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STUDIES ON TRYPANOSOMA RANGELI TEJERA, 1920 X-ITS COMPARISON WITH TRYPANOSOMA CRUZI CHAGAS, 1909. INFECTION IN DIFFERENTS STAGES OF RHODNIUS PROLIXUS STAL, 1859

ESTUDIOS SOBRE TRYPANOSOMA RANGELI TEJERA, 1920 X-COMPARACION CON INFECCIONES POR TRYPANOSOMA CRUZI CHAGAS, 1909, EN DIFERENTES ESTADIOS DE RHODNIUS STAL, 1859

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ABSTRACT

The comparison among specimens of **Rhodnius prolixus**, at different stages of development, exposed to infections by **Trypanosoma rangeli** and **Trypanosoma cruzi**, revealed that the bugs exposed to T.

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cruzi showed a significant higher susceptibility to infection than that observed in the groups exposed to T. **rangeli** or to the mixture of both parasites.

The similar mortality rate recorded in uninfected and T. **cruzi** infected bugs confirms that this parasite is harmless to **R. prolixus.** On the other hand, T. **rangeli** produces its pathological effect although the bugs ingest a relatively low number of flagellates, suggesting the existence of a direct relation between the amount of ingested parasites and the mortality produced. In mixed infection, T. **cruzi** does not inhibit the harmful effect of T. **rangeli** on **R. prolixus.**

The life cycle of **R. prolixus** is not affected by high infection of T. **cruzi** or low infection by T. **rangeli,** However, when bugs ingested a high amount of flagellates of T. **rangeli** in mixed infections, the time to reach the adult stage in relation to controls, was significantly longer.

The epidemiological significance of the present results, is discussed.

KEYWORDS:
Trypanosoma rangeli, Trypanosoma cruzi, Rhodnius prolixus, mixed infection.

RESUMEN

La comparación entre especímenes de **Rhodnius prolixus,** en los diferentes estadios de desarrollo, expuestos a infecciones por **Trypanosoma rangeli** y **Trypanosoma cruzi,** reveló mayor susceptibilidad a la infección en los ejemplares expuestos a T. **cruzi** que en los expuestos a T. **rangeli** o a la mezcla de ambos parásitos.

El porcentaje similar de mortalidad registrado en especímenes infectados con T. **cruzi** y sus respectivos controles, confirma la inocuidad de éste para **R. prolixus.** Por otra parte, T. **rangeli** produjo efectos patológicos aun cuando los triatominos ingirieron un bajo número de flagelados, sugiriendo una relación directa entre la cantidad de parásitos ingeridos y la mortalidad producida. En infecciones mixtas, T. **cruzi** no inhibe el efecto patológico de T. **rangeli** en **R. prolixus.**

La duración del ciclo de vida de **R. prolixus** no fue afectado por fuertes infecciones de T. **cruzi** o ligeras de T. **rangeli.** Sin embargo,

cuando los triatominos ingirieron altas cantidades de flagelados de **T. rangeli**, el tiempo para alcanzar el estadio adulto fue significativamente mayor.

Se discute la significación epidemiológica de los presentes resultados.

PALABRAS CLAVES:
Trypanosoma rangeli; Trypanosoma cruzi; Rhodnius prolixus; Infección mixta.

INTRODUCTION

In the New World two types of human trypanosomiase have been described. That caused by **Trypanosoma (Schizotrypanum) cruzi** Chagas, 1909 known as Chagas'disease and the provoked by **Trypanosoma (Tejeraia) rangeli** Tejera, 1920.[1]

Despite the well known differences in the morphology, biology and behaviour in their hosts between **T. cruzi** and **T. rangeli**, it is not unusual to find mixed infections in **Rhodnius prolixus** in the endemic áreas of Chagas'disease, where this insect acts as a common vector for both parasites.[2]

In the present work, carried out under experimental conditions, some aspects of the parasite-vector relationship, are studied. This includes the models: **T. cruzi-R.prolixus; T. rangeli-R. prolixus** and the association of both parasites in the same vector, in order to obtain information that could be used in the interpretation of these models in nature. The aspects studied include the comparison of the susceptibility, mortality and time of permanence per instar when different stages of development of **R. prolixus**, were exposed to single or mixed infections by both parasites.

MATERIALS AND METHODS

Triatomine-bugs: The specimens of **Rhodnius prolixus** used in this study were obtained from the colony of the Faculty of Science, University of Los Andes, Merida, Venezuela.

Stock of parasites: The "Y" strain (M/HOM/Br/50/"Y") of **T. cruzi** isolated by Silva & Nussenzweig[3] and the isolate (M/CAN/VE/82/DOG-82) of **T. rangeli**[2], were used.

Infection of mice used as source of parasites: White mice were inoculated whith metacyclic-forms of T. **cruzi** obtained from the hindgut of infected **R. prolixus.** These mice were used to feed bugs when they showed a high parasitaemia level. Mice were infected whith T. **rangeli** by the bite of bugs with salivary glands infection, a condition previously detected using the method of salivation on glass slides.[4]

Infection of R. prolixus: Groups of 50 specimens of each of the stages of **R. prolixus**, were fed on mice infected whith T. **cruzi** which showed parasitaemias between 1.500 to 3.000 Tryps/mm^3 Similary, 50 specimens of each instar nymphs and 20 adults, were fed on T. **rangeli** infected mice showing parasitaemias of about 40 Tryps/mm^3 The parasitaemia in mice was estimated by the method described by Pizzi[5] and modified by Brener.[6] One more group made up of 50 specimens of each of the different stages, was exposed to a mixed infection. In this case, an artificial feeding system consisting of a latex membrane containing equal parts of culture forms (90% epimastigotes) of T. **rangeli** and T. **cruzi** mixed with fresh blood, was used. The number of flagellates contained in each membrane oscillated from 1.000 to 5.000 forms/mm^3, estimated in Neubauer chamber.

During the experiments, each group was exposed to infection only once.

In all the experimental treatments described, each group of exposed instar (from I to adult) had its respective uninfected control consisting of 20 specimens of **R. prolixus** fed on clean mice.

After engorging, bugs were placed individually in 3.5 cm x 5 cm plastic vials with a piece of filter paper and covered with gauze held with a rubber band. Each vial containing a bug was identified with a code which was used to follow its life history.

All the 1.230 specimens were kept in an automatic "Percival" incubator, regulated at 25°C,75% relative humidite and 12:12 h light: dark periods. They were fed on clean mice every 15-21 days until the end of the experiments.

Details of the methods of observation of infected bugs, records of deaths and time of moulting, as well as the detection of infection in the gut, haemolymph and salivary glands in dead and surviving bugs, have been indicated in a previous paper of this series.[7]

Statistical Analysis: To determine the statistical significance of susceptibility and mortality, the Chi square (x^2) test following the comparison of proportions from nany samples[8] and for two independent samples, was used. To establish the significance of the time of permanence per instar among controls and exposed-bugs, the Student "t" test, was used. The comparison of the time of permanence per instar among the 3 treatments used in this study, was carried out by means of an Analysis of Variance, following the two-two multiple comparison method. The computational work was performed in microcomputers with software adapted by Marquez.[9]

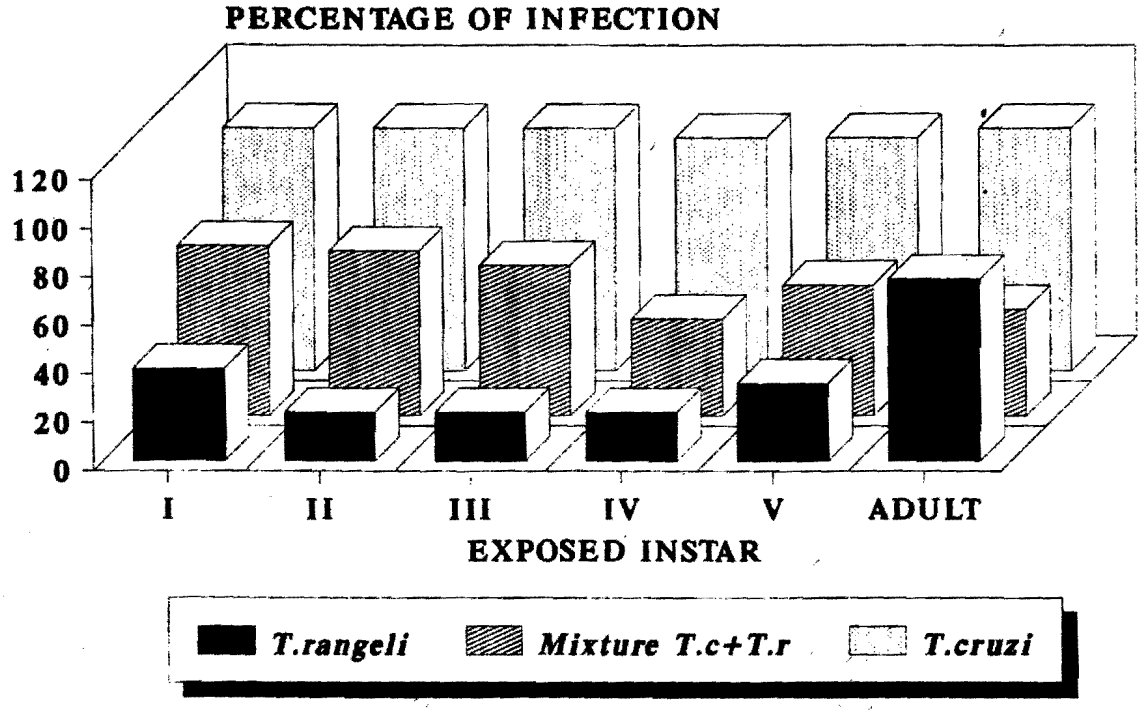
RESULTS

Susceptibility and mortality in different stages of R. prolixus exposed to the infection by T. cruzi and T. rangeli.

From 300 specimens exposed to T. **cruzi**, 296 (98.6%) were positive. The range of infection varied from 96% in IV and V instars to 100% in the other stages. Statistical comparison among the different instars showed no significant differences, indicating a similar susceptibility in all the stages (Fig. 1). 80 out of 270 (29.6%) bugs exposed to T. **rangeli** were infected, being the percentage of infection per instar as follows: 38% in I;20% in II, III and IV;32% in V and 75% in adults. The comparison of infection showed a higher susceptibility in the adult than in any other nymphal stage, with a statistically significant difference ($x^2 = 28.6$; $P < 0.001$). No significant difference was observed when the nymphal stages were compared. The proportion of infected bugs per exposed instar is presented in Fig. 1.

When 300 bugs were artificially fed with an infected meal containing a similar high number of flagellates of T. **cruzi and T. rangeli**, 169 (56.3%) of them showed positive results for parasites. Of these, in 140 (83%) both species were detected; in 14 (8%) only T. **cruzi** and in 15 (9%) only T. **rangeli** was observed. The range of the mixed infection varied from 40% in the bugs exposed during the IV instar to 70% in those fed ad I nymphal stage. The statistical comparison among the insects infected at different instars, revealed the existence of two suscep-

Fig.1.SUSCEPTIBILITY OF THE STAGES *OF Rhodnius prolixus TO* INFECTION *BY Trypanosoma cruzi AND T.rangeli*



tible groups one made up of the 3 first instars and the other of the rest of the stages ($x^2=15.8$; $P<0.05$). Fig. 1 shows the percentage of infection in each instar exposed to the mixture of parasites.

The statistical comparison of the susceptibility among bugs exposed to T. cruzi, T. **rangeli** and the mixture of them, revealed significant differences, being higher in the bugs exposed to **T. cruzi** than in those exposed to T. **rangeli** or to the mixture of both parasites ($x^2=293.8$; $P<0.001$). Similar results were observed when compared at any particular exposed instar. When the bugs exposed to T. **rangeli** and the mixed infection were compared a higher susceptibility in the latter, was detected ($P<0.001$).

In relation to mortality, 16 out of 300 (5.3%) specimens exposed to T. **cruzi** died during the course of the infection, while from the 120 uninfected control bugs a 7.5% mortality was observed. The statistical comparison of the total mortality and the recorded deaths in each instar, between controls and T. **cruzi** infected bugs, revealed no significant differences. Details on the observed mortality in controls and infected grous per instar, are shown in Table I. In the bugs exposed to T. **rangeli**, a total mortality of 9.3% was observed (Table I). This figure was statistically higher than detected in the uninfected control bugs ($x^2=4.57$; $P<0.05$). The observed mortality between unexposed and mixed infection-exposed bugs, is shown comparatively in Table I. The statistical analysis revealed significant differences ($x^2=48.58$; $P<0.001$) at any stage of development. The more affected instars were II, III and adults, which showed a range of mortality near to 50%. The comparison with the less affected group, revealed highly significant differences ($x^2=21.7$; $P<0.001$).

The statistical comparison of the mortality observed in the bugs exposed to the treatments, revealed significant differences ($x^2=131.03$; $P<0.001$), indicating a higher mortality in the specimens exposed to mixed infection than exposed solely to T. **cruzi** or T. **rangeli**.

Relation infection-mortality and localization of infection:

Dissections carried out on the 25 bugs which died during the course of infection by T. **rangeli**, revealed the presence of parasites in 12 of

TABLE I

MORTALITY PER INSTAR IN Rhodnius prolixus EXPOSED TO Trypanosoma cruzi, Trypanosoma rangeli AND MIXED INFECTION AT DIFFERENT NYMPHAL STAGES.

EXPOSED INSTAR	No. deaths/No. Exposed (% Mortality)					
	T. cruzi	CONTROL	T. rangeli*	CONTROL	T. cruzi + T. rangeli**	CONTROL
I	5/50 (10)	4/20 (20)	6/50 (12)	1/20 (5)	16/50 (32)	1/20 (5)
II	0/50 (0)	2/20 (10)	2/50 (4)	2/20 (10)	24/50 (48)	0/20 (0)
III	2/50 (4)	0/20 (0)	4/50 (8)	0/20 (0)	26/50 (52)	0/20 (0)
IV	4/50 (8)	1/20 (5)	3/50 (6)	0/20 (0)	14/50 (28)	1/20 (5)
V	3/50 (6)	0/20 (0)	10/50 (20)	0/20 (0)	9/50 (18)	0/20 (0)
ADULT	2/50 (4)	2/20 (10)	0/20 (0)	0/20 (0)	26/50 (52)	2/20 (10)
TOTAL	16/300 (5.3)	9/120 (7.5)	25/270 (9.3)	3/120 (2.5)	115/300 (38.3)	4/120 (3.3)

* Low number of parasites.

** High number of parasites.

them (48%). From these, 11 (92%) had parasites in the gut,7 (58%) in the haemolymph and 6 (50%) showed flagellates in the salivary glands. Similar analysis made in those triatomine-bugs dead as a consequence of the mixed infection, revealed parasites in 77%. In general, the presence of both **T. cruzi** and **T. rangeli** was detected in a high proportion (94%) in the gut, independently of the instar in which they were exposed to infection. In the haemolymph and the salivary glands, flagellates were observed in 55% and 37%, respectively. Unlike those observed in the gut, the infections detected in the haemolymph and salivary glands were exclusively due to **T. rangeli**. As can be observed, when specimens of **R. prolixus** were infected with a mixed infection, no flagellates of **T. cruzi** were detected outside the gut. Moreover, when dissections were carried out in 300 specimens infected only with **T. cruzi**, invasion to the haemolymph or salivary gland was never detected.

Time to reach the adult stage: Daily observations carried out on the 750 specimens of **R. prolixus** exposed, in each of the instar nympha, to the infection by **T. cruzi**, **T. rangeli** and the mixture of both parasites, allowed a precise estimation of the time of permanence per instar in each particular bug, and consequently the time to reach the adult stage. From 3 groups of 250 each, 236 (94%), 225 (90%) and 161 (64%), respectively reached the adult stage (Table II).

A statistical comparison between unexposed and **T. cruzi** exposed bugs, revealed no significant differences. In those bugs exposed to **T. rangeli**, only the group infected in the III instar showed a longer time than the controls to become adult, with a statistically significant difference ($t=2.53$;D.F:66; $P<0.02$). In relation to the specimens exposed to a mixed infection, the statistical analysis revealed highly significant differences ($P<0.001$) in the time of transformation to adult when compared with controls.

The analysis of variance carried out among the 3 treatments used in this study, revealed no significant differences in the time to reach the adult stage between. **T. cruzi** and **T. rangeli** exposed bugs. However, when these were compared with those bugs exposed to the mixed infection, significant differences were detected ($P<0.05$).

TABLE II.- Time of permanence/instar in **Rhodnius prolixus** exposed to **Trypanosoma cruzi, Trypanosoma rangeli** and mixed infection at different nymphal stages.

Exposed nymphal stage	Mean time (± SD) in days of permanence/instar in: **T. cruzi** exposed/control-bugs					
	I	II	III	IV	V	TOTAL time to become ADULT
I	12±3	16±3	18±4	20±5	49±5	115±17
	10±1	16±1	18±5	20±5	48±20	112±18
II		9±1	19±5	20±4	49±19	97±20
		9±1	19±4	20±5	52±18	100±20
III			11±2	19±3	49±20	79±20
			12±2	22±9*	43±20	77±24
IV				21±9	43±15	64±19
				24±13	48±15	72±13
V					38±12	38±12
					43±10	43±10
	T. rangeli # exposed/control-bugs.					
I	9±1	18±5	17±1	23±4	40±13	107±15
	10±4	16±4	19±8	23±5	38±10	106±15
II		18±7***	19±5	22±6	46±22	105±27
		10±2	18±7	21±4	50±20	99±21

III			21±7 ***	22±6 *	44±14	87±16 **
			11±1	18±1	47±15	76±15
IV				25±10	41±14	66±15
				25±10	41±21	66±23
V					38±18	38±18
					32±6	32±6

T. cruzi + T. rangeli # # exposed/control-bugs.

I	23±9 ***	16±5	19±8	25±11	49±18 *	132±23 ***
	12±3	18±4	17±6	20±3	38±11	105±16
II		28±7	21±10	22±9	40±11	111±19
		8±1 ***	19±3	19±3	46±15	92±15 ***
III			25±5 ***	26±8 **	40±12	91±13 ***
			12±1	19±6	38±12	70±13
IV				29±7 ***	41±11	70±9 ***
				17±2	40±11	57±11
V					54±29 **	54±29 **
					38±9	38±9

Statistical significance:

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

DISCUSION

The manifestations observed in **R. prolixus** as consequence of **T. rangeli**-infection have not been reported for this species when infected with **T. cruzi**. For this reason, in the present study the comparison of the course of infection in different stages of **R. prolixus** exposed to either of these parasites or their combination is made with the intention of producing some results that can help in the interpretation of the vector-parasite ralationship, and the hope of throwing some light on its epidemiological implications.

In relation to the susceptibility to infection observed in **R. prolixus** exposed to the 3 experimental treatments, significant differences among them were detected. These values ranged from 98.6% in the group exposed to **T. cruzi** to 29.6% in those exposed to **T. rangeli**, with an intermediate average (56%) in the bugs exposed to mixed infection.

The high susceptibility observed in the specimens exposed to **T. cruzi**, reveals the great adaptation of this parasite to **R. prolixus**, a species that is considered its principal vector in Venezuela[10], due to the close association observed over the time in endemic areas. This claim is here supported by the perceptage of susceptibility obtained in each of the stages, which ranged from 96% to 100%.

The relative low susceptibility recorded in the bugs exposed to **T. rangeli**, could be probably explained for the scanty number of parasites ingested from the donor mice, which in general exhibited parasitaemias of about 40 tryps/mm^3. This affirmation appears to be supported by the results reported by Añez et al[2] who working with the same isolate of the parasite and triatomine from the same origin as those used in this study, detected 41% susceptibility to infection in bugs fed on mice with parasitaemias of 600 tryps/mm^3, indicating that the quantity of parasites ingested is an essential factor for the establishment of a **T. rangeli**-infection in **R. prolixus**. Analysing the above results in the epidemiological context, it is possible to find a certain relation with that observed in nature. In fact, the vertebrate hosts naturally infected whith **T. rangeli** usually show occult or inapparent parasitaemias[11], very similar to those detected in our donor mice, while natural infections caused by **T. cruzi**, at least during the acute phase, always show a higher number of circulating parasites in thier hosts than that observed for

T. rangeli, as actually happened in our experimental work. Therefore, it is not surprising to have recorded 98% susceptibility in bugs exposed to **T. cruzi** against 29% in those exposed to **T. rangeli**. These observations find support in the results of Sousa & Johnson[12] who working under natural conditions in Panama reported that in wild bugs infections by **T. cruzi** were theree times more frequent than those by **T. rangeli**, the same difference observed in our experimental models.

The susceptibility observed in the bugs exposed to the mixed infection, was higher than detected in the **T. rangeli**-exposed groups, but lower than in the bugs exposed to **T. cruzi**, with statistically significant differences ($P < 0.001$). The probable reason in the case of **T. rangeli**, is the high number of parasites ingested by the insects when offered the infective meal. However, in the case of **T. cruzi**, it is possible to speculate that the observed reduction in the bugs susceptibility could be due to an effect caused by the simultaneous infection by **T. rangeli**, which as is known, can provoke an increase of the haemolymphatic cells[13] which when invading the intestinal wall[14] are able to phagocyte flagellates of **T. cruzi** unable to survive inside the haemocytes as **T. rangeli** does[15], causing a partial loss of infection for **T. cruzi** in some specimens originally infected. Although this argument is speculativa, it may not be ignored in the interpretations on the behaviour of **R. prolixus** with mixed infections by **T. cruzi** and **T. rangeli**. On the other hand, it is clear that more investigations to explain the physiological and/or immunological reasons for this phenomenom, are required.

The individual examination of specimens exposed to the mixed infection allowed the observation of each species of parasites and their forms in different regions of the body. So, metacyclic forms of **T. cruzi** were observed at the rectum of the infected bugs, while the same forms for **T. rangeli** were only detected in the haemolymph and salivary glands. This observation appears to have epidemiological importance since the same bug can simultaneously transmit to the vertebrate host the infection by **T. cruzi** and **T. rangeli**. The former by contaminative via, by depositing infected faeces on mucous or sites of easy penetration, and the latter by inoculating the metacyclics when probing in the skin[16] or directly into the blood vessels during the ingestion (17;16).

The similar mortality observed in specimens exposed to **T. cruzi** and that detected in the uninfected controls, indicates that this parasi-

te was not responsible for the deaths during the course of the infection. Analysing these results in the context of Garnhams's opinion[18], who stated that the determination of the pathogenicity produced by the parasites in its insect vectors is only possible through an increase mortality, it is concluded that **T. cruzi** does not have a pathological effect on **R. prolixus**, independently of the number of parasites ingested by the bugs. On the contrary, when bugs of the same species were fed on **T. rangeli**-infected mice showing a low parasistaemia, a significantly higher mortality than in the controls was detected ($P < 0.05$). This fact allows us to incriminate T. **rangeli** for the majority of deaths, supporting the observations of previous workers (2;7;13;17;19).

These results suggest that **T. rangeli** produces its pathological effect on **R. prolixus**, although the bugs ingest a relativel low number of flagellates during the infective meal and also suggest that there is a direct relation between the amount of ingested parasites and the mortality produced (i.e. The fewer the parasites ingested the lesser the mortality). The latter is particular true in the context of this work, in which average mortalities of 9% and 38% were observed when bugs ingested a low (in single infection) and a high (in mixed infection) amount of parasites, respectively. These observations could be of use for a better interpretation of what actually happens in nature, in places where **T. rangeli** circulates and where, as is known, the reservoirs of this parasite generally show occult parasitaemias[11]. This obviously demonstrates thar under natural conditions the tendency of bugs is to ingest few parasites, allowing us to speculate that if the same pathological effect observed under laboratory conditions occurs in nature, the percentage of mortality in wild bugs infected with T. **rangeli** could possible be similar to that detected in the present work, when specimens of **R. prolixus** ingested a low amount of blood forms of this parasite.

The above leads us to conclude that the mortality observed in bugs exposed to mixed infection was undoubtedly a consequence of the parhological effect of **T. rangeli**, demostrating not only the harmlesness of **T. cruzi** to **R. prolixus**, but also that this parasite does not inhibit the pathogenicity of **T. rangeli**. This fact, could have not inhibit the pathogenicity of **T. rangeli** according to Añez et al.[2] could act as a regulator of the population of **R. prolixus**. However, this claim

must be taken cautiously due to wath was demonstrated here: that T. **rangeli** has an intensely harmful effect only when the bugs ingest a high amount of parasites, a condition that appears to be not very frequent in nature, judging by the inapparent infections commonly detected in the reservoirs of this parasite.

From the foregoing, it is clear that more investigation under natural conditions is necessary to elucidate the real effect of T. **rangeli** on the populations of **R. prolixus**, and to explore the possibility of using this parasite as a type of biological control as suggested by previous workers[2-20].

The comparison of the time to reach the adult stage among the instar nymphs of **R. prolixus** exposed to single and mixed infection with the controls, revealed that groups infected with a high number of flagellates of T. **cruzi** and few amount of blood forms of T. **rangeli**, did not show significant differences. However, in the sepecimens exposed to the mixed infection in which the bugs ingested a high number of flagellates of T. **rangeli**, a highly significant difference ($P < 0.0001$) in relation to control was detected. Although the effect of T. **rangeli** on the life cycle of **R. prolixus**, has been studied and confirmed by various workers (2;19;21), it is possible to affirm that the delay in the time of moulting occurs only when high amount of flagellates are ingested by the bugs during the infective meal. This suggests the existence of a direct relation between the amount of parasites producing the infection and its effect on the bug's life cycle (i.e. The higher the number of ingested flagellates the longer the time of development).

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REFERENCES

1. AÑEZ, N. Studies on **Trypanosoma rangeli** Tejera, 1920. IV. Reconsideration of its systematic position. Mem. Inst. Oswaldo Cruz, 77 (4): 405-415, 1982.

2. AÑEZ, N., NIEVES, E. & CAZORLA, D. Studies on **Trypanosoma rangeli** Tejera, 1920. IX. Course of infection in different stages of **Rhodnius prolixus**. Mem. Inst. Oswaldo Cruz, 82 (1): 1-6,1987.

3. SILVA, L.H.P. & NUSSENZWEIG, V. Sobre uma cepa de Trypanosoma cruzi altamente virulenta para o camundongo branco. Folia Clin. et Biol., 20: 191-208, 1953.

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

5. PIZZI, T. La inmunología de la Enfermedad de Chagas. Tesis. Univ. Chile. Santiago, 1957.

6. BRENER, Z. Contribução ao estudo da terapeutica experimental da doença de Chagas, Thesis from. the Faculty of Pharmacy, University Minas Gerais, Belo Horizonte, Brasil, 1961.

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

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

9. MARQUEZ, V. Análisis de varianza en microcomputadores (sistema computacional para métodos estadísticos). Monografía. Publicación 86-01. Universidad de Los Andes, Inst. Estadist. Aplicada & Computac., Facultad de Economía, Mérida, Venezuela, 250 pp, 1986.

10. DER/UCV/UC. Enfermedad de Chagas. Bol. Dir. Malariol. & San. Amb., 15 (6): 286-298, 1975.

11. GARNHAM, P.C.C. The significance of inapparent infections in Chagas'disease and other forms of trypanosomiasis. Mem. Inst. Oswaldo Cruz, 75 (1-2): 181-188, 1980.

12. SOUSA, O.E. & JOHNSON, C.M. Prevalence of Trypanosoma cruzi and Trypanosoma rangeli in triatomines (Hemiptera: Reduviidae) collected in the republic of Panama. Am. J. Trop. Med. & Hyg., 22[1]: 18-23, 1973.

13. GOMEZ, I. 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, 1967.

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

15. RIBEIRO, R.D. & PRADO JUNIOR, J.C. Ciclo extraintestinal do Trypanosoma cruzi em triatomineos. Rev. Fac. Odont. Ribeirao Preto, 23 (1): 33-36, 1986.

16. AÑEZ, N. Studies on Trypanosoma rangeli Tejera, 1920. I. Deposition, migration and growth of T. rangeli in two mammals. Parasitological Topics sp. Publ. Soc. Protozool. Allen Press, Kansas, 1: 19-25, 1981.

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

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

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

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

21. TOBIE, E.J. Experimental transmission and biological comparison of strains of **Trypanosoma rangeli**. Exp. Parasitol. 11 (1): 1-9, 1961.