

ABSTRACT

Trypanosoma evansi parasite is the causative agent of trypanosomiasis (surra) in camels and other livestock with devastating economic consequences in many parts of the world, including Kenya, Vietnam, Brazil, China, Indonesia and the Philippines. The trypanosome is mechanically transmitted by tabanids and *Stomoxys* biting flies. Two strains of the parasite (A and B) have been documented to date where Type A strains is established across all *T. evansi* areas whereas Type B has been reported both in Kenya and Ethiopia. *T. evansi* has been emerging in non-endemic areas and infecting new hosts. It is also thought that *T. evansi* is under-diagnosed and the level of infection is greater than frequently reported. Investigations were conducted to establish 1) levels of virulence between various strains of *T. evansi* in the mouse model, 2) evolutionary origins of *T. evansi* in Kenya and 3) spatial expansion of *T. evansi* to other endemic regions of the world, in relation to *T. brucei brucei*, *T. b. rhodesiense* and *T. equiperdum* parasites, respectively. The absence of the tools that distinguish these strains of the parasite necessitate in-depth assessment of the key virulence indicators. The phylogenetic relationship in the various parasite strains enriched the current understanding of how *T. evansi* evolved and adapted to various regions. In a laboratory-based study, groups of male Swiss white mice were each inoculated with 17 isolates of *T. evansi* (Types A and B) collected from surra endemic countries (10 isolates from Kenya and 7 from South America and Asia). Following infection, the infected animals were monitored for parasitaemia, however, all groups of mice were monitored for live body weight, packed cell volume (PCV) and survivorship over the experimental period of 60 days (endpoint, above which all remaining animals were euthanized). In addition, DNA was isolated from 107 (41 *T. evansi*, 51 *T. b. brucei* and 15 *T. b. rhodesiense*) isolates, most of which were collected from Kenya and stored at the KETRI Cryobank. Individual DNA samples were genotyped with 15 polymorphic microsatellite markers to quantify levels and patterns of genetic diversity. Using the same microsatellite for the spatial expansion study, DNA was further extracted from an additional 11 isolates classified as *T. equiperdum* and 8 *T. evansi* were added to the already generated data (107 isolates) making a total of 132 isolates genotyped. Data was analyzed using one-way ANOVA in comparison of means of individual isolates from parameters recorded while for the genetic studies, distinct genetic clusters were identified using structure and Principal Component Analysis (PCA). Results of survivorship demonstrated three virulence categories; high virulence (0-10 days), moderate (11-30 days) and low virulence (31-60 days). Only one isolate was identified as Type B, depicting high virulence in mice. Differences in survivorship, PCV and bodyweights between isolates were significant and correlated with $P < 0.05$. The genotyping placed *T. evansi* isolates into at least two distinct *T. brucei* genetic units. Allelic richness within clusters of all isolates ranged from 2.10 to 3.86 indicating the lowest genetic diversity in cluster that contains both *T. b. brucei* and *T. b. rhodesiense*, but not *T. evansi* and the highest genetic diversity in cluster that contains *T. b. brucei*, *T. b. rhodesiense*, and *T. evansi* spanning a wide range of heterozygosity. The data also demonstrated that the origin of *T. evansi* isolates was from multiple *T. brucei* strains of different genetic backgrounds, implying 1) independent origins of *T. evansi* from *T. brucei* strains and 2) repeated events of acquisition of mechanical transmission of *T. evansi*, hence escape from obligate link with tsetse fly vectors. For spatial expansion the mean allelic richness was highest at 7.58 in *T. b. brucei*, lowest at 4.63 in *T. evansi*, and intermediate at 7.35 and 6.19 in *T. b. rhodesiense* and *T. equiperdum*, respectively. The study suggests that one isolate of *T. equiperdum* is genetically distinct from other *T. evansi* and *T. equiperdum* isolates; it thus represents an important addition to the emerging panel of new isolates. Overall, these findings suggest 1) region-specific differential virulence between *T. evansi* isolates, and 2) potential epidemiological implications of the genotyping results underpinned by probability of *T. brucei* strains from different genetic backgrounds becoming causative agent's livestock disease 3) the findings also challenge the taxonomic rank of species for these parasites and have important implications in the epidemiology, diagnostics and treatment of trypanosomiasis.

