

The majority of *Trypanosoma evansi* can be detected using diagnostic tests based on the variant surface glycoprotein (VSG) of *Trypanosoma evansi* Rode Trypanozoon antigen type (RoTat) 1.2. Exceptions are a number of *T. evansi* isolated in Kenya. To characterize *T. evansi* that are undetected by RoTat 1.2, we cloned and sequenced the VSG cDNA from *T. evansi* JN 2118Hu, an isolate devoid of the RoTat 1.2 VSG gene. A 273 bp DNA segment of the VSG gene was targeted in PCR amplification for the detection of non-RoTat 1.2 *T. evansi*. Genomic DNA samples from different trypanosomes were tested including 32 *T. evansi*, 10 *Trypanosoma brucei*, three *Trypanosoma congolense*, and one *Trypanosoma vivax*. Comparison was by PCR amplification of a 488 bp fragment of RoTat1.2 VSG gene. Results showed that the expected 273 bp amplification product was present in all five non-RoTat 1.2 *T. evansi* tested and was absent in all 27 RoTat 1.2-positive *T. evansi* tested. It was also absent in all other trypanosomes tested. The PCR test developed in this study is specific for non-RoTat 1.2 *T. evansi*.