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STUDIES ON *TRYPANOSOMA RANGELI* TEJERA, 1920. IX. COURSE OF INFECTION IN DIFFERENT STAGES OF *RHODNIUS PROLIXUS*

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Frequent individual observations od different stages of Rhodnius prolixus *exposed to* Trypanosoma rangeli, *revealed a higher susceptibility to infection in the bugs exposed during the two first instars. The mortality rate in infected bugs was significantly higher than in controls, indicating that the parasite was responsible for the majority of deaths. An analysis of the mortality distribution, per instar, is presented.*

Statistical analysis of deaths among the different infected instars, showed that T. rangeli *produces its pathological effect in any stage of* R. prolixus *independently of its susceptibility to the parasite.*

The survival to adult decreased in all the infected instar bugs.

A significant longer time to reach the adult stage was observed in the infected bugs when compared with controls, excepting for specimens exposed in the third instar.

The epidemiological significance of the present results is discussed.
Key words: *Trypanosoma rangeli – Rhodnius prolixus* infection

The pathogenicity of *Trypanosoma rangeli* for its triatomine-vectors has been confirmed and studied both under natural and experimental conditions by various workers (Grewal, 1957; Tobie, 1965; Gomez, 1967; Marinkelle, 1968; Watkins, 1971; D'Alessandro, 1976; Añez, 1984).

Recently, Añez (1984) reported that when first instar nymphs of *Rhodnius prolixus* and *R. robustus* were experimentally exposed to *T. rangeli*-infection, the survival of the infected bugs to the adult stage, decreased significantly in relation to the uninfected control-bugs. He also concluded that in *R. prolixus* the most affected instars were the first, second and fifth, where a higher mortality was observed, while in *R. robustus* a progressive increase of the mortality from the first to fifth instar, was mortality from the first to fifth instar, was detected. However, the pathogenicity of *T. rangeli* as measured by overall mortality was the same in both species of bugs.

In order to know the effect of *T. rangeli*-infection on each of the instar nymphs and its further development, groups of specimens of *R. prolixus* at different nymphal stages were exposed experimentally to a blood meal infected with a fresh Venezuelan isolate of the parasite. This work was carried out following frequent individual observations on the infected bugs and its control, until they reached the adult stage or died. In this paper details are given of the susceptibility of each instar to the infection, the mortality rate and its distribution per instar, the relation infection-survival and the time of permanence in each instar nymph.

MATERIALS AND METHODS

Triatomine-bugs – The origin and maintenance of our colony of *Rhodnius prolixus*, have been described in previous papers (Añez, 1981; Añez & East, 1984).

Stock of Trypanosoma rangeli – DOG/82, isolated by one of us (N.A.) by xenodiagnosis of infected dogs in Trujillo State, Venezuela, and maintained by bug-mouse-bug passages. Bugs with salivary gland infection, detected by the method of salivation on glass slides were used to infect white mice as previously described (Añez, 1980, 1984).

Infection of Triatomine-bugs – Groups of 30 specimens of the first, second, third, fourth and adult stages and 35 of the fifth instar of *R. prolixus*, were allowed to feed on infected mice, which exhibited an average parasitaemia of 521 Tryps/mm^3; 508 Tryps/mm^3; 467 Tryps/mm^3; 470 Tryps/mm^3; 1.125 Tryps/mm3 and 468 Tryps/mm^3; respectively. To estimate the quantity of infected blood ingested, all the bugs were weighed before and after feeding.

R. prolixus, first, second and fifth instar nymphs and adult stages in groups of 10 and third and fourth instar nymphs in groups of 20 were fed on clean mice and kept as uninfected controls.

Engorged bugs were placed individually in

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3.5 cm x 2 cm glass vials, maintained at 26°C and 75% relative humidity and fed on clean mice every 15 to 21 days until the end of the experiments.

Occurred deaths, time of moulting and detection of infection in dead or surviving bugs, were recorded as indicated in a previous publication (Añez, 1984). The time of permanence (TP) of each instar was estimated as the difference between the time of moulting (TM) to an indicated instar and the time of moulting to the next stage (i.e. TPII = TMIII-TMII).

Statistical analysis – To determine the statistical significance of susceptibility, mortality, relation mortality-infection and survival-infection, a CHi square (x^2) test following the comparison of proportions from many samples (see Fleiss, 1973) was used. To establish the level of significance of the time of permanence per instar, the student T test, was used.

RESULTS

Susceptibility of developmental stages of R. prolixus *to the infection by* T. rangeli – Observations on different instar nymphs of *R. prolixus* exposed to a *T. rangeli* infected meal, revealed that the range of infection varied from 16.6% at the III instar to 66.6% at I and II nymphal stages (Table I). Statistical comparison of infected bugs among the different stages, showed the existence of two groups susceptible to *T. rangeli:* one made up of the two first instars and the other of the rest of nymphal stages (Table I).

TABLE I

Susceptibility of the various stages of *Rhodnius prolixus* to infection by *Trypanosoma rangeli*

R. prolixus Instar	No. Infected / No. Exposed	% Infected *
I	20/30	66.6 *a*
II	20/30	66.6 *a*
III	5/30	16.6 *b*
IV	7/30	23.3 *b*
V	14/35	40.0 *b*
Adult	10/30	33.3 *b*
Total	76/185	41.08

*Proportions marked with the same letter showed no significant differences ($p > 0.05$)

– Statistical significance between groups *a* and *b* ($x^2 = 6,37$; $P < 0.05$).

TABLE II

Mortality distribution per instar in *Rhodnius prolixus* exposed to *Trypanosoma rangeli* at different nymphal stages

No. of specimens and exposed nymphal stage		No. (%) of deaths/ instar					Total No. (%) of deaths/ exposed instar	Uninfected Control
		I	II	III	IV	V		No. deaths/ instar (%)
30	I	3 (10)	1 (3.3)	2 (6.6)	1 (3.3)	2 (6.6)	9 (30)	0/10 (0)
30	II		2 (6.6)	3 (10)	0 (0)	3 (10)	8 (26.6)	0/10 (0)
30	III			3 (10)	0 (0)	2 (6.6)	5 (16.6)	2/20 (10)
30	IV				2 (6.6)	4 (13.3)	6 (20)	1/20 (5)
35	V					4 (11.4)	4 (11.4)	0/10 (0)
30	Adult						4 (13.3)	0/10 (0)
Total	185						36 (19.45)	3/80 (3.75)

TABLE III

Relation of mortality among *Trypanosoma rangeli* – infected *Rhodnius prolixus* and localization of infection in different instars.

Instars / Bugs	No. Deaths / No. Infected	(% Mortality)	No. (%) of dead bugs with infection in:		
			Gut	Haemolymph	Salivary Glands
I	8/20	(40.0)	8 (100)	7 (87.5)	7 (87.5)
II	7/20	(35.0)	7 (100)	4 (57.1)	0 (0.0)
III	3/5	(60.0)	3 (100)	0 (0.0)	0 (0.0)
IV	4/7	(57.1)	4 (100)	2 (50.0)	2 (50.0)
V	3/14	(21.4)	3 (100)	2 (66.6)	2 (66.6)
Adult	1/10	(10.0)	1 (100)	0 (0.0)	0 (0.0)
Total	26/76	(34.2)	26 (100)	15 (57.6)	11(42.3)

TABLE IV

Relation infection-survival to adult stage in *Rhodnius prolixus* infected with *Trypanosoma rangeli* at different instars.

Instars	No. Infected / No. survivors	(% infected survivors)	No. (%) of survivors with infection in:		
			Gut	Haemolymph	Salivary Glands
I	12/21	(57.1)	12 (100)	5 (41.6)	5 (41.6)
II	13/22	(59.0)	13 (100)	2 (15.3)	2 (15.3)
III	2/25	(8.0)	1 (50)	1 (50.0	0 (0.0)
IV	3/24	(12.5)	3 (100)	3 (100)	3 (100)
V	11/31	(35.4)	11 (100	1 (9.0)	1 (9.0)
Total	41/123	(33.3)	40 (97.5)	12 (29.2)	11 (26.8)

Mortality rate and mortality distribution per instar in R. prolixus *exposed to* T. rangeli *at different nymphal stages* – From 185 specimens exposed to the infection, 36 (19.45%) died during the time of observation, while in uninfected control bugs only a 3.75% mortality, was observed. Statistical comparison of mortality between unexposed and *T. rangeli*-exposed bugs, revealed significant differences ($x^2 = 9,76$; $P < 0.005$).

Although the percentage of observed mortality varied from 30% in specimens exposed during the I instar to 11.4% in those exposed in the V nymphal stage, no statistical significance was obtained when deaths among the different exposed instar were compared.

The distribution of mortality to each exposed instar until their transformation to adult, is shown in Table II.

The proportional comparison of the mortality occurred among specimens of the same instar, which were exposed at different stages of development, revealed no statistical significance in the II, III and V instar nymphs. However, in the case of the IV instar the statistical analysis showed significant differences ($x^2 = 32.94$; $P < 0.001$), which can be due to the non-occurrence of deaths when the II and III stages were exposed (see Table II).

Mortality among R. prolixus *infected with* T. rangeli *different instars and detection of infection in dead bugs* – Table III shows details on the number of deaths that occurred in relation to the infected instars and the detection of infection in the gut, haemolymph and salivary glands of bugs infected at different developmental stages.

Statistical comparison of the proportion of occurred deaths among the different infected instars, revealed no significant differences.

The mean time in which deaths occurred varied from 35.6 days in the group of bugs infected in the V instar to 88 days in those exposed during the IV stage. Deaths were recorded as early as 6 days in I instars and as late as 192 days in V instars that were exposed to infection when they were at I nymphal stage.

Survival to adult stage in T. rangeli-*infected* R. prolixus *and localization of infection* –

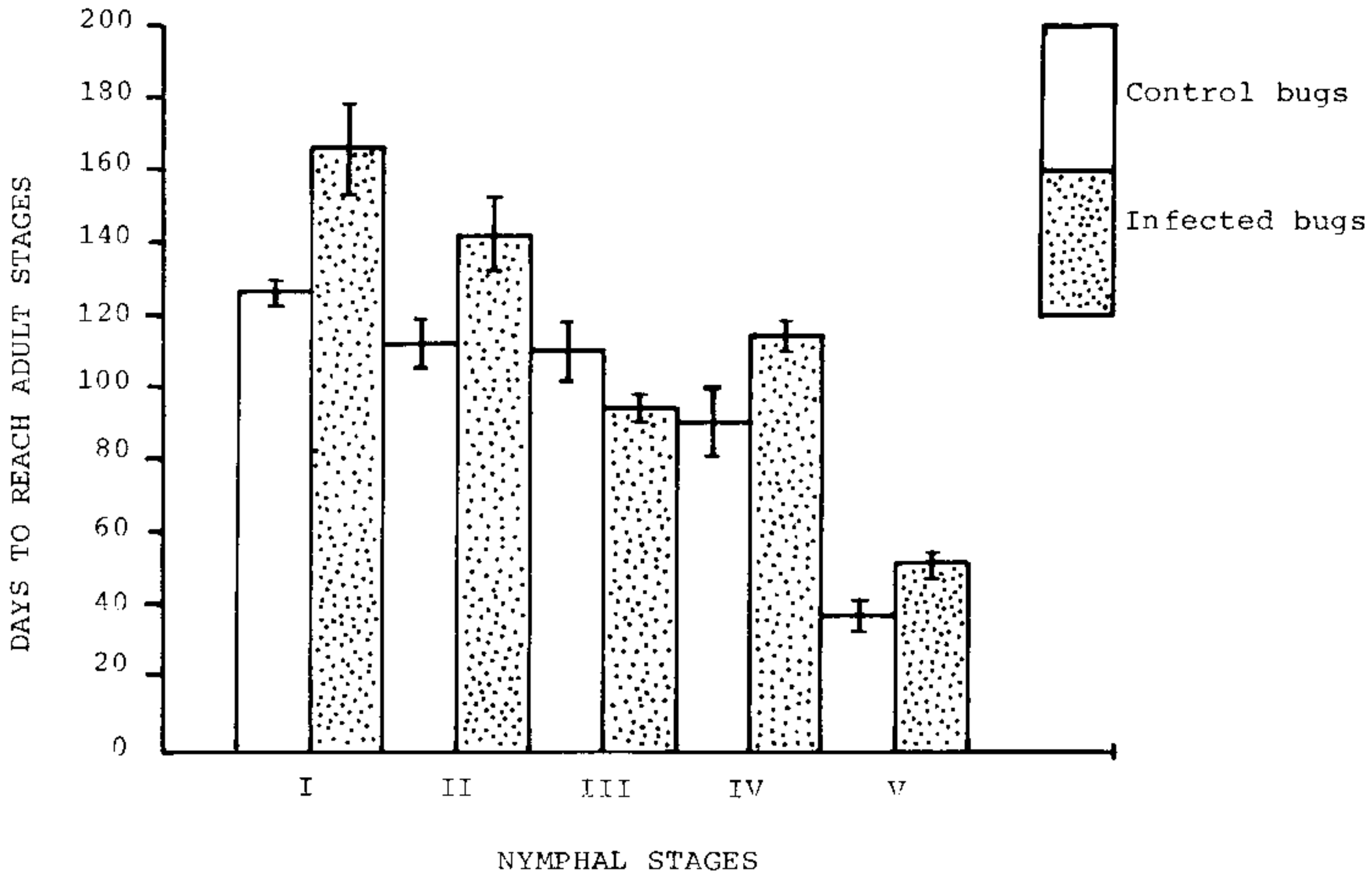

Fig. 1: comparison of time to reach the adult stage between specimens of *Rhodnius prolixus* uninfected and infected by *Trypanosoma rangeli* at different instars.

When groups of specimens from the I to the V instars were exposed to *T. rangeli*-infection, survival to adult stage decreased to 70%, 73.3%, 83.3%, 80% and 88.5%, respectively. Statistical comparison of these proportions showed no significant differences.

In Table IV details are given on the percentages of infection and the detection of parasites in the gut, haemolymph and salivary glands of survivor-bugs.

Time of permanence per instar in R. prolixus *exposed to* T. rangeli *at different developmental stages* – The comparison of the time spans to reach the adult stage between uninfected and *T. rangeli*-infected *R. prolixus*, revealed that apart from the specimens exposed during the III instar, all the other groups exhibited a longer time than that detected in the uninfected control bugs. The differences being statistically significant ($P < 0.001$). See Fig. 1.

DISCUSSION

In the present study, when specimens of *R. prolixus* at different nymphal stages were exposed to *T. rangeli*, a 41% susceptibility to infection was detected, resulting the most susceptible those bugs exposed during the two first instars, where a 66% infection was observed.

Considering that all the bugs were fed on mice with a similar level of parasitaemia and not all of them acquired the infection, it is possible to assume that those who escape from infection were able to develop an individual resistance, which allowed them to destroy the parasite and survive without infection. This speculation finds support in the opinion of Steinhaus (1949) and Garnham (1964) who stated that is not uncommon in susceptible species of insects that many individuals may escape from infection. This phenomenom has been categorized as an individual immunity (Weathersby, 1975).

Although the physiological or immunological mechanisms to explain the individual resistance of *R. prolixus* to *T. rangeli*-infection are unknown, our results on susceptibility to infection appear to indicate that the stages from the III to adult are more resistant than the two first instars.

Feliciangeli de Piñero & Torrealba (1977) and Rabinovich et al. (1979) demonstrated the persistence of an enlarged middle section in the age pyramid of *R. prolixus* both under field and domiciliary conditions. Moreover, Uribe (1927) and Feliciangeli et al. (1980) reported III and IV instar nymphs of *R. prolixus* surviving from 5 to 7 months under fast condition. Analysing the results obtained by these authors in the context of the present work, it is possible to explain the enlargement in the middle section of the age pyramid of *R. prolixus* in nature. III

and IV instars of *R. prolixus* are on the one hand included in the group less susceptible to *T. rangeli*-infection, which has been demonstrated to be lethal to this species of bug and on the other, they are able to resist starvation for long periods, during the time and under conditions according to Tejera (1920) they can lose the infection by *T. rangeli.*

Determination of pathogenicity of protozoa in their insect-vectors, is usually possible only through an increased mortality (Garnham, 1955). In the model *T. rangeli-R. prolixus* the pathogenic effect of the parasite was first demonstrated by Grewal (1956), being later repeatedly confirmed both naturally and experimentally (Tobie, 1965; Marinkelle, 1968; Añez, 1984).

In this work when different nymphal stages of *R. prolixus* were exposed to *T. rangeli*, 19.45% of them died during the course of the infection. This figure is significantly higher than that detected in the uninfected control bugs, indicating that parasite is responsible for the major number of occurred deaths. The fact that no significant differences were observed after comparing the occurred deaths among the different exposed instars, shows that *T. rangeli* is able to produce its pathological effect in any stage of *R. prolixus*, independently of its susceptibility to the parasite. This could have an enormous epidemiological importance, considering that the elimination of a part of the infected population of *R. prolixus* could diminish the possibility of transmitting not only *T. rangeli* but also *T. cruzi* in the endemic areas of Chagas' disease, where this insect acts as a common vector for both parasites. Moreover, Marinkelle's demonstration (1968) that the same pathological effect of *T. rangeli* observed in experimentally infected *R. prolixus* is detected in nature, lead us to believe that *T. rangeli* could actually act as a regulator of the population of this vector. This statement appears to be supported by D'Alessandro (1976) who affirms that the pathogenic effect of *T. rangeli* and the high mortality produced in this vectors, is a type of biological control of triatomine populations.

RESUMO

Observações individuais sobre diferentes estádios de *Rhodnius prolixus* expostos ao *Trypanosoma rangeli*, revelaram uma maior susceptilidade à infecção nos espécimens expostos durante os dois primeiros estádios. A taxa de mortalidade nos triatomíneos infectados foi significativamente maior que nos controles, indicando que o parasito foi responsável pelo maior número das mortes. Apresenta-se uma análise da distribuição da mortalidade por estádio.

A análise estatística das mortes ocorridas entre os diferentes estádios infectados, indica que o *T. rangeli* produz seu efeito patológico em qualquer estádio de *R. prolixus*, independentemente da sua susceptibilidade ao parasito.

A sobrevida até adulto decresceu em todos os estádios dos triatomíneos infectados. Excetuando-se os espécimens expostos no terceiro estádio, quando foram comparados com os controles, observou-se nos triatomíneos infectados um significativo retardo para alcançar o estádio adulto.

Discute-se o significado epidemiológico dos presente resultados.

Palavras-chave: *Trypanosoma rangeli* – *Rhodnius prolixus* – infecção

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REFERENCES

AÑEZ, N., 1980. Detection of *Trypanosoma rangeli* by salivation of infected *Rhodnius prolixys* on glass slides. *Ann. Trop. Med. & Parasitol., 74* (5): 561-562.

AÑEZ, N., 1981. Studies on *Trypanosoma rangeli* Tejera, 1920. I. Deposition, migration and growth of *T. rangeli* in two mammals. *Parasitological Topics* Sp. Publi. No. 1: 19-25. Soc. Protozool. Allen Press. Kansas.

AÑEZ, N., 1984. Studies on *Trypanosoma rangeli* Tejera, 1920. VII. Its effect on the survival of infected triatomine bugs. *Mem. Inst. Oswaldo Cruz, 79* (2): 249-255.

AÑEZ, N. & EAST, J. S., 1984. Studies on *Trypanosoma rangeli* Tejera, 1920. II. Its effect on feeding behaviour on triatomine bugs. *Acta Tropica, 41*: 93-95.

D'ALESSANDRO, A., 1976. Biology of *Trypanosoma (Herpetosoma) rangeli* In: *Biology of the Kinetoplastida,* Lumsden, W.H.R. and Evans, D.A. ed. Academic Press, London.

FELICIANGELI de PIÑERO, D. & TORREALBA, J. W., 1977. Observaciones sobre *Rhodnius prolixus* (Hemiptera Reduviidae) en su biotopo silvestre *Copernicia tectorum. Bol. Direc. Malariol. San. Amb., 17* (3): 198-205.

FELICIANGELI, M. D.; RABINOVICH, J. & FERNANDEZ, E., 1980. Resistencia al ayuno en triatominos (Hemiptera, Reduviidae) venezolanos. I. *Rhodnius prolixus* Stal. *Rev. Inst. Med. Trop. São Paulo, 22* (2): 53-61.

FLEISS, J. L., 1973. The comparison of proportions from many samples. In: *Statistical Methods for rates and proportions.* John Wiley ed. New York. London. Sydney. Toronto.

GARNHAM, P. C. C., 1955. The comparative pathogenicity of protozoa in their vertebrate and invertebrate hosts. 5th Symp. Soc. Gen. Microbiol., 191-206.

GARNHAM, P. C. C., 1964. Factors influencing the development of protozoa in their arthropodan hosts. 2nd Symp. Brit. Soc. Parasitol., 33-50.

GOMEZ, I., 1967. Nuevas observaciones acerca de la acción patógena del *Trypanosoma rangeli* Tejera, 1920 sobre *Rhodnius prolixus* Stal, 1859. *Rev. Inst. Med. Trop. São Paulo, 9* (1): 5-10.

GREWAL, M. S., 1956. Studies on the "occult" trypanosomes. Ph.D. Thesis of University of London.

GREWAL, M. S., 1957. Pathogenicity of *Trypanosoma rangeli* Tejera, 1920, in the invertebrate host. *Exp. Parasitol., 6:* 123-130.

MARINKELLE, C. J., 1968. Pathogenicity of *T. rangeli* for *R. prolixus* Stal in nature. *J. Med. Ent., 5* (4). 497-499.

RABINOVICH, J.; LEAL, J.A. & FELICIANGELI de PIÑERO, D., 1979. Domiciliary biting frequency and blood ingestion of the Chagas' disease vector *Rhodnius prolixus* Stal (Hemiptera: Reduviidae) in Venezuela. *Trans. R. Soc. Trop. Med. & Hyg.; 73* (3): 272-283.

STEINHAUS, E. A., 1949. *Principles of Insects Pathology.* New York: McGraw-Hill Book Co.

TEJERA, E., 1920. Un nouveau flagelle de *Rhodnius prolixus Trypanosoma (ou crithidia) rangeli* n. sp. *Bull. Soc. Pathol. Exot., 13:* 527-530.

TOBIE, E. J. 1965. Biological factors influencing transmission of *Trypanosoma rangeli* by *Rhodnius prolixus. J. Parasitol., 51* (5): 837-841.

URIBE, C., 1927. On the biology and life history of *Rhodnius prolixus* Stal. *J. Parasitol., 13*: 129-137.

WATKINS, R., 1971. Histology of *Rhodnius prolixus* infected with *Trypanosoma rangeli. J. Invert. Pathol., 17*: 59-66.

WEATHERSBY, A. B., 1975. The haemocoel as barrier to parasite infection in insects. In: *Invertebrate immunity.* Maramorosch, K. & Shope, R. E. ed. Academic Press New York. San Francisco. London.