Book of abstracts

GERI 2015

Genes, Ecosystems and Risk of Infection

21-23 April 2015

Aquila Atlantis Hotel,
Heraklion, Crete, Greece

www.geri2015.edenext.eu

Identifying the last bloodmeal of questing wood tick nymphs (Ixodes ricinus L.) by DNA amplification: three approaches tested

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Tick-borne disease risk can be modelled more accurately when the main hosts of the most common European tick vector, I. ricinus, are known for a given area. However, I. ricinus is known to feed on more than 300 species in Europe, and estimating their relative importance from field observations (which includes live-trapping of hosts to count ticks) is time-consuming and likely to be highly biased, since individual ticks spend only a few days a year feeding. On the other hand, I. ricinus ticks are easily collected from vegetation while questing; therefore, more recent studies have focussed on using molecular markers in these individuals to identify their last bloodmeal. Here we present three different protocols that we optimized to detect bloodmeal sources and discuss the quality of the results: Reverse Line Blot Hybridization (RLBH), Next Generation Sequencing (NGS) and, for the first time, High Resolution Melting Analysis (HRMA). Regarding RLBH and NGS, we managed to limit contamination and increase the quality of results, but some uncertainty remained. Instead, using HRMA, we showed that with six newly designed host-group primers (Muroidea, Soricidae, Passeriformes, Canidae, Caprinae, Artiodactyls), we could successfully amplify 20 target host species and genera. When first tested on a limited number of questing nymphs, the new protocol showed high sensitivity (bloodmeal sources were identified in 65.4 % of nymphs), reliable mixed bloodmeal identification and high identification success (35 out of 42 amplified bloodmeals were identified to genus or species), and low contamination levels. In order to improve the cost-effectiveness and productivity of the HRMA protocol, we then automated both the extraction method and PCR reaction setup for an additional 741 nymphs. Although mean sensitivity decreased to 21.5 % (159/741 nymphs), identification success and contamination control were maintained. HRMA results confirm the importance of Apodemus spp. as larval hosts (58/173 bloodmeals), but also indicate that the domestic dog, C. l. familiaris (37/173) may support larval populations. In conclusion, we present the pros and cons of these and other published techniques and the prospects for improvements. We also discuss the epidemiological implications of the results.