*Trypanosoma evansi* is a haemoflagellate of veterinary importance that can infect most mammals but is generally more severe in camels and horses. It causes surra, a disease of great economic importance in Africa, Asia and South America. The standard laboratory method for diagnosis of surra is to demonstrate and identify trypanosomes in the blood of the infected animal. Because parasitological techniques have low sensitivity, alternative methods are used to complement parasite detection. Serological methods are based on variable antigens of the trypanosome. *T. evansi*, like all African trypanosomes, has a surface coat composed of the variant surface glycoprotein (VSG) which acts as a defence against general innate immunity and against acquired immunity directed at invariant surface antigens. To counter specific antibodies against the VSG, trypanosomes undergo antigenic variation, the change to expression of a different VSG during which, distinct trypanosome populations are produced termed variable antigen types (VATs). Predominant VATs (pVATs) tend to appear regularly during the early stages of infection and can be used as diagnostic antigens. The *T. evansi* pVAT designated RoTat 1.2 was originally isolated from a buffalo in Indonesia and has been shown to be expressed in all *T. evansi* examined to date. CATT/*T. evansi*, an antibody detection test based on RoTat 1.2 VSG, has shown high specificity in various geographical regions of the world; false negative results have been observed but not investigated. No previous use of CATT/*T. evansi* has been reported in Kenya. Consequently, this study aimed to investigate the suitability of RoTat 1.2 VAT as a diagnostic antigen for *T. evansi* in camels in Kenya. In the survey conducted, 2227 camels were screened of which 2038 belonged to nomadic pastoralists in *T. evansi* endemic Isiolo District of Eastern Province. For comparison, parasite detection was used as the reference method and Suratex for the detection of circulating antigen. Results showed that CATT/*T. evansi* detected 35 of 51 parasitologically positive camels (68.6% sensitivity), and Suratex® 58.8% (30/51). Both tests were highly specific (100%). The overall prevalence was 2.3% (51/2227) by parasite detection, 32.2% (327/1017) by CATT/*T. evansi* and 19.6% (188/961) by Suratex®. It was concluded that CATT/*T. evansi* and Suratex® were able to detect aparasitaemic infections rapidly and were more sensitive than parasitological methods in revealing the true extent of trypanosomosis in a herd. A follow-up was conducted to investigate the false negative results of CATT/*T. evansi*.

Three possible explanations were considered for the false negative results by CATT/*T. evansi*: firstly, in early infections, antibody levels are too low to be detected. Secondly, the RoTat 1.2 VSG gene may be absent in some *T. evansi* isolates. Thirdly, the RoTat 1.2 VSG gene may be present but not expressed. Serological examination included immune lysis, direct agglutination, immunofluorescent antibody test (IFAT) and Western blot. The presence of the RoTat 1.2 VSG gene in the genome of the *T. evansi* trypanosomes was determined using two PCR tests, one targeting a 488-bp fragment and the other a 205 bp fragment of the RoTat 1.2 VSG gene. Expression of the RoTat 1.2 VSG was determined by Western blot analysis using specific anti RoTat 1.2 VAT antibody made in mouse. Results showed that of the 16 *T. evansi* isolates not detected by CATT/*T. evansi*, four lacked the RoTat 1.2 VSG gene and did not express isoVATs of RoTat 1.2. This group, designated Group 1, comprised JN 2118Hu, JN 6512Muh and JN 6524
Muh, JN 4306Moh and the reference *T. evansi* KETRI 2479. Group 2 *T. evansi* contained the RoTat 1.2 VSG gene not expressed. In Group 3, the RoTat 1.2 VSG gene was present and expressed, but no explanation was immediately found for CATT/*T. evansi* failing to detect this group of *T. evansi*. To characterize *T. evansi* that are undetected by RoTat 1.2, the VSG cDNA from *T. evansi* JN 2118Hu was cloned and the nucleotide sequence determined. A region of the sequence lacking similarity with already known trypanosome sequences was identified by GenBank homology search. A PCR test, targeting a 273-bp segment of JN 2118Hu gene, was developed which specifically identified all five non-RoTat 1.2 *T. evansi* tested. No amplification was observed in any of the 27 RoTat 1.2 positive *T. evansi* tested. All 10 *T. brucei* tested negative. In conclusion, this study showed that the diagnostic antigen RoTat 1.2 is specific for *T. evansi*, but lacks the potential to detect all *T. evansi*. Clearly, the RoTat 1.2 VSG gene may be sufficient, but not necessary to identify a parasite at *T. evansi*. This study has provided valuable information for the future development of a practical serological test for the sensitive and specific detection of *T. evansi* infections.