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A COMPARISON OF THE ISOENZYMES OF *TRYPANOSOMA (DUTTONELLA) VIVAX* ISOLATES FROM EAST AND WEST AFRICA

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Abstract—FASOGBON A. I., KNOWLES G. and GARDINER P. R. 1990. A comparison of the isoenzymes of *Trypanosoma (Duttonella) vivax* isolates from East and West Africa. *International Journal for Parasitology* **20**: 389–394. The genetic diversity in 13 stocks and clones of *Trypanosoma vivax* from East and West Africa was compared by isoenzyme analysis. The Ugandan and West African stocks and clones showed a very high degree of genetic similarity to each other but they differed from the Kenyan stocks and clones. Two haemorrhagic stocks, IL 2337 (Galana, Kenya) and IL 3067 (Bamburi, Kenya), showed a high degree of similarity in enzyme banding patterns in electrophoresed preparations. One of the Kenyan stocks, M1D 627, differed in most of its enzyme banding patterns from all the other stocks and clones used.

INDEX KEY WORDS: *Trypanosoma (Duttonella) vivax*; isoenzyme analysis; ruminant-restricted; rodent-adapted; characterization; similarity indices; genetic diversity; genetic groups; zymodeme.

INTRODUCTION

Trypanosoma vivax is a most important protozoan parasite of livestock in both West and East Africa (Anosa, 1983), and *T. vivax* is prevalent in cattle in South America (Hoare, 1972). There is a need on both continents, however, to distinguish this parasite from other pathogenic trypanosomes and to evaluate the genetic diversity within such a widely dispersed species.

Isoenzyme analysis of the *T. brucei* species complex has shown that *T.b. gambiense* is genetically distinct from *T.b. brucei*, and that domestic livestock are reservoirs of *gambiense* sleeping sickness (Gibson, Marshall & Godfrey, 1980). Gibson *et al.* (1980) have also observed genetic differences between stocks of *T. brucei* isolated from East and West Africa. Similarly, based on stocks sampled from different climatic zones, the species *Trypanosoma congolense* has been found to comprise several genetically distinct groups (Young & Godfrey, 1983; Gashumba, Baker & Godfrey, 1988). Isoenzyme analysis of *T. cruzi* has also been useful in revealing peridomestic transmission of certain stocks (Miles, Toye, Oswald & Godfrey, 1977).

Comparable studies to those on *T. brucei, T. congolense* and *T. cruzi* have not been undertaken with *T. vivax* and it is unclear whether *T. vivax* is like the other salivarian species of trypanosomes in comprising distinct genetic groups. However, a sample of field isolates of *T. vivax* from Nigeria was analysed for polymorphisms in alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) and preliminary evidence for subspeciation was obtained (Kilgour, Godfrey & Na'isa, 1975). The patterns of ALAT and ASAT in field isolates from Nigeria remained stable over a period of 3 years (Kilgour & Godfrey, 1977). However, since the electrophoretic variation in only two enzymes was investigated little could be deduced about the degree of genetic diversity within the *T. vivax* species. Later, Murray (1982) examined nine stocks of *T. vivax* and found electrophoretic variation of three enzymes. Allsopp & Newton (1985) extended the number of enzymes used to characterize *T. vivax* to 13. They identified nine zymodemes and, in contrast to Murray's observations, only two stocks were found to be similar.

It is important to discover the extent of the phenotypic differences amongst stocks of *T. vivax* from geographically separate parts of Africa because the pathology caused by the organism is generally more severe in West Africa (Hoare, 1972). It is also of interest to ascertain whether particular phenotypes could be correlated with parameters such as antigenicity or rodent-infectivity.

Techniques which have been used for the characterization of trypanosomes include detection of isoenzyme polymorphisms, the detection of repetitive DNA sequences which are specific for particular genetic groups of trypanosomes (ole-MoiYoi, 1987), and serological techniques (Nantulya, Musoke, Rurangirwa, Saigar & Minja, 1987). We present here an isoenzyme analysis of *T. vivax* stocks and clones, and discuss our results in the context of the geographical distribution of phenotypes between East and West Africa.

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MATERIALS AND METHODS

Trypanosome stocks/clones. The derivation of the *Trypanosoma vivax* stocks/clones used is given in Table 1. Boran cattle and goats were infected with different stocks or clones of *T. vivax* by intravenous (i.v.) inoculation of a cryopreserved stabilate. Parasitaemia was detected by wet blood film examination, and the haematocrit centrifugation technique (HCT) (Woo, 1970). Irradiated mice (C_3H strain) were inoculated either i.v. or intraperitoneally (i.p.) with rodent-infective or rodent-adapted stocks or clones. Trypanosomes were purified on columns of DEAE-cellulose (Lanham & Godfrey, 1970), and lysates of trypanosomes were prepared and stored in liquid nitrogen (Gibson, Mehlitz, Lanham & Godfrey, 1978).

Calculation of similarity indices. To directly compare pairs of stocks, similarity indices were calculated using the following formula:

$$\text{Similarity index} = \frac{\text{No. of bands common to both zymodemes,}}{\text{Total no. of bands in both zymodemes}}$$

$$\text{i.e. } S = \frac{z}{x+y} \times 100.$$

Where S = similarity index, x = no. of bands in zymodeme X, y = no. of bands in zymodeme Y and z = no. of enzyme bands common to zymodemes X and Y (Gibson *et al.*, 1980). The similarity index was expressed as the total number of enzyme bands (for the designation of bands, see below) common to two zymodemes divided by the total number of bands in both zymodemes, and expressed as a percentage. Where bands were absent for enzymes of one stock, the corresponding bands for that enzyme in the other stock of the pair were not used for the calculation of similarity indices. The data were further examined by cluster analysis. Similarity indices were calculated by the method of Sokal & Michener (1958) according to the formula:

$$S = \frac{2a \times 100}{2a + b + c}$$

where a = no. of bands in common between two stocks, b = no. present in one stock but not the other, c = no. present in the second stock, but not in the first. The indices were clustered using the BoLA-PC computer programme (Kemp, Gettinby, King & Teale, 1987) which employs the centroid method of clustering. The cluster relationships were depicted in a dendrogram.

TABLE 1—ORIGIN OF *T. vivax* STOCKS AND CLONES

Stock no.	Original isolate: Designation	Origin	Year of isolation	Raised in
IL 2337	KETRI 2392	Galana, Kenya	1978	Steer
IL 3067	–	Bamburi, Kenya	1986	Steer
IL 2292*	KETRI 2375	Likoni, Kenya	1978	Steer
IL 2969*	–	Kilifi, Kenya	1982	Steer
MID 627§	–	Muhaka, Kenya	1985	Steer
IL 2932	EATRO 2058	Teso, Uganda	1972	Mice
IL 7E10	EATRO 1184	Teso, Uganda	1968	Steer
IL V-25†	EATRO 1262	Lugala, Uganda	1969	Goat
IL 2038‡	EATRO 1262	Lugala, Uganda	1969	Mice
IL 3184*	–	Ibadan, Nigeria	1985	Goat
IL 2946	EATRO 1721	Vom, Nigeria	1970	Goat
IL 2160	Y 486	Zaria, Nigeria	1973	Mice
IL 3200*	ITC 016	Buruku, The Gambia	1987	Steer

* Clone: stocks IL 2292 and IL 2969 were cloned *in vitro* while stocks IL 3184 and IL 3200 were cloned in goats (for further details of these stocks and the techniques of cloning, see Vos, 1989, thesis cited above).

† Before rodent adaptation.

‡ After rodent adaptation.

§ Ref. Knowles *et al.*, 1988.

EATRO, East African Trypanosomiasis Research Organization, Tororo, Uganda (now UTRO, Uganda Trypanosomiasis Research Organization).

IL (Abbreviation of ILRAD), International Laboratory for Research on Animal Diseases.

ITC, International Trypanotolerance Centre, The Gambia.

KETRI, Kenya Trypanosomiasis Research Institute.

Enzyme electrophoresis. Enzyme electrophoresis was carried out on thin layer starch-gels. Lysates of trypanosomes were adsorbed onto cotton threads which were then inserted into the gel, by a method first described by Wraxall & Culliford (1968), and used for trypanosomes by Kilgour & Godfrey (1973) and Bagster & Parr (1973). Electrophoretic variations in the following enzymes were investigated: malate dehydrogenase (MDH, E.C. 1.1.1.37), malate dehydrogenase, decarboxylating NADP (malic enzyme, ME, E.C. 1.1.1.40), peptidase (substrate: L-leucylglycylglycine, PEP 1, E.C. 3.4.11), peptidase (substrate: L-leucyl-L-alanine, PEP 2, E.C. 3.4.11), aspartate aminotransferase (ASAT, E.C. 2.6.1.1.), alanine aminotransferase (ALAT, E.C. 2.6.1.2), phosphoglucomutase (PGM, E.C. 2.7.5.1), glucose-6-phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49), aconitate hydratase (ACON, E.C. 4.2.1.3), mannose-6-phosphate isomerase (MPI, E.C. 5.3.1.8), isocitrate dehydrogenase (ICD, E.C. 1.1.1.42), L-threonine-3-dehydrogenase (TDH, E.C. 1.1.1.103), hexokinase (HK, E.C. 2.7.1.1).

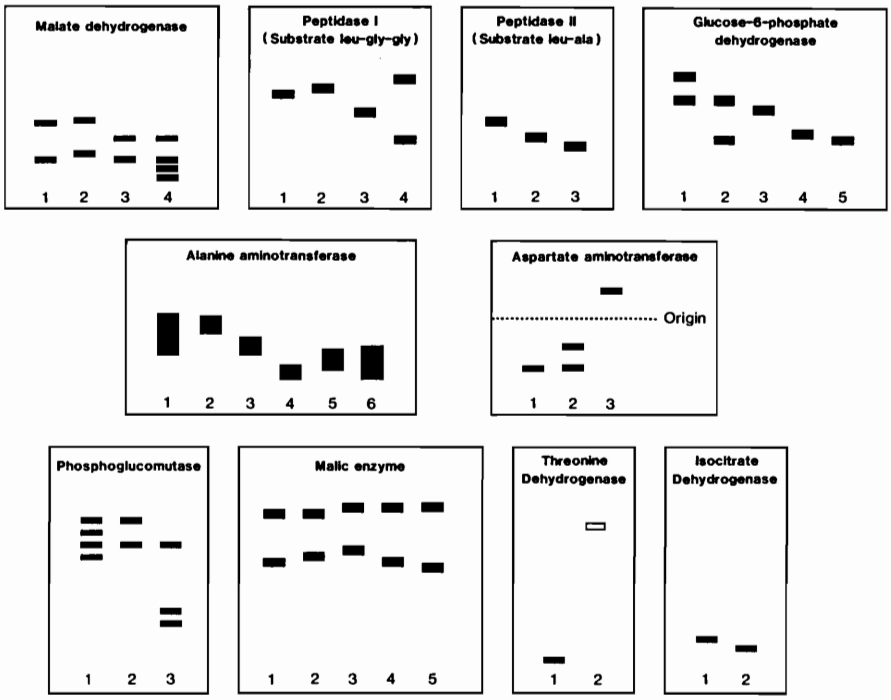

FIG. 1. Banding patterns of polymorphic enzymes. (▬), Strong band; (▭), weak band. Designation of bands follows the usual convention, and each pattern (combination of bands) is designated by an arabic numeral. Anode is the top of the gel; cathode the bottom. The isoenzymes of aspartate aminotransferase (ASAT) migrated towards either the anode or the cathode, while the electrophoretic variants of all the other enzymes migrated towards the anode.

RESULTS

The enzymes ACON, MPI and HK were electrophoretically invariant. The enzymes, MDH, ME, PEP1, PEP2, ASAT, ALAT, PGM, G6PDH, ICD and TDH were electrophoretically variant. The banding patterns for these polymorphic enzymes are given in Fig. 1.

The enzyme profiles for each stock are given in Table 2. Only two stocks were found to be identical in their electrophoretic patterns. The numbering of the bands follows the usual convention which has been used by several authors (Gibson *et al.*, 1980; Murray, 1982; Allsopp & Newton, 1985; Knowles, Betschart, Kukla, Scott & Majiwa, 1988; Gashumba *et al.*, 1988). Each band is designated by a lower case letter, from the lowest to the highest (not shown in the figure); each pattern (combination of bands) is designated by an arabic numeral (Fig.1).

The similarity indices (*S*) for all possible pairs were calculated and the results are presented in Table 3. The *S* values of pairs of stocks from West Africa and Uganda were very similar ranging from 60 to 100%. In contrast, the *S* values from comparisons between stocks originating from West Africa and Uganda, and from Kenya were low. The results of the cluster analysis are shown in Fig. 2. The dendrogram further demonstrates that there is a high degree of isoenzymic similarity between the tested stocks from Uganda, Nigeria and The Gambia; that four of the Kenyan stocks form a group different from the West African group; but that the stock from Muhaka, Kenya, shows only low similarity to either of these groups, and forms a group apart.

DISCUSSION

In the present study, we have compared the differences between the isoenzyme profiles of stocks of *T. vivax* from East and West Africa. The sample of isolates from West Africa and Uganda showed marked similarities with each other, with a median *S* value of 88.5. However, these isolates showed low similarity with stocks from Kenya, the median *S* value being 23.5. Thus, our sample could be divided into two: a Kenyan group, and a Ugandan and West

TABLE 2—CHARACTERIZATION OF STOCKS AND CLONES OF *T. vivax* BY ISOENZYME POLYMORPHISMS

Country stock	MDH	ME	PEPI	PEPII	ASAT	ALAT	PGM	G6PDH	ACON	MPI	ICD	TDH	HK
Kenya IL 2337	1	1	1	1	1	1	1	1	1	–	1	–	1
Kenya IL 3067	1	1	2	1	2	1	1	2	1	–	1	–	1
Kenya IL 2292*	1	2	1	1	2	2	2	1	1	–	1	–	1
Kenya IL 2969*	1	1	1	1	2	1	2	3	1	–	1	–	1
Kenya M1D 627	2	3	3	2	?¶	3	–	–	–	–	1	–	1
Uganda IL 2932	3	5	4	3	3	6	3	5	1	1	2	2	1
Uganda IL 7E10	3	5	4	3	3	6	3	5	1	1	2	2	1
Uganda IL V-25†	4	4	4	3	3	6	3	5	1	1	2	?	1
Uganda IL 2038 ‡	3	5	4	3	3	6	3	5	1	1	2	1?	1
Nigeria IL 3184*	3	5	4	3	3	4	–	–	–	–	–	–	?
Nigeria IL 2946	3	5	4	3	3	5	3	4	–	–	2	–	1
Nigeria IL 2160	3	4	4	3	3	6	3	4	1	1	2	2	1
The Gambia IL 3200*	-§	5	4?‖	3	–	6	–	–	–	–	2	–	?

* Clone; † before rodent adaptation; ‡ after rodent adaptation; § no staining occurred; ‖ bands were very weak; ¶ bands were not strong enough for characterization.

TABLE 3—SIMILARITY INDICES FOR EACH POSSIBLE PAIR OF STOCKS

	Kenyan					Ugandan				West African			
Stocks	1 IL 2337	2 IL 3067	3 IL 2292	4 IL 2969	5 M1D 627	6 IL 2932	7 7E10	8 V-25	9 IL 2038	10 IL 3184	11 IL 2946	12 IL 2160	13 IL 3200
1. Kenya IL 2337		86	79	81	22	24	24	29	24	12	19	24	0
2. Kenya IL 3067			71	79	22	29	29	33	29	11	23	29	0
3. Kenya IL 2292*				77	22	25	25	24	25	11	20	25	0
4. Kenya IL 2969*					22	26	26	30	26	11	21	32	0
5. Kenya M1D 627						21	21	19	21	13	21	21	20
6. Uganda IL 2932							100	90	100	88	87	90	100
7. Uganda IL 7E10								90	100	88	87	90	100
8. Uganda IL V-25†									90	67	75	90	80
9. Uganda IL 2038 ‡										88	87	89	100
10. Nigeria IL 3184*											88	75	75
11. Nigeria IL 2946												87	80
12. Nigeria IL 2160													90
13. The Gambia IL 3200*													

For explanation of *, †, ‡, see legend to Table 2.

African group. Four isolates from Kenya showed a high degree of similarity to each other. However the stock from Muhaka, M1D 627, was dissimilar, and may be a representative of a different genetic group within Africa.

Stocks IL 7E10 and IL 2932 were identical in their enzyme polymorphisms. This observation is interesting because, although both were isolated from Teso, Uganda, they were isolated at different times, 1968 and 1972, respectively. The similarity in their isoenzyme patterns suggests a common genetic origin. On the other hand, stock IL V-25 (Lugala, Uganda) and IL 2038 (a rodent-adapted stock of IL V-25) showed differences for MDH and ME. Although band pattern 3 of MDH was present in all the Ugandan and West African stocks and clones, stock IL V-25 gave two additional bands (Fig. 1). The reason for the absence of the two extra MDH bands in the rodent-adapted stock IL 2038 is unknown, although it could have arisen as a result either of an alteration (probably a mutation) in the genome of stock IL V-25, or a selection of one parasite type from a mixture, during rodent passage.

Whilst the enzymes, ACON, MPI and HK were apparently electrophoretically invariant, we cannot deduce that these enzymes would be diagnostic for *T. vivax* because the bands obtained from some stocks were not strong enough for characterization, and no enzymatic activity was observed for others. Earlier work done by Murray (1982) and Allsopp & Newton (1985) suggested that ICD, which they observed to be invariant, could be diagnostic for *T. vivax*. However, from our results, ICD was observed to be electrophoretically variant, and it is probable that polymorphic enzymes will be less useful than monomorphic for the identification of *T. vivax*.

We have used two haemorrhagic *T. vivax* stocks, IL 2337 (Galana, Kenya) and IL 3067 (Bamburi, Kenya). These stocks showed greater than 80% similarity in their banding patterns, differing only in the banding

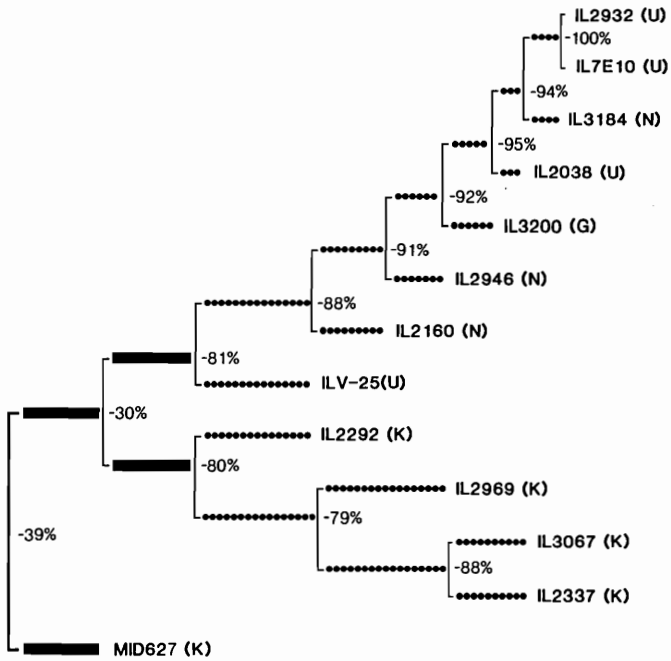

FIG. 2. Clustering of the *T. vivax* isolates by the centroid method according to their isoenzyme banding patterns. The *T. vivax* stocks are denoted by their numbers and countries of origin (U, Uganda; N, Nigeria; G, The Gambia and K, Kenya). Percentages indicate the degree of relationship between pairs of stocks or between groups of stocks. The division of the major groupings is denoted by the heavy bars.

patterns for PEP1, ASAT and G6PDH. We also observed that clones IL 2292 (Likoni, Kenya) and IL 2969 (Kilifi, Kenya) possess a high degree of similarity to each other, differing only in their banding patterns for three enzymes. The clone from Likoni also has patterns for most of the enzymes studied which are present either in one or both of the haemorrhagic stocks. This observation is not surprising since KETRI 2388, which is a forbear of IL 2292, had originally been found to be haemorrhagic (Allsopp & Newton, 1985), but seems to have lost this characteristic after extensive passage (Gardiner, unpublished).

We have used three rodent-infective or rodent-adapted stocks from East and West Africa; IL 2932 (Teso, Uganda), IL 2038 (Lugala, Uganda) and IL 2160 (Yakawada, Nigeria). These stocks showed the same banding patterns for seven out of the 13 enzymes examined, the differences in the non-identical patterns were due mostly to the presence of additional bands for some of the stocks since they all had several bands in common. Due to the limited number of rodent-infective or rodent-adapted stocks used, it is not possible to adduce whether the similarities observed are the result of geographical factors or rodent infectivity. This uncertainty arises because, on comparison of their isoenzyme profiles, these rodent-infective or rodent-adapted stocks also bear a high degree of similarity in banding pattern to the ruminant-restricted stocks from Uganda and West Africa. More rodent-infective or rodent-adapted stocks from different parts of Africa need to be analysed for their isoenzyme patterns to determine if a correlation exists between these parameters. It is noteworthy that, to date, it has not been possible to adapt any of the Kenyan *T. vivax* stocks to rodents (Gardiner, unpublished).

Some of the results obtained in this study can be compared with results from earlier work: three banding patterns were obtained for ASAT, and ASAT 1 in this study corresponded to Kilgour & Godfrey's (1977) ASAT 1 while ASAT 2 was a mixture of Kilgour & Godfrey's (1977) ASAT 1 and 3. Four banding patterns were obtained for MDH, three of which corresponded to Allsopp & Newton's (1985) MDH 1, 2 and 3. Similar to observations made by Murray (1982), ME showed the greatest variation but five banding patterns were obtained, one of which corresponded to Murray's (1982) ME II (Fig. 1).

A split into East and West African groups of *T. vivax*, comparable to that reported here, has been demonstrated on the basis of their antigenic relatedness (Vos, G.J., unpublished Ph.D. thesis, University of Utrecht, Utrecht, The Netherlands, 1989; Vos & Gardiner, in press). In those studies it was observed that there was cross-reactivity, in reciprocal immune lysis tests, between stocks and clones from

Uganda, Nigeria and The Gambia, but there was no cross-reactivity between stocks and clones from these three countries with the Kenyan stocks. However, within the Kenyan sample, the stocks from Galana and Bamburi were completely lysed but showed no cross-reactivity with other stocks used. Similarly, the stocks from Likoni and Kilifi were observed to cross-react with each other but not with any of the other stocks.

In addition, a DNA probe, lambda gDIL-10, derived from the genome of ILDat 1.2 (a clone of the same stock as IL 2160 from Nigeria), hybridized strongly with parasites from West Africa, but weakly or not at all with *T. vivax* stocks from Kenya (ILRAD, 1988). It has further been observed that the Ugandan stocks we have analysed for enzyme polymorphisms also hybridized with DNA probes derived both from Kenyan and Nigerian *T. vivax* (Kukla, Majiwa, Young, Moloo & ole-MoiYoi, 1987; discussed by Gardiner, 1989). The collected data suggest that the Ugandan stocks may share features with both the East and West African groups but are generally more similar to the West African isolates than to the Kenyan stocks.

It will be of interest to ascertain if the reason for the geographical demarcation of *T. vivax* stocks is due to climatic or vectorial influences or such factors as the distribution of the wildlife reservoir and host preference.

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