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A preliminary comparison of *Trypanosoma simiae* and *T. congolense* by isoenzyme electrophoresis

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Summary

Trypanosoma simiae, although similar to *T. congolense* in morphology and mode of development in the tsetse vector, is regarded as a separate species mainly because it is highly pathogenic to the domestic pig and fails to infect rodents. To establish whether the two species are distinct biochemically, we compared by isoenzyme electrophoresis 2 isolates of *T. simiae* with 7 stocks of *T. congolense,* together with one of *T. brucei.* All isoenzyme patterns of the 6 enzymes examined differed in *T. simiae* from *T. congolense* and *T. brucei* stocks. This supports the designation of *T. simiae* as a separate species. However, a comparison involving a much larger collection of *T. simiae* is necessary in order to get conclusive evidence.

Key words: *Trypanosoma simiae; T. congolense;* isoenzyme electrophoresis.

Introduction

Trypanosoma simiae is classified together with *T. congolense* in the subgenus *Nannomonas* (Hoare, 1972) because of the similarity of morphological characteristics and mode of development in the tsetse vector. However, *T. simiae* is regarded as a separate species because it causes a rapidly fatal disease in the domestic pig. *T. simiae* also causes a severe form of trypanosomiasis in camels and in monkeys (see Hoare, 1972); rodents are refractory to infection.

T. simiae was first described as a *T. congolense*-like trypanosome from a monkey, hence the «simiae» misnomer (see Hoare, 1972). Later the trypano-

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some was positively associated with a devastating porcine trypanosomiasis (Hoare, 1936). As with other *congolense*-like trypanosomes, controversy continued over alternative names for the trypanosome causing the pig disease. The problem of the nomenclature of *T. simiae* is discussed in detail by Hoare (1936, 1972) and Stephen (1966).

Since a wide variation occurs in the range of pathogenicities within the species *T. congolense,* it can be argued that *T. simiae* is merely a strain of *T. congolense* which is virulent for the pig (Godfrey, 1977). For example Chardome and Peel (1967) describe two variants of *T. congolense* that are restricted to pigs, and a strain of *T. simiae* causing a chronic disease in pigs.

To help clarify this issue we have compared *T. simiae* and *T. congolense* by isoenzyme electrophoresis. This approach has been extensively used for other African trypanosomes where the problem of subspeciation arises due to their morphological similarity (e.g. Gibson et al., 1980; Tait et al., 1984). Unfortunately, only two stocks of *T. simiae* could be examined because of the difficulties of isolating *T. simiae* in the field and the impossibility of growing the parasite in laboratory rodents. However, the isolates were compared with a number of *T. congolense,* in which the extent of enzyme polymorphism is now better known (Young and Godfrey, 1983; Gashumba, unpublished).

Materials and Methods

The trypanosome stocks and their histories are shown in Table 1. Enzyme extracts of *T. simiae,* prepared in Kenya, were from infections of stocks originally isolated at Ukunda, on the Kenyan Coast in 1970. Both *T. simiae* extracts were taken from pigs at peak parasitaemia, while the *T. congolense* enzyme extracts were from mice. *T. congolense* stocks of wide isoenzyme variation were used for comparison, using the preparative methods and thin-layer starch-gel electrophoresis as described by Young and Godfrey (1983). However, no stocks were cloned. Six enzymes were examined: alanine aminotransferase (ALAT, E.C. 2.6.1.2); malate dehydrogenase (MDH, E.C. 1.1.1.37); glucose phosphate isomerase (GPI, E.C. 5.3.1.9); phosphoglucomutase (PGM, E.C. 2.7.5.1) and two peptidases (PEP1, substrate: L-leucylglycyl glycine, and PEP2, substrate: L-leucyl L-alanine, E. C. 3.4.11).

Table 1. Brief histories of the trypanosome stocks

Stock	Species	Host	Country	Year
JG 8	*T. congolense*	*Glossina pallidipes*	Kenya	1983
1/148 FLY	*T. congolense*	cow	Nigeria	1960
EATRO 1617	*T. congolense*	*Glossina brevipalpis*	Uganda	1970
WG 46	*T. congolense*	cow	Kenya	1980
TSW 99	*T. congolense*	pig	Liberia	1977
WG 81	*T. congolense*	goat	Kenya	1981
GAM 2	*T. congolense*	cow	Gambia	1977
JG 12	*T. brucei*	*Glossina pallidipes*	Kenya	1983
EATRO 1786	*T. simiae*	*Glossina austeni*	Kenya	1970
EATRO 1806	*T. simiae*	*Glossina brevipalpis*	Kenya	1970

Results

The results are presented diagrammatically in Fig. 1, and a zymogram for MDH is shown in Fig. 2.

It can be seen that isoenzymes differed in mobility between a variety of *T. congolense* isolates and the two *T. simiae* samples examined: *T. brucei* was also different. Limited data on five other enzymes (threonine dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, malic enzyme, nuclease hydrolase and aspartate aminotransferase) showed similar differences between these two isolates of *T. simiae* and two reference *T. congolense* stocks.

The results support the designation of *T. simiae* as a separate species, since it is as different from *T. congolense* isoenzymically as *T. congolense* itself is different from *T. brucei* or *T. vivax* (Young, 1980). Although *T. congolense* isoenzyme patterns vary considerably, stocks always share bands (Young and

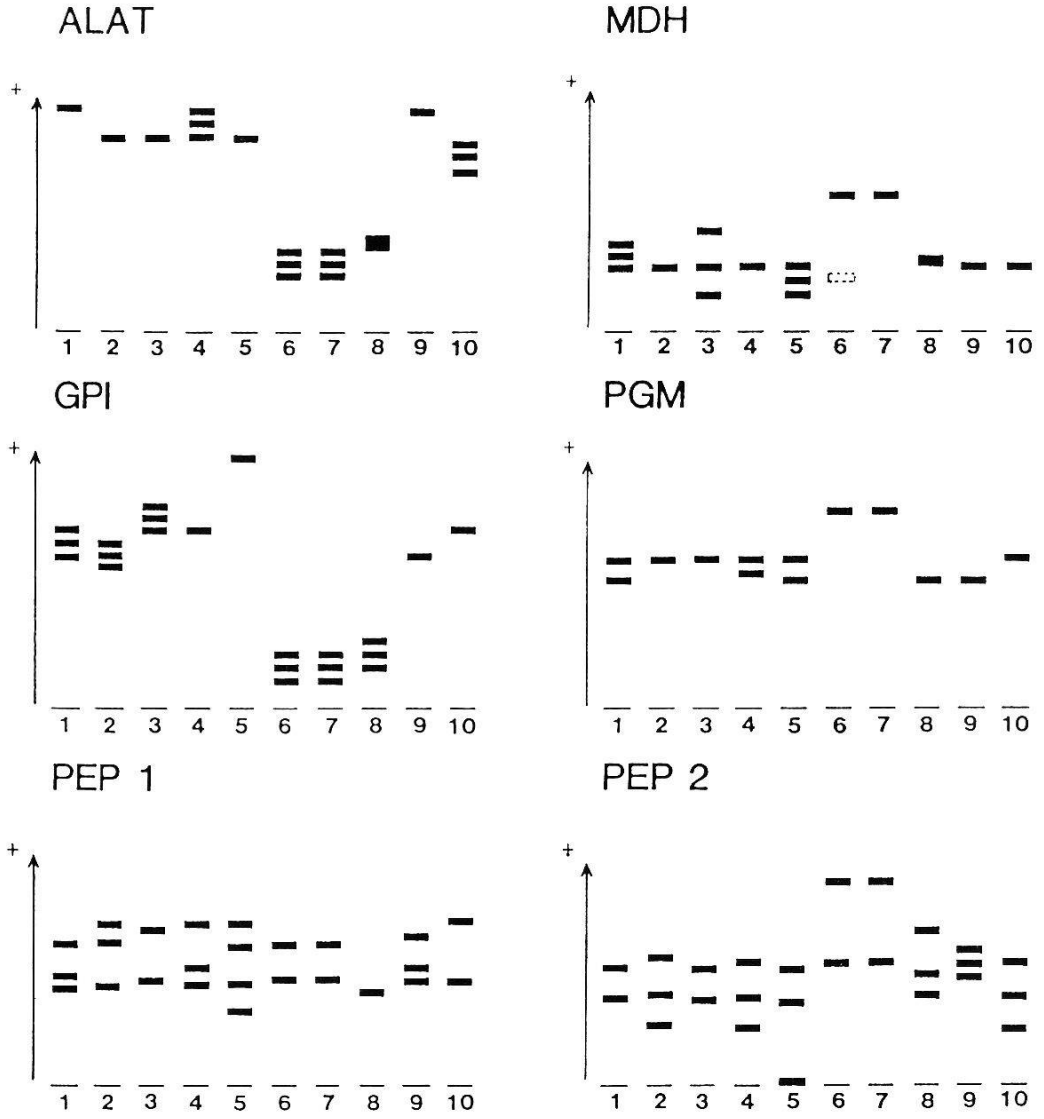

Fig. 1. Diagrams of zymograms of six enzymes in 10 stocks: 1–5 and 9, 10 are *T. congolense;* 6. 7 are *T. simiae;* 8 is *T. brucei.* 1. JG 8; 2. 1/148 FLY; 3. EATRO 1617; 4. WG 46; 5. TSW 99; 6. EATRO 1786; 7. EATRO 1806; 8. JG 12; 9. WG 81; 10. GAM 2.

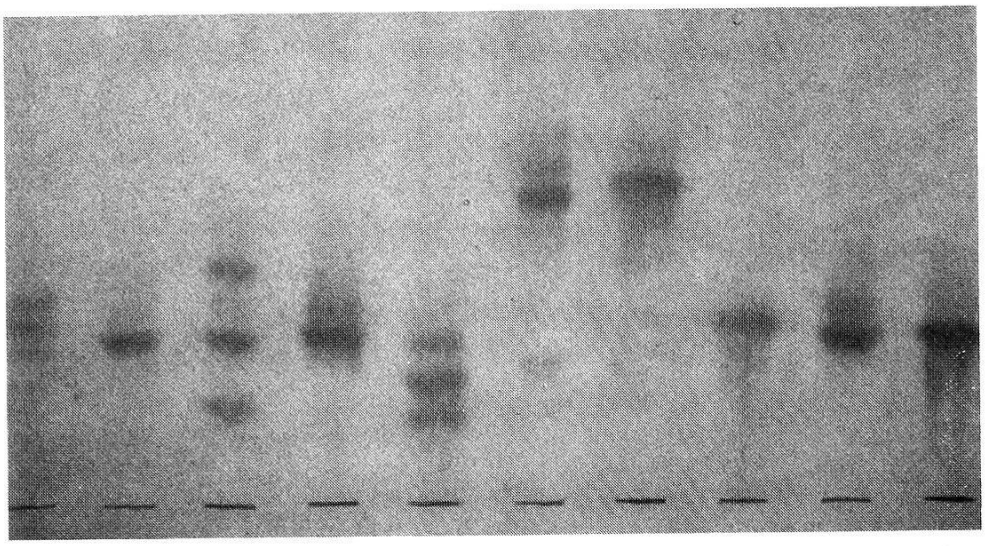

Fig. 2. Zymogram of MDH. Position of samples as in Fig. 1. The weak anodic bands associated with the strong ones are probably secondary isoenzymes (Harris and Hopkinson, 1976), and are not included in the diagram. However, a faint cathodic component of MDH also appeared in *T. simiae* EATRO 1786.

Godfrey, 1983), whereas *T. simiae* has a totally different set of isoenzyme bands for ALAT, MDH, GPI and PGM. However, isoenzyme results alone, especially from only two samples, cannot be conclusive (Miles et al., 1984). Behavioural data on *T. simiae* already exist, which is why it has maintained species status, and this is now supported by the isoenzyme results.

It will be necessary to carry out more extensive investigations, not only of enzyme heterogeneity in *T. simiae,* but also on behavioural and epidemiological characteristics in order to determine reliably its taxonomic status. Certainly *T. simiae* is genetically quite distinct from *T. congolense.*

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