA method). G= group*

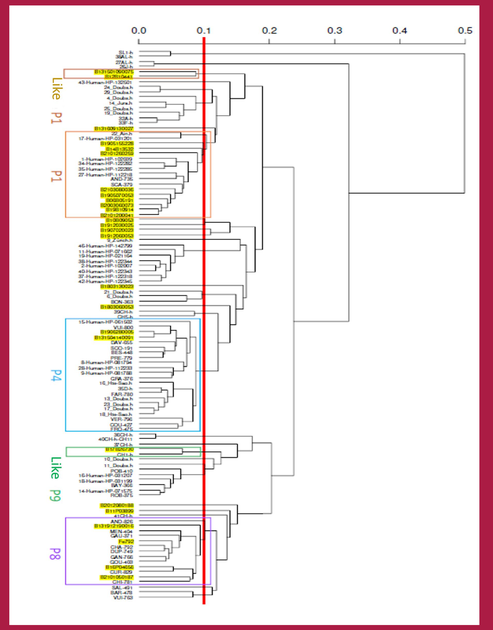

***Figure 3**: Dendrogram representing the twenty six Belgian strains among other European strains from the EWET data collection. Belgian strains are highlighted in yellow (except Fe792 which is a French reference strain)(Knapp J. et al, 2020) . P=profile*

- **Figure 4** represents the map of Belgium with different profiles found in different Belgian regions in reference to EWET profiles. We can see that no clear relationship can be established between the different profiles found and the geographical region of residence of the patient. However P1 and "P1 like" profiles are essentially found in South West Belgium.

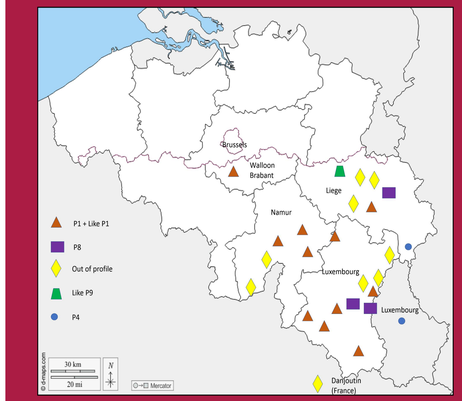

***Figure 4**: Map representing the geographical repartition of the analysed strains (Fe792 which is a French reference strain, is not included)*

CONCLUSIONS

The EmsB microsatellites analysis allowed to genotypically characterize Em clinical specimens isolated in Belgium for the first time. Twenty six strains have been treated by EmsB method in this work and will be further published. This study highlights that some Belgian samples share the same genotypic profile already found in other European countries but that heterogenetic diversity exist in Belgium. Some profiles are unique and differ from other European ones. No clear correlation can be established between the geographical location of the patient and the belonging to a given profile. Information about trips and risk factors experimented by patients have to be obtained to go further in the comparison between epidemiology and parasite genetic diversity.

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