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Veterinary Parasitology 76 (1998) 149–152

veterinary parasitology

Short communication

Biometrical alterations of *Trypanosoma evansi* isolate in laboratory rodents

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Received 8 October 1996; accepted 2 January 1997

Abstract

Biometrical alterations in a horse *Trypanosoma evansi* isolate were observed when passaged in laboratory rodents. The major parasite transformation observed was the increase in the total length as a consequence of an increase in some parasite measurements. These transformations probably occurred as a consequence of a host change from horse to rodents. No kinetoplastic forms were observed. © 1998 Elsevier Science B.V.

Keywords: Trypanosoma evansi; Horse; Rat; Mouse; Morphology; Biometrical alteration; Strain selection

1. Introduction

Studies of the ecology and epidemiology of parasites and parasitic diseases have demonstrated marked differences in morphologic aspects of parasites. Brener (1975) reported on form–function relationships, with morphology representing a marker of peculiar physiological, biochemical and pathogenic behaviour. According to Gill (1977) *Trypanosoma evansi* is a monomorphic trypanosome found exclusively in the slender intermediate forms.

Strains from different geographical regions appear to be morphologically indistinguishable. According to John et al. (1992), a useful adjunct to the examination of the morphology of trypanosomes, observed on stained slides, relies on the biometrical evaluation. *T. evansi* has been described as being monomorphic, although in additional studies many strains have presented stumpy forms on rare occasions (Losos, 1980).

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PII S0304-4017(97)00006-X

Stephen (1986) reported that variations in measurements may occur in the trypanosomes depending on the host species and may even vary in the same host from day to day. This study recorded biometrical alterations from a horse isolate during successive syringe passages in laboratory rodents.

2. Materials and methods

CPAP-A isolate from an infected horse was used for this study. This isolate was maintained by random syringe passage in mice and rats. Blood smears of the passages were examined after staining with Giemsa stain. The horse isolate was identified based on morphological and biometrical data. Biometrical studies were carried out using an eyepiece micrometer as described by Hoare (1972). A Carl Zeiss light microscope with a total magnification of 1000 × was used for this purpose.

The measured data of the primary isolate and passages number 10, 15, 50 and 180 were taken in 100, 50, 50, 50, and 50 observations from thin smears, respectively. In each case, five sets of smears were made for the biometrical study and the observations were made on different trypanosomes. The data were analyzed statistically using a *T*-test.

Parasite measurements were designed as follows: PK, distance from posterior end to kinetoplast; KN, from kinetoplast to middle of nucleus; PN, from posterior end to middle of nucleus; NA, from nucleus to anterior end; F, free flagellum length; T, total length including free flagellum; PN/NA, nuclear index.

3. Results

The measured data are given in Table 1. For statistical analysis, results were organized in three groups: (a) comparison between primary isolate and passage 10 in rodents; (b) comparison between passages in the rodent host; (c) comparison between passages 10 and 180 in the rodent host.

Table 1
Measurements (means (SD) μm) of a horse *T. evansi* in thin smears of primary isolate and syringe passages

	PK	KN	PN	NA	F	T	PN/NA
Isolate	–	–	6.46 (0.56)[a]	5.07 (1.2)[a]	5.63 (1.32)[a]	17.16 (1.61)[a]	1.35 (0.37)[a]
Passage 10	–	–	10.63 (2.11)[b]	9.45 (1.55)[b]	7.48 (2.03)[b]	27.42 (2.82)[b]	1.14 (0.25)[b]
Passage 15	–	–	10.56 (1.32)[b]	7.7 (1.06)[c]	6.8 (1.61)[c]	25.25 (3.15)[c]	1.39 (0.27)[a]
Passage 50	–	–	10.04 (2.53)[b]	9.06 (1.07)[b]	10.63 (3.1)[d]	29.74 (5.03)[d]	1.12 (0.28)[b]
Passage 180	–	–	9.06 (2.06)[c]	7.19 (2.14)[c]	8.66 (3.58)[e]	24.92 (5.24)[c]	1.31 (0.29)[a]

Values by column followed by different letters are statistically distinct ($P < 0.001$). PK, distance from posterior end to kinetoplast; KN, from kinetoplast to middle of nucleus; PN, from posterior end to middle of nucleus; NA, from nucleus to anterior end; F, free flagellum length; T, total length including free flagellum; PN/NA, nuclear index.

In Group (a) a highly significant increase in PN, NA, F and T was observed ($P < 0.001$). In Group (b) statistically significant reductions in PN were not observed from passages 10 to 15 or from 15 to 50. The reductions in NA, F, and T measurements from passages 10 to 15 were highly significant ($P < 0.001$). NA, F, L and T increases were highly significant from passages 15 to 50. Statistically significant reductions in PN, NA, F and T were observed from passages 50 to 180. In Group (c) highly significant reductions in PN, NA and T were observed from passages 10 to 180.

Morphological alteration was observed as a gradual increase in undulating membrane in stumpy forms and a more tapered posterior end in the rodent host. The stumpy forms were occasionally observed among the predominant presence of slender intermediate forms. No kinetoplastic forms were observed from the isolate or passages in rodents.

4. Discussion

The data provides evidence that biometrical variations occurred in NA, F, T and PN/NA parasite regions in the rodent host. The major transformations observed were the increases of PN, NA, F and T regions after the host change.

Studies on strain selection of *Trypanosoma cruzi* through maintenance in laboratory rodents have discussed the representativeness of laboratory strains compared with populations that circulate in nature (Deane et al., 1984a). We believe that the transformations observed in this study probably occurred as a consequence of adaptation from horse to rodent host. This is possible because variations in measurements may occur in the trypanosomes depending on the host species and may even vary in the same host from day to day (Stephen, 1986). However, like any parasite that is passaged for a long time, *T. evansi* isolates change in their characteristics over time. This is probably because we end up selecting for a faster growing population (T.W. Jones, personal communication).

Although trypanosomes with the posterior end tapered were more predominant in rodents than in the horse, no significant differences in the morphological aspects were observed after the host change. Nevertheless our data showed that significant biometrical alterations occurred in this isolate in the rodent host. The parasite conserved the same basic morphology found in the horse. No significant parasite morphological differences were found in the rodents from day to day. The parasite measurements in the rodents were always larger than the primary isolate. According to Hoare (1972) the nucleus is located in the anterior part of trypanosome when PN/NA > 1. However, our data showed that the nucleus was always located in the anterior part of parasite in rodents and the horse.

In another study we observed that primary isolates showed a varied prepatent period from 12 to 43 days when passaged to laboratory rodents in comparison with the short prepatent period from 2 to 4 days showed after several passages. Generally, the first passages of primary isolates showed low parasitemia and after several passages an increase was observed (unpublished data). As there is a form–function relationship, and the morphology represents a marker of peculiar physiological, biochemical and pathogenic behaviour (Brener, 1975), we believe that the biometrical variations of

isolate observed in this study occurred as an effort to adapt in the rodent host. On the other hand, the primary isolate may consist of a number of different subpopulations differing among them in zymodeme patterns, VATs (variable antigenic types), measurements, etc. However, based on strain selection studies (Deane et al., 1984a,b), the laboratory rodents could have selected *T. evansi* subpopulations with different mean measurements (27.42, 25.25, 29.74 and 24.92 μm in T) from those found in the horse (17.16 μm in T), through syringe passages. The long prepatent period observed during the first passages of primary isolate may indicate corroborative evidence of strain selection.

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