The evaluation of host antibody response to *Ixodes ricinus* as an indicator of exposure and disease risk

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Antibodies against tick salivary protein antigens are potential markers of tick exposure. In a systematic review of *Ixodes spp.* salivary proteins and host-response, we selected calreticulin as a candidate antigen with which to study the antibody response to *I. ricinus* bite. The aim was to determine if human antibody responses to calreticulin detect exposure to ticks, and whether bioinformatic prediction may be used to develop a peptide-based immunoassay.

I. ricinus calreticulin (Genbank ID: AAR29958.1) was expressed as a recombinant protein (rCT). Linear B-cell epitopes were predicted using IEDB tools and non-linear epitopes using Ellipro and Discotope. Putative cross-reacting B-cell epitopes were screened using protein BLAST search. Overlapping 15amino acid oligopeptides were synthesized to cover predicted diagnostic epitopic regions. Sera were obtained from human subjects with confirmed tick bite within 48h of exposure and also at 3 month follow up. Negative control sera were obtained from a commercial biobank.

Overall subjects exhibited significantly increased IgG and IgM responses to rCT compared to negative control after a recent tick bite in ELISA studies; IgG responses were further enhanced three months after a tick bite. Immunoblots confirmed IgG and IgM reactivity against *I. ricinus* salivary gland proteins and rCT, with calreticulin being the most dominant and consistent antigen in crude salivary gland extracts as confirmed by LC-MS/MS. Bioinformatic analysis of *I. ricinus* rCT identified two putative B-cell epitopic regions. After eliminating peptide sequences that exhibited potential cross reactivity against host proteins and those of other haematophagous taxa, nine oligopeptides (15-mer) were synthesized. The

performance of the individual and pooled oligopeptides is being compared to that of rCT in the optimization of a tick bite immunoassay.

A platform for a tick (*I. ricinus*) bite immunoassay was established using ELISA and the specificity of the response confirmed by western blot. This assay refines previous work in individuals challenged with *I. scapularis* and offers the potential for monitoring tick exposure at the individual and population level in both high-risk groups and the wider population.