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Research Note

ANALYTIC MORPHOMETRY OF THE *TRYPANOSOMA CRUZI* (BOLIVIA STRAIN) FORMS FOUND IN THE INTESTINE OF *RHODNIUS PROLIXUS*

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Chagas' disease affects about 24,7 million people in the American Continent with a risk population of 60 million people (C. J. Schofield, 1985, *Br. Med. J., 41:* 187-194). *Trypanosoma cruzi,* the causative agent of Chagas'disease, utilizes triatomine bugs for part of its life cycle. *Rhodnius prolixus* being one of the most important vectors (D. H. Molyneux & R. W. Ashford, 1983, p. 161-181. In Taylor and Francis (eds), London).

There have been attempts to correlate the morphologic and biological properties of different *T. cruzi* strains with their pathogenic properties (F. G. Wallace, 1966, *Exp. Parasitol., 18:* 124-193; C. A. Hoare, 1972, p. 360. In B. S. P. Oxford-Edinburg; S. G. Andrade, 1974, *Rev. Pat. Trop., 1:* 65-121; K. Vickerman, 1974, p. 161-198. In Ciba Foundation Symposia). This led us to propose a morphometric study of *T. cruzi* in the vector *R. prolixus* to look for morphometric parameters which could facilitate the typification of different strains. Such a study is very difficult to carry out precisely with classical visual microscopy techniques; however, the use of an image analysis system such as the IBAS-I can eliminate all the possible errors due to subjectivy of the observer and the lack of exactitude inherent in classical measuring methods, and yield reliable quantitative data more susceptible to statistical analysis. In this way we intended to add new data useful for the characterization of a given *T. cruzi* strain.

We have studied the development of a *T. cruzi* strain from Bolivia (Bolivia strain) in the gut of *R. prolixus,* carrying out an exhaustive and detalied morphometric study through planimetry using the IBAS-I (Image Basic Analytic System) analyzing 16 morphometric parameters and extracting the relevant statistical data.

We have take 11 lots of 5 nymphs each one, alimented on infected white mice. Knowing its parasitemia, we have obtained the number of parasites ingested per insect, by the method of Pizzi & Prager (1952, *Bol. Chil. Parasitol., 7:* 20-21) modified by Brener (1962, *Rev. Inst. Med. Trop. São Paulo, 4:* 389-396), being this number of 10^4. The intestine of the infected batches of vectors were dissected after 28 days of infection and smears were made in order to detect all the forms existing in the preparation. The total number of parasites in the whole gut at this time averaged 56.304/nymph. We prepared slides with all the forms of the parasite observed in the intestine of *R. prolixus:* short-epimastigotes, long-epimastigotes and metacyclic trypomastigotes. Thirty slides were taken from each of the expressed forms. These slides were then amplified with a Leitz-Wetzlar amplifier, type PRADO-Universal 1:2,5/90 mm from which drawings were made.

To avoid the possibility of introducing significant errors while measuring the parameters, the results obtained by two different observers on different days were compared. No significant differences were shown to exist to an accuracy level of 90%.

The parameters measured in each form under study are shown in Table. Ence measured these parameters, we obtained the values of the average and typical deviation for each of the forms employing the image analyzer IBAS-I.

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TABLE

Comparative measures (μm) of the *Trypanosoma cruzi* forms (Bolivia strain) in the intestine of *Rhodnius prolixus*

Studied parameters	Short epimastigotes $\bar{X}$	S.D.	Sm	Long epimastigotes $\bar{X}$	S.D.	Sm	Sphaeromastigotes $\bar{X}$	S.D.	Sm	Metacyclic $\bar{X}$	S.D.	Sm
L	27,45	7,52	1,37	39,57	3,42	0,62	14,78	5,89	1,07	26,80	3,53	0,64
F	14,17	4,97	0,90	14,59	5,75	1,05	5,50	2,55	0,46	6,28	1,48	0,27
NP	6,05	1,60	0,29	12,56	1,43	0,26	4,84	3,09	0,56	9,30	2,00	0,36
NA	7,21	2,74	0,50	12,32	5,10	0,93	4,78	2,68	0,48	11,20	2,95	0,54
AN	3,68	2,15	0,39	5,77	1,13	0,20	3,15	0,59	0,10	4,08	1,05	0,19
PN	7,08	2,05	0,37	10,04	1,27	0,23	6,70	0,81	0,14	10,09	1,50	0,27
AC	18,42	7,59	1,38	48,64	12,45	2,27	25,23	11,09	2,02	21,33	5,50	1,00
PC	26,45	6,24	1,14	49,42	11,49	2,09	19,21	5,28	0,96	39,32	5,91	1,08
AK	1,28	0,63	0,11	1,15	0,21	0,39	2,27	0,71	0,12	1,91	0,73	0,13
PK	4,32	1,23	0,22	4,18	0,38	0,06	5,92	1,02	0,18	5,20	1,42	0,25
A	2,00	0,66	0,12	2,59	0,51	0,09	4,86	0,71	0,12	1,68	0,42	0,07
NK	1,77	0,42	0,07	2,90	0,68	0,12	3,59	1,03	0,18	6,69	2,24	0,40
N/C	0,19	0,07	0,01	0,13	0,05	0,01	0,14	0,05	0,01	0,17	0,04	0,01
IN	0,94	0,41	0,07	1,20	0,62	0,11	1,49	1,28	0,23	0,90	0,35	0,06
LC	13,28	3,28	0,60	24,97	6,22	1,13	9,28	3,78	0,69	20,52	3,63	0,66
F/C	1,07	0,27	0,05	0,65	0,34	0,06	0,51	0,34	0,06	0,32	0,10	0,02

L: total body length; F: length flagellum; NP: nucleus-posterior extremity; NA: nucleus-anterior extremity; AN: nuclear surface; PN: nuclear perimeter; AC: cytoplasmic surface; PC: cytoplasmic perimeter; AK: kinetoplast surface; PK: kinetoplast perimeter; A: cellular width; NK: nucleus-kinetoplast; N/C: ratio nucleus/cinetoplast; IN: nuclear index; LC: cytoplasmic length; F/C: ratio flagellum/cytoplasm.

Among the different forms of *T. cruzi* found in the intestine of *R. prolixus,* the long epimastigotes showed the largest total length with a length of 39,57 μm, the sphaeromastigotes being the shortests with a measure of 14,78 μm. There were no great differences among the medium length of the short and metacyclic forms, which spanned the intermediate spectrum, although the short epimastigotes showed the largest variability in length (S. D. of 7,52 μm).

The flagellums of the epimastigotes nearly tripled in length those of the other two forms, measuring 14,17 (short epimastigotes) and 14,59 μm (long epimastigotes) versus 6,28 and 5,50 μm in the metacyclic and sphaeromastigotes respectively. Since the total length was measured as the sum of the cytoplasm plus the flagellum length, we can describe it more accurately in terms of the ratio flagellum/cytoplasm. This ratio was considerably greater in the short epimastigote forms, where the cytoplasm almost equalled the flagellum in length (1,07 F/C), while in the other forms the total length was mainly due to the cytoplasm.

The cytoplasmic and nuclear dimensions are largest in the long epimastigotes, although the greatest width is found in the sphaeromastigotes. However the nucleus/cytoplasm ratio was largest in sphaeromastigotes and metacyclic forms, also, in these forms the kynetoplastic area was approximately double that in the epimastigotes. From our results it seems that the kinetoplast is largest in the sphaeromastigotes forms, shortly followed by the metacyclic forms, both in length and perimeter. However, if we consider the ratio of kinetoplast area to cytoplasm area, we find the same precedence order previously reported (C. Brack, 1968, *Acta Tropica, 25:* 289-356; R. Milder, 1976, *W. H. O. Sci. Publ. 318:* 132-134). The largest dimensions of kinetoplast may be due to its polyenergetic nature (Vickerman, 1974, *loc. cit.*), possibly reflecting an underlying change in the metabolic needs. This change must obviously be closely correlated with the morphologic differences found along the evolution of *T. cruzi.* The existence of a great genetic variability among the different stages has been reported, mostly observed in the nuclear DNA (D. M. Engman et al., 1987, *Mol. Biochem. Parasitol., 22:* 115-123).

The nuclear index showed that metacyclic and short epimastigote forms are posteronuclear while the other forms are mostly anteronuclear. The nucleus showed largest dimensions in the long epimastigotes, forms

that have raised an interesting controversy as to their biological significance. The metacyclic forms also showed a larger nucleus. These measures may be reflecting a largest nuclear activity in these forms.

It has been noted thus supporting that the pathogenicity of trypanosomes may be related with the nuclear index (C. A. Hoare, 1972, *loc. cit.*). We have found a nuclear index of 0,90 in the infective metacyclic forms of this strain of *T. cruzi.*

As far as we know, this is the first morphometrical study of *T. cruzi* evolutive forms in the intestine of *R. prolixus* aided by an image analysis system, hence guaranteeing the objectivity and precision of the results. We have measured an exhaustive series of morphometrical parameters, some of them never measured before, extracting them relevant statistical data.

These data have a real interest for classifying some features of the biological cycle of *T. cruzi* forms in the vector as well as the future development of diagnosis techniques or therapeutical and prophylactic experiments. These data could allow the microscopical differentiation of different strains based on their morphology directly from the xenodiagnostic preparation. We have shown significant differences between blood and culture forms of *T. cruzi* (Cali strain) (J. A. de Diego et al., 1991, *Ann. Parasitol. Hum. Comp., 66:* 3-8). Now, we are studying the morphometry of other *T. cruzi* strains in the intestine of *R. prolixus* to know with a multivariant assay if some morphometrical parameter is helpful in Chagas' disease diagnosis and/or epidemiological studies. To achieve this, more data are necessary accurately describing other strains of *T. cruzi* and the relevant differences among them. This could also lead to the detection of morphological differences that could constitute the substrate of the differences found in the pathoclinical findings described for them, hence serving as a useful tool for the diagnosis, prognosis and orientation of the treatment of Chagas' disease patients.

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