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A COMPARATIVE STUDY OF THE BEHAVIOR OF VENEZUELAN AND BRAZILIAN STRAINS OF TRYPANOSOMA (SCHIZOTRYPANUM) CRUZI IN THE VENEZUELAN INVERTEBRATE HOST (RHODNIUS PROLIXUS)

Servio URDANETA-MORALES and Irma G. RUEDA

SUMMARY

The behavior of a Venezuelan strain ("E.P." strain) of **Trypanosoma (Schizotrypanum) cruzi** in the local race of the vector **Rhodnius prolixus** is studied and compared with the behavior of a Brazilian strain ("Y" strain) in the same local race of insect. A review is made of the results of investigations made in other countries into the factors that influence the adaptation, development, and multiplication of this parasite in the digestive tract of triatomids. A detailed analysis of the results of the present investigation reveals that the local strain of **T. cruzi** is better adapted to develop in the local vector, **R. prolixus**, than is the Brazilian strain of the parasite. Therefore, it is concluded that one of the important factors in the development of **T. cruzi** is the adaptation of particular strains to the vectors found in the same geographical area.

INTRODUCTION

The ecology of **Rhodnius prolixus** and its role as the local vector of **Trypanosoma (Schizotrypanum) cruzi** in Venezuela have been elucidated by the works of TORREALBA [30]; GAMBOA [8,9,10]; GOMEZ-NUÑEZ [11,12]; GOMEZ-NUÑEZ et al. [13,14]; PIFANO et al. [23,24].

Many clinical and experimental investigations upon the behavior of this parasite in vertebrate hosts have been carried out [16,21, 25,29,32]. However, few studies have been made on the factors affecting the invertebrate host/parasite relationships of Venezuelan strains of **R. prolixus** and **T. cruzi**; to our knowledge, such data as are available are incidental observations from studies undertaken for other purposes.

WOOD [33] observed that, in natural conditions, high temperatures increased the numbers of trypomastigotes of **T. cruzi** in the intestine of reduviids, a result confirmed in the laboratory PHILLIPS [19]. PIFANO [25] noted that there was apparently no direct relation between the level of parasitaemia in an infected vertebrate and the number of flagellates produced in a vector that had fed upon it, as some triatomids did not acquire infections from vertebrate hosts with many **T. cruzi** in the blood. PHILLIPS & BERTRAM [20] reported similar results, noting individual differences in the susceptibility of the insects to infection by the flagellate.

RYCKMAN [27] carried out elegant experiments to show that, in nature, there is a better adaptation between the parasite and the invertebrate host when they are from the same geographical area. MAEKELT [15] and BRENER [2] reported that there are genetic factors in the insects that condition their susceptibility to infection.

URDANETA-MORALES [31] demonstrated that the type of blood ingested by **R. prolixus** (bird, mammal, or poikilotherm) had no evident effect on the development and multiplication of **T. cruzi** in the vector. PERLOWA-GORA-SZUMLEWICZ [18] emphasized the need of determining whether the age and sex of

Instituto de Zoología Tropical. Facultad de Ciencias. Universidad Central de Venezuela, Caracas, Venezuela

the invertebrate host should be taken into consideration with respect to susceptibility to infection, as they are in the mammal host.

The present work was undertaken to investigate the influence of the above mentioned factors, with a view to comparing the behavior of local and foreign strains of **T. (S.) cruzi** in a local strain of **R. prolixus.**

MATERIALS AND METHODS

The vectors employed were from our laboratory strain of **Rhodnius prolixus,** maintained for more than 20 years in the laboratory and derived from insects captured in the state of Guárico, Venezuela; they are kept at 29°C and 70-80% relative humidity.

Two strains of **T. cruzi** were employed; the local strain, denominated "E.P.", was isolated by TORREALBA [29] from a human case in Tinaquillo, state of Cojedes, Venezuela; the other, Brazilian, strain denominated "Y", was isolated by SILVA & NUSSENZWEIG [28]. Both strains were maintained by intraperitoneal inoculation into white mice every 10 or 12 days.

To avoid the possibility of confusion of the **T. cruzi** with natural infections of flagellates in the insects, such as that reported by CERISOLA et al. [3] of **Blastocrithidia triatomae** infecting **Triatoma infestans** reared in the laboratory, the intestinal contents of 30 insects were extracted by the technique of DIAS [6], mixed with 0.85% saline, examined fresh under phase contrast, and stained by the technique of COTRIM & RAMALHO [5].

The insects were infected by allowing them to engorge on mice showing high parasitaemias: an average of 8.5 x 10^6 trypanosomes/ml blood for the "E.P." strain, and 8.0 x 10^6 trypanosomes/ml for the "Y" strain, by haemocytometer count.

The following experiments were carried out in parallel with both strains of the parasite:

Investigation of the "prepatent period" of infection of T. cruzi in R. prolixus

Twenty-five insects of each of the first three instars and 20 each of the 4th and 5th instars were infected as above with each strain of the parasite. At 24-hour intervals from the infecting blood meal, pools of spontaneously expelled feces from each of the instars were diluted with 0.05 ml of sterile 0.85% saline and examined microscopically to determine the first expulsion of living flagellates.

Investigation of the number of fecal flagellates and percentage of metacyclic forms for each instar and sex of R. prolixus

For each strain of **T. cruzi,** 25 bugs each of the lst, 2nd, and 3rd stages, 20 bugs each of the 4th and 5th instars, 10 adult females and 10 adult males were infected in the usual manner. The fecal counts (number of motile flagellates/ml feces/insect) were made with a haemocytometer on two occasions, the first on spontaneously expelled feces 6 days after the infecting blood meal, and the second on the intestinal content obtained by dissection according to the technique of DIAS [6], immediately after the molt to the next instar. The latter material was also made into smears, for estimation of the percentage of metacyclic forms, expressed as the number of metatrypomastigotes/500 flagellates counted at 1000 X.

Study of temperature effect on the adaptation, development, and multiplication of T. cruzi in the vector

A total of 80 insects of each of the first three instars was divided into 4 lots of 20 each, of which one lot was kept at each of the following temperatures: 20°C, 25°C, 29°C, and 35°C, at 60-80% relative humidity. Six days after the infective blood meal, and immediately after the next molt each lot was examined as in the above experiment to determine the progress of the infection at each of the four temperatures.

Study of the production of metatrypomastigotes in the course of the developmental cycle of R. prolixus

Fifty lst instar nymphs were infected as described above. Four months later, their feces were examined, and at the time of molting to the 5th instar, those insects whose feces

were negative were dissected for determination of the presence of the parasite in the intestinal contents.

Study of the relationship between the parasitaemia of the vertebrate host and the density of the flagellates in the intestinal content of R. prolixus fed upon it

Two experiments were carried out in this study:

1) Ten 5th instar bugs and 10 adults of each sex were infected by allowing them to engorge upon mice with parasitaemias of 8.5 x 10^6 trypanosomes/ml of blood of the "E.P." strain. The insects were dissected individually at the times of molt or oviposition, respectively (12-17 days after the blood meal) to determine the number of flagellates/ml intestinal content/insect.

2) Two lots of 60 lst instar insects each were infected by engorging on mice which had parasitaemias of 12 x 10^6 and 4.4 x 10^6 trypanosomes/ml of blood, respectively. Ninety-six hours later, and thereafter at intervals of 2-3 days up to the 25th day, pools of the intestinal contents of samples of the insects were examined to determine the density of the parasites.

RESULTS

Observations upon the intestinal material of uninfected R. prolixus reared in the laboratory

No flagellates were seen in the intestinal contents of uninfected bugs reared in the laboratory, either in fresh material examined under phase contrast or in stained smears. It was therefore concluded that the laboratory strain of insects used in the experiment was free of intestinal flagellates.

Observations upon the "prepatent period" of T. cruzi infections in R. prolixus

The first flagellates were expelled in the feces between 48 and 72 hours after the infecting blood meal when the vertebrate host was infected with the "E.P." strain of **T. cruzi** (Table I), while the first flagellates appeared 3 to 6 days after an infecting meal on a host with the "Y" strain. The scanty parasites observed in the incipient infection of the insect vector were sphaeromastigotes and epimastigotes; amastigotes were rarely seen.

Observations upon numbers of intestinal flagellates and percentage of metatrypomastigotes of T. cruzi in R. prolixus, by instar and sex

Table II shows that 6 days after the insects were permitted to engorge upon a mouse infected with the Brazilian "Y" strain of **T. cruzi,** flagellates were detectable in the feces of the 2nd stage only, at very low density (4.06 flagellates/ml feces). In contrast, the insects fed upon mice with the Venezuelan "E.P." strain showed far denser infections in the same period of time (144 flagellates/ml feces in the 4th instar).

In Table III, it may be seen that, in bugs infected with the Venezuelan strain of **T. cruzi,** the average number of parasites encountered increases with the age of the insect, so that the lowest number of parasites is found in the lst instar nymphs, increasing gradually through the stages until the highest infections are found in the adults. On the other hand, with the "Y" strain, it does not appear to be any significant influence of the age of the insect on the level of infection.

There is an impression of better development of the parasites of the "E.P." strain in adult female insects than in adult males, although the differences in number of flagellates observed in the two sexes are not statistically significant.

Table IV indicates that metatrypomastigotes were present in the feces of all instars at the moment of molting to the next stage, and in adult females at the time of oviposition, in the case of insects infected with the "E.P." strain, although both molting and oviposition took place at an average of 13 days after the blood meal inciting these phenomena. In contrast, the table shows that the metacyclic forms were not seen in the feces of insects infected with the Brazilian strain.

Observations upon the influence of temperature on the development of infections of T. cruzi in R. prolixus

Comparison of the data given in Tables V, VI and VII emphasizes the high densities of flagellates in the feces of all three instars of **R. prolixus** infected with the "E.P." strain of **T. cruzi** in contrast to the low densities of the "Y" strain in the same three stages kept at the same constant temperatures.

The Venezuelan strain appears to develop best at the lower temperatures (20 and 25°C) in the 1st instar; in the 3rd stage the higher temperatures (29 and 35°C) produce the highest densities of intestinal flagellates; in the 2nd stage, both temperature ranges were equally favorable for development of the parasites.

The Brazilian "Y" strain, on the contrary, appeared to develop best at the lower temperatures (20 and 25°C) and, judging from the data on hand, it would appear that the density of the parasites decreased as the temperature was raised.

The relatively high temperature of 35°C did not noticeably affect the colonization of the insect's digestive tract by the "E.P." strain, while it was definitely limiting factor for the "Y" strain, especially in the 3rd instar.

Production of T. cruzi metatrypomastigotes in R. prolixus four months after infection

First instar nymphs of **R. prolixus,** fed upon mice infected with the Venezuelan "E.P." strain or the Brazilian "Y" strain took 4 months to develop to the 5th instar. At this time, the stained feces of the insects were examined to determine the percentage of metatrypomastigotes in the intestinal flagellates. The results were clear: 31% metacyclic forms for the Venezuelan strain as compared with 0.66% metatrypomastigotes for the Brazilian strain.

Influence of the level of the parasitaemia of the vertebrate host on production of fecal flagellates in R. prolixus ("E.P." strain)

TABLE I

«Prepatent period» of infection by **Trypanosoma (S.) cruzi** in the 5 larval stages of **Rhodnius prolixus**

Stage	«E.P.» Strain	«Y» Strain
I	48 hrs.	5 days
II	48 hrs.	4 days
III	72 hrs.	6 days
IV	48 hrs.	3 days
V	48 hrs.	3 days

TABLE II

Fecal infections (No. flagellates/cubic mm. feces/insect) observed in **R. prolixus** 6 days after being fed upon mice parasitized with **T. cruzi**

Stage	«E.P.» Strain	«Y» Strain
I	7.76	0
II	26.18	4.06
III	70.25	0
IV	143.75	0
V	67.5	0

Note: Adults did not defecate

TABLE III

Behavior of **T. (S.) cruzi** in each of the 6 instars of **R. prolixus**

Instar	«E.P.» Strain				«Y» Strain			
	(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)
I	20	377.95	265.83	628.0	16	41.88	30.0	48.75
II	12	840.08	731.67	914.17	17	5.15	2.5	9.0
III	18	540.14	275.0	913.33	14	4.11	2.5	7.5
IV	12	1176.46	666.25	2317.5	11	5.68	1.67	16.25
V	11	1201.77	95.0	2770.0	11	14.32	7.5	22.5
adult males	10	1504.75	85.0	2902.5				
adult females	11	1825.68	477.5	3982.5				

(a) = number of insects examined
(b) = average number flagellates/ml intestinal content/insect
(c) = minimum
(d) = maximum

TABLE IV

Percentage of metacyclic forms of **T. cruzi** observed in smears of intestinal contents taken at times of molting and oviposition

Strain	Instar						
	I	II	III	IV	V	males	females
«E.P.»	0.6	0.2	0.4	1.0	0.4	0.6	0.4
«Y»	0	0	0	0	0	—	—

TABLE V

Average number of **T. cruzi** flagellates/ml feces/insect/temperature 6 days post-infection

Instar	«E.P.» Strain				«Y» Strain			
	Temperature							
	20°C	25°C	29°C	35°C	20°C	25°C	29°C	35°C
I	8.44	11.88	7.76	—	1.67	0	0	—
II	11.75	18.89	26.18	125.25	13.25	0	4.06	2.5
III	29.69	43.33	70.25	20.0	1.11	1.54	0	2.0

—: no defecation

TABLE VI

Influence of temperature upon **T. cruzi** infections in **R. prolixus** expressed as number of flagellates/ml intestinal content/insect

Temperature	«E.P.» Strain Instar			
		I	II	III
20°C	(a)	10	12	15
	(b)	673.91	711.25	492.67
	(c)	605.63	590.0	430.83
	(d)	766.88	837.5	630.0
25°C	(a)	14	14	13
	(b)	600.18	1018.36	424.81
	(c)	297.5	812.5	306.67
	(d)	1135.0	1171.25	584.17
29°C	(a)	20	12	18
	(b)	377.95	840.08	540.14
	(c)	265.83	731.67	275.0
	(d)	628.0	914.17	913.33
35°C	(a)	11	12	12
	(b)	484.32	801.17	750.21
	(c)	313.13	638.33	510.0
	(d)	617.5	1027.5	1050.0

(a) = number of insects examined
(b) = mean number flagellates/ml intestinal content/insect
(c) = minimum
(d) = maximum

TABLE VII

Influence of temperature upon **T. cruzi** infections in **R. prolixus**, expressed as number of flangellates/ml intestinal content/insect

Temperature	«Y» Strain	Instar		
		I	II	III
20°C	(a)	16	12	13
	(b)	472.66	373.96	1.92
	(c)	400.0	315.0	0
	(d)	512.7	461.67	6.25
25°C	(a)	15	14	13
	(b)	162.67	10.18	29.62
	(c)	128.13	2.5	1.25
	(d)	255.0	23.33	66.25
29°C	(a)	16	17	14
	(b)	41.88	5.15	4.11
	(c)	30.0	2.5	2.5
	(d)	48.75	9.0	7.5
35°C	(a)	6	15	12
	(b)	22.5	65.17	0
	(c)	8.33	24.17	0
	(d)	36.67	105.83	0

(a) = number of insects examined
(b) = mean number of flagellates/ml intestinal content/insect
(c) = minimum
(d) = maximum

Table VIII and Fig. 1 indicate that the level of parasitaemia of the vertebrate host has no apparent influence on the production of fecal parasites in the invertebrate host, at least for the "E.P." strain. It is interesting to note that a parasitaemia of 4.4 x 10^6 produced nearly double the number of fecal protozoans in **R. prolixus**, compared with a host parasitaemia of 12 x 10^6.

Analysis of Table IX appears to indicate that, not only is there no evident relation between the parasitaemia of the vertebrate host and the level of infection in the invertebrate host, but that there are wide variations of susceptibility to infection among the individual insects. Fifth instar nymphs fed upon host with high parasitaemias had fecal counts ranging between 95 and 2770 flagellates/ml intestinal content; adult males and females expelled feces containing 85-2902.5 flagellates/ml and 477.5-3982.5 flagellates/ml, respectively.

DISCUSSION

Of the host-parasite relationships of American trypanosomiasis, one of the least studied in Venezuela has been the capacity of the local strains of **Trypanosoma (Schizotrypanum) cruzi** to establish infections in the local triatomids.

WOOD [33] emphasized that the seasons of high environmental temperatures (28-35°C) were more favorable for the formation of metacyclic forms in the intestine of **T. protracta** than seasons of low temperature (22-23°C). PHILLIPS [19], in laboratory studies on **R. prolixus**, found that the higher the temperature, the sooner the vector expelled infective forms of **T. cruzi** in the feces. NEVES [17] reported that temperatures between 23°C and 27°C were optimal for production of metatrypomastigotes in the feces of **T. infestans, T. brasiliensis, R. prolixus** and **Panstrongylus megistus**, and that at 36°C, metatrypomastigotes and epi-

TABLE VIII

Infections in 1st instar larvae of **R. prolixus** fed upon mice with different parasitaemias of **T. cruzi** («E.P.» strain), expressed in mean number of flagellates/ml feces/insect

Days post-infection	Parasitaemias	
	12 x 10^6 t/ml	4 x 10^6 t/ml
4	+	+
6	0.75	5.25
9	7.08	10.83
12	13.0	32.5
15	11.07	0.83
18	9.25	14.5
20	3.75	13.12
22	5.71	4.17
25	6.67	12.5

t/ml = trypanosomes/ml blood

TABLE IX

Results of individual examinations of the intestinal contents of 10 insects each of the 5th instar, adult males and adult females fed upon mice with a parasitaemia of 8.5 x 10^6 t/ml, trypanosomes of the «E.P.» strain of **T. cruzi**

Number flagellates/ml intestinal content/insect		
5th Instar	Adult male	Adult female
1155	697.5	2287.5
240.0	2902.5	3982.5
1430.0	305.0	3745.0
2770.0	85.0	477.5
2295.0	2455.0	2460.0
1952.0	2390.0	1197.5
95.0	762.5	750.0
270.0	2722.5	647.5
1680.0	302.5	1215.0
177.5	2425.0	857.5

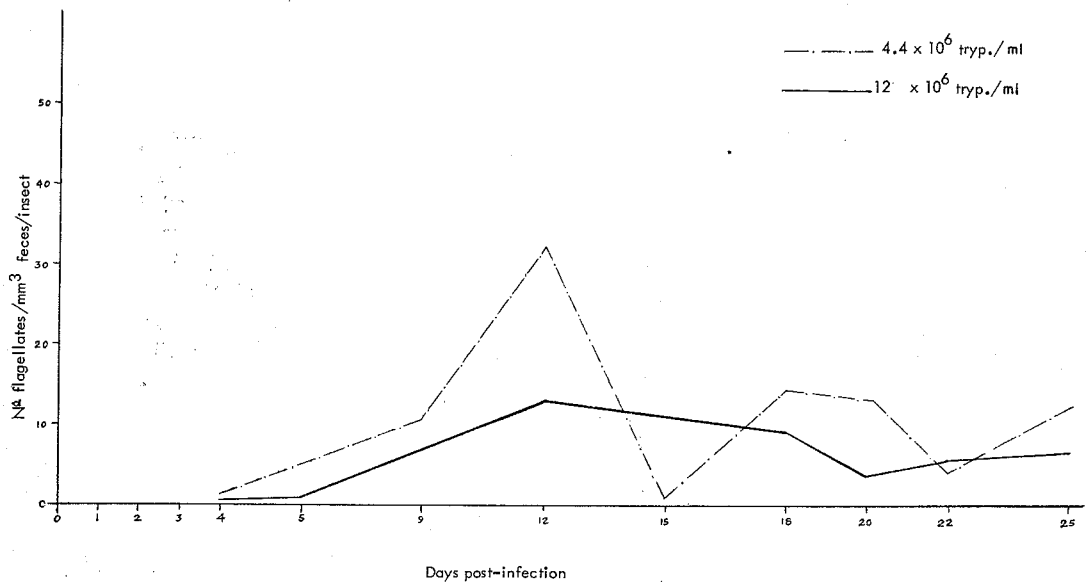

Fig. 1 — Relation between level of parasitaemia (4.4 x 10^6 and 12 x 10^6 tryp./ml) of **T. cruzi** («E.P.» strain) and fecal production of flagellates in **R. prolixus** (1st instar nymphs)

mastigotes were very scanty or completely absent. In our experiments with the Venezuelan "E.P." strain, the densities of flagellates were of approximatelly equal magnitude at the two ranges of temperature employed; however, there was a superimposed age effect: both "E.P." and "Y" strains developed best in the 1st instar at the lower range; the "E.P." strain developed equally well in the 2nd instar at both ranges of temperature; and the higher range was more favorable for development of this strain in the 3rd stage.

It should be noted that the local "E.P." strain developed and multiplied well in the Venezuelan **R. prolixus** at the elevated temperatures of 29 and 35°C; at the latter temperature there were appreciable quantities of intestinal protozoans in all three instars employed, in contrast to the results of NEVES [17]. Also, insects infected with the "E.P." parasites produced high levels of metacyclic forms in the feces (31%) when maintained for 4 months at 29°C.

There appears to be a correlation between the instar of the triatomid and the density of fecal parasites obtained (Tables II and III). There was a steady increase in flagellate level from the 1st stage to the adult female. This result may have important epidemiological implications, inasmuch as ZELEDON [34], working with **R. prolixus, T. infestans,** and **T. dimidiata,** obtained a higher average number of defecations from the first species, a result similar to that found by PIPPIN [26], when working with **R. prolixus, T. sanguisuga,** and **T. gerstaeckeri.**

DIAS [7] noted that the Brazilian triatomids showed higher levels of infection with local strains of **T. cruzi** than did Venezuelan insects; RYCKMAN [27] found North American triatomids (**T. protracta**) more susceptible to North American strains of **T. cruzi** than to South American strains, and that South American triatomids (**T. infestans**) were more susceptible to the South American strains of the parasite. CERISOLA et al. [4] obtained positive xenodiagnosis of chronic human cases of Chagas's disease in Argentina most frequently with the Argentine triatomid **T. infestans,** followed by **T. phyllosoma** and **R. prolixus,** and that xenodiagnosis of the same cases with triatomids of different geographic origin (**T. dimidiata, P. herreri,** and **R. pallescens**) were negative. ZELEDON [34], marking xenodiagnosis in Costa Rica, concluded that the local strains of **T. dimidiata** was more susceptible to infection by the Costa Rican strains of **T. cruzi** than foreign **R. prolixus** or **T. infestans.**

The results of our comparative study of the behavior of the Venezuelan and Brazilian strains of **T. cruzi** in a Venezuelan strain of **R. prolixus** have led us to the conclusion that the highest incidence of infection of the vector and the best development of the parasite occur when both are from the same geographical area; the Venezuelan vector showed a consistently higher susceptibility to the Venezuelan parasite than to the Brazilian one.

Of 28 insects infected with the Venezuelan "E.P." strain, none were negative 4 months later, while 14 of 34 insects allowed to feed on mice parasitized with the Brazilian strain were negative after this period of time. Another indication of the better physiological adaptation of the local parasites to the Venezuelan vector was the high percentage of metatrypomastigotes in the feces of bugs infected with the "E.P." strain (31%), as against only 0.66% of metacyclic forms with the Brazilian strain.

Although we believe that the best parasite-vector combination is achieved when both are from the same geographical locality, the influence of individual differences among the vectors can not be neglected (URDANETA-MORALES [31]) since, as mentioned above, some 41% of Venezuelan bugs fed upon mice with a high parasitaemia (8.5×10^6 trypanosomes/ml blood) of the Brazilian "Y" strain did not retain an infection throughout the course of their development. This lends support to the supposition of PHILLIPS & BERTRAM [20] and BRENER [2] that, in a given lot of insects, a certain number will be refractory to infection.

In addition, the variability of susceptibility to infection in individual insects is illustrated by the examination of the intestinal contents of 30 bugs which were allowed to engorge to repletion on blood with a high parasitaemia of the "E.P." strain (Table IX). The densities of intestinal parasites in these insects ranged from 85 to 3982.5 flagellates/ml of intestinal content, in spite of having been fed on the same mouse.

The relatively greater capacity of the local strain to infect Venezuelan **R. prolixus,** in comparison to the Brazilian strain, might be explained by the greater degree of physiological adaptation of the Venezuelan **T. cruzi** to the conditions offered in the gut of the local vector; the Brazilian strain of the parasite would then be better adapted to the characteristic Brazilian hosts (**T. infestans, T. sordida, T. maculata, T. brasiliensis, T. rubrovaria, P. geniculatus,** and **P. megistus**) than to **R. prolixus,** which has never been found naturally infected in Brasil (BARRETO [1]).

RESUMEN

Estudio comparativo del comportamiento de una cepa venezolana con una cepa brasileña del Trypanosoma (Schizotrypanum) cruzi **en el vector venezolano** (Rhodnius prolixus)

URDANETA-MORALES, S. & RUEDA, I. G. — A comparative study of the behavior of Venezuelan and Brazilian strains of **Trypanosoma (Schizotrypanum) cruzi** in the Venezuelan invertebrate host (**Rhodnius prolixus**). **Rev. Inst. Med. trop. São Paulo 19**:241-250, 1977.

Se estudia el comportamiento de una cepa venezolana de **Trypanosoma (Schizotrypanum) cruzi** (cepa "E.P.") en **Rhodnius prolixus** local y se lo compara con una cepa de orígen brasileño (cepa "Y").

Se hace un recuento de las investigaciones que se han realizado en otros países para tratar de determinar los factores que influyen en la adaptación, evolución y multiplicación de este parásito en el tracto intestinal de los triatominos; en base a ésto, los autores analizan detalladamente los resultados obtenidos por ellos los cuales demuestran que, se logró una mejor adaptación parasitaria entre las cepas venezolanas de **T. (S.) cruzi** y la del triatomino empleado, por lo cual se concluye que, entre otras causas, uno de los factores responsables de este comportamiento es el hecho de que las cepas de **T. cruzi** deben estar adaptadas en la naturaleza a determinados vectores de la misma área geográfica.

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