

Genetic diversity among *Trypanosoma (Duttonella) vivax* strains from Zambia and Ghana, based on cathepsin L-like gene

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Abstract – Understanding the evolutionary relationships of *Trypanosoma (Duttonella) vivax* genotypes between West Africa and Southern Africa can provide information on the epidemiology and control of trypanosomiasis. Cattle blood samples from Zambia and Ghana were screened for *T. vivax* infection using specie-specific PCR and sequencing analysis. Substantial polymorphism was obtained from phylogenetic analysis of sequences of cathepsin L-like catalytic domains. *T. vivax* from Ghana clustered together with West African and South American sequences, while *T. vivax* from Zambia formed one distinct clade and clustered with East African and Southern African sequences. This study suggests existence of distinct genetic diversity between *T. vivax* genotypes from West Africa and Zambia as per their geographical origins.

Key words: Trypanosomiasis, *Trypanosoma vivax*, Zambia, Ghana.

Résumé – **Diversité génétique des souches de *Trypanosoma (Duttonella) vivax* de Zambie et Ghana, basée sur le gène similaire à la cathepsine L.** Comprendre les relations évolutives des génotypes de *Trypanosoma (Duttonella) vivax* d'Afrique de l'Ouest et du Sud peut fournir des informations sur l'épidémiologie et le contrôle de la trypanosomiase. Des échantillons de sang de bétail de Zambie et du Ghana ont été testés pour l'infection à *T. vivax* en utilisant une PCR spécifique à l'espèce et en analysant les séquences. Un polymorphisme substantiel a été obtenu par une analyse phylogénétique des séquences de domaines catalytiques similaires à la cathepsine L. *T. vivax* du Ghana était groupé avec les séquences d'Afrique de l'Ouest et d'Amérique du Sud, alors que *T. vivax* de Zambie formait un clade distinct et était groupé avec des séquences d'Afrique de l'Est et du Sud. Cette étude suggère l'existence d'une diversité génétique distincte des génotypes de *T. vivax* d'Afrique de l'Ouest et de Zambie en fonction de leurs origines géographiques.

Food and Agriculture Organization (FAO) has estimated that the problem of trypanosomiasis costs Africa about US\$4.5 billion per year, which includes losses in agricultural production, perennial expenditure on trypanocidal drugs, and other local intervention schemes in attempts to control trypanosomiasis [13, 19]. Of major importance is *T. vivax* infection that predominantly affects cattle, buffalo, goats, sheep, and wild bovinds [7, 18]. *T. vivax* is a hemoprotozoan parasite found in Africa, central and South America. In Africa the parasite is transmitted both cyclically by *Glossina* spp. and mechanically by tabanids and other biting flies, while in South America only mechanical transmission occurs [3, 22, 26, 29]. Certain cattle

and goat breeds in Africa are trypanotolerant while others are highly susceptible to *T. vivax* infection.

T. vivax infection in small ruminants from Africa and South America has been reported to result in a variety of clinical outcomes ranging from acute to chronic or subclinical disease; the course of infection varying depending on the parasite strain, endemicity, and the species and breed of the ruminant host [2, 4, 5, 10]. West African *T. vivax* strains are believed to be more pathogenic compared to East African strains, however, severe hemorrhagic outbreaks, with high mortality levels, have been periodically reported in East Africa [6, 21]. South American *T. vivax* strains have been shown to have close genetic similarity to West African strains [7, 14], re-affirming the historical theory that *T. vivax* was imported into the

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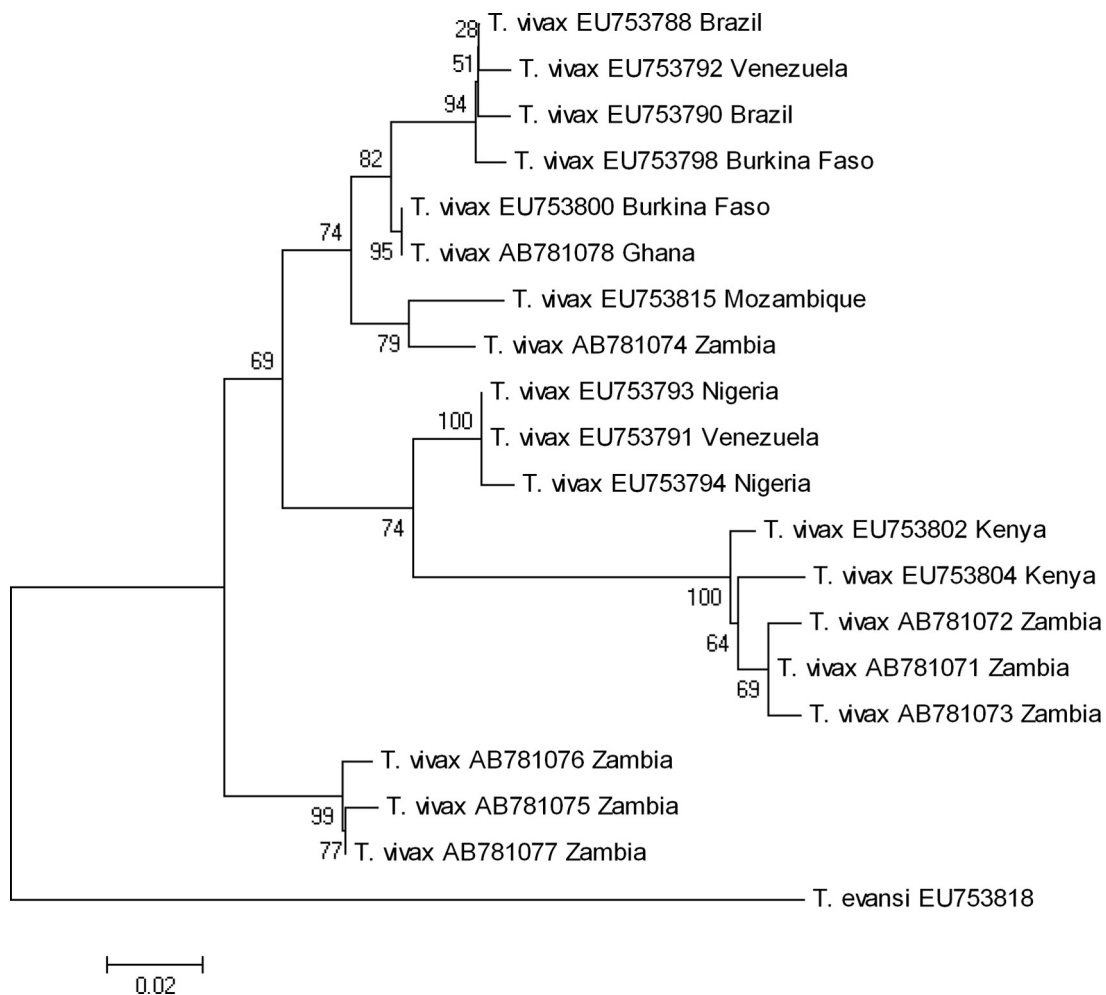


Figure 1. Phylogeny based on Neighbor-joining method using catL-like catalytic domain sequences from West African (Ghana) and Southern African (Zambia) isolates of *T. vivax*. The numbers correspond to percentage of bootstrap support values derived from 1000 replicates. Comparison with GenBank sequences with their accession nos. for South American, Southern African, East African, and West African *T. vivax* is indicated.

New World through infected cattle from West Africa [18]. Although *T. vivax* is considered to be an important salivarian trypanosome species because of its wide distribution, pathogenicity to cattle, and its relatively high infection rates in tsetse, it remains highly neglected in the scientific literature. This is attributed to the species being notoriously difficult to work with as isolates are not easily adapted to culture or grown in standard laboratory animals [15]. Few studies that have investigated the genetic diversity in *T. vivax* have typically focused on comparison between isolates from across Africa and South America [1, 11].

The genomes of trypanosomes consist of multiple copies of catL-like genes that vary depending on species. Cathepsin L-like (catL-like) enzymes are cysteine proteases involved in the pathogenicity, immunity, cell differentiation, infectivity, and metabolism of trypanosomes [8]. The potential role of cysteine proteases in the life cycle and pathogenesis of trypanosomes can be elucidated by knowledge of their evolutionary relationships and identification of species-specific molecules [20, 25]. In this study, we characterized sequences of cathepsin L-like genes of *T. vivax* circulating in Ghana (West Africa) and

Zambia (Southern Africa) and compared their relationship by phylogenetic analysis.

A total of 100 bovine blood samples from Petauke district in Zambia (Latitude: $-14^{\circ} 14' 28.5246''$ and Longitude: $31^{\circ} 19' 11.4564''$) were screened for *T. vivax* infection using the species-specific PCR (designated TviCatL-PCR) employing primers TviCatL1 and DTO155 [8]. Thereafter, *T. vivax*-positive samples ($n = 11$) were randomly selected and subjected to PCR amplifying approximately 500 bp of cathepsin L-like gene using primers DTO154 and DTO155 [8]. The bovine blood samples from Adidome and Koforidua in Ghana, which were positive for *T. vivax* in a previous study [24], were also included in this study ($n = 11$). PCR was conducted using Amplitaq Gold[®] 360 reagent (Applied Biosystems, Foster City, CA) with 35 cycles of amplification to minimize PCR errors. The amplified products were cloned into a pGEM-T vector (Promega, Madison, WI) and sequenced using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems) and an ABI Prism 3130X genetic analyzer according to the manufacturers' instructions. The DNA sequences obtained

were submitted to the DNA Data Bank of Japan (DDBJ) (<http://www.ddbj.nig.ac.jp>) under Accession Nos. AB781071 to AB781078.

Among 100 cattle tested, 32 samples were positive for *T. vivax* infection (data not shown). The infection rate (32%) was comparable to that observed in Ghanaian cattle (25.3%) in our previous study [24]. Seven different sequences were obtained from 11 Zambian samples, while all the 11 samples from Ghana had an identical sequence. A phylogenetic analysis revealed that *T. vivax* from Ghana clustered together with those from West Africa (Burkina Faso) and South America (Brazil and Venezuela) (Figure 1). *T. vivax* from Zambia were assigned to three different clades, forming one distinct clade.

Genetic diversity of *T. vivax* has been reported to be limited [27]. It is, however, essential to know its true diversity considering the differences in disease outcome, diagnosis, response to drug, and resistance to chemotherapeutic treatments [17]. Clustering of Ghanaian *T. vivax* with those from West Africa and South America is in agreement with previous findings [11, 12, 14], corroborating the hypothesis that *T. vivax* was introduced into South America with bovines imported from West Africa [11, 12, 16, 18]. *T. vivax* from central Africa is reported to share molecular features with both the East and West African isolates [14, 15]. Previously, other workers [16, 7, 23, 28, 14, 11] illustrated a complex structure of *T. vivax* populations corroborating the high genetic divergence between West and East African *T. vivax* strains. This has been revealed according to morphological, immunological, pathological, molecular features, and behavior in the tsetse fly and mammalian hosts.

An attempt to characterize *T. vivax* using a panel of eight microsatellites [9] failed to amplify any positive alleles from our *T. vivax*-positive samples (data not shown). Such failure has been attributed to a population whose parasitemia is below the threshold for detection by single-locus PCR [9]. Additionally, there exist high levels of genetic diversity within this species, particularly in East Africa, and primer sets used for microsatellite genotyping were designed from genomic sequences from a West African strain. The diverse genotypes in East Africa and the need to identify novel genotypes require use of “generic” primers, designed to amplify DNA from a wide range of trypanosome species [17]. In conclusion, we revealed the genetic diversity of *T. vivax* genotypes from Ghana (West Africa) and Zambia (Southern Africa).

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