

A direct card agglutination test for *Trypanosoma evansi*, CATT/*T. evansi* based on the predominant variable antigen-type (pVAT) RoTat 1.2 was evaluated previously in the field in Isiolo District, Kenya. Sixteen out of 51 (31.4%) parasitologically positive camels were negative by the antibody detection test. In the present study, trypanosomes isolated from the camels were analysed in an attempt to determine the cause of the false negative results of CATT/*T. evansi*. A total of 20 field isolates comprised 16 stocks from camels that were negative by CATT/*T. evansi*, and 4 from CATT/*T. evansi*-positive camels. In addition, 15 known *T. evansi* and four *T. brucei* were used as reference. Purified DNA samples were tested using an established RoTat 1.2-based polymerase chain reaction (PCR) that yields a 488 bp product for the specific detection of *T. evansi*. Antibodies to RoTat 1.2 variant surface glycoprotein (VSG) were used in Western blotting to detect RoTat 1.2 VSG linear epitopes. Results of PCR and Western blot showed that the 16 stocks isolated from CATT/*T. evansi*-negative camels fell into three groups. In Group 1, both the RoTat 1.2 VSG gene and the VSG were absent in three stocks. In five trypanosome stocks in Group 2, the RoTat 1.2 VSG gene was detected, but Western blot was negative indicating absence of the expressed VSG. Five other stocks containing the RoTat 1.2 VSG gene were also in this group. The RoTat 1.2 VSG gene was detected and Western blot was positive in all four trypanosome stocks in Group 3. All four stocks from CATT/*T. evansi*-positive camels contained the RoTat 1.2 VSG gene and the expressed VSG. The reference *T. evansi* KETRI 2479 lacked the RoTat 1.2 VSG gene and there was no immune reactivity detected by Western blot. The rest of the reference *T. evansi* stocks examined contained the RoTat 1.2 VSG gene. All the four *T. brucei* samples examined were negative by PCR and Western blot. In conclusion, this study showed that the RoTat 1.2 VSG gene was absent from some *T. evansi* trypanosomes in Kenya.