

Application of SHERLOCK detection for epidemiological surveys of Animal African Trypanosomiasis

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Animal African trypanosomiasis (AAT) is a disease caused by parasites of the genus *Trypanosoma*. After the successful development of a diagnostic test for human African trypanosomiasis, we have adapted the CRISPR-based detection toolkit SHERLOCK (Specific High-sensitivity Enzymatic Reporter unlocking) for trypanosomatid parasites responsible for AAT. SHERLOCK first amplifies nucleic acid using recombinase polymerase amplification (RPA), which is then combined with the Cas13a nuclease for RNA target recognition via specific guides (crRNAs) and a fluorescent reporter linked to a quencher. Target sequence recognition by Cas13a results in promiscuous ribonuclease activity which cleaves the fluorescent reporter, emitting fluorescence used for detection. To test the applicability of this technique in the field, we analysed 360 domestic animal samples (sheep, goat, pig and dog) from two surveys in Cameroon and Côte d'Ivoire. The preliminary results of this pilot study show that the SHERLOCK4AAT method is able to detect and discriminate between trypanosome species involved in multiple infections with a high sensitivity, especially in blood samples. In Côte d'Ivoire, we determined that approximately 60% of the Trypanozoon-positive blood samples collected on free-ranging pigs were co-infected with *T. congolense*, while no *T. vivax* infections were detected. We are now focussing on further improving the sensitivity of the assay and developing a multiplex version for species discrimination in a single test.