A new high-throughput analysis to screen *Ehrlichia ruminantium* in field ticks

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In order to improve sample screening capacity of *Ehrlichia ruminantium* in ticks, an automatic DNA extraction method for *Amblyomma* ticks and a new QPCR targeting *E. ruminantium* *pCS20* region, *pCS20* Sol1 QPCR, were developed. A comparison between the new *pCS20* Sol1 QPCR, a previously published *pCS20* Cow™ QPCR and the gold standard nested *pCS20* PCR have been carried out. *pCS20* Sol1™ QPCR was highly sensitive (up to 2.4 copies per sample) and specific (16 *E. ruminantium* strains detected and no cross-reaction with close-related species). We showed that Sol1 QPCR and *pCS20* nested PCR had similar sensitivity and specificity however there was limited risk of contamination and timeless process for *pCS20* Sol1 QPCR. In parallel, a tick 16S^{Syb} rRNA QPCR was successfully developed for DNA quality control. DNA yield and quality from ticks extracted automatically, using 16S^{Syb} rRNA QPCR were high. 16S^{Syb} rRNA QPCR results (n=671 ticks) showed also a good reproducibility of automatic DNA extraction with a mean Ct=23+/-3. The whole method of screening, including automatic DNA extraction and *pCS20* Sol1 QPCR, demonstrated a limit of detection between 6 and 0.6 copies per sample. It was also reproducible with restricted standard deviation of Ct (+/-1.3) for samples at the limit of detection. The development of a new automatic DNA extraction and QPCR allows for improving tick sample processing and *E. ruminantium* diagnostic capacities using high throughput methods. More widely, the automatic DNA extraction based on DNA/RNA virus kit

and the new DNA quality control method, $16S^{Syb}$ rRNA QPCR, can then be used for screening other bacterium and virus and for other tick species.