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STUDIES ON *TRYPANOSOMA RANGELI* TEJERA, 1920. VII – ITS EFFECT ON THE SURVIVAL OF INFECTED TRIATOMINE BUGS

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The pathological effects of Trypanosoma rangeli *on* Rhodnius prolixus *and* R. robustus, *and the relation of mortality to infection, were studied under laboratory conditions. Frequent observations revealed that when the first instar nymphs of* R. prolixus *and* R. robustus *were infected with* T. rangeli, *survival of the bugs during the stages of development to the adult stage decreased. This decrease was statistically significant when compared with uninfected control-bugs, indicating that* T. rangeli *is pathogenic for both species of triatomine. In* R. prolixus *the most affected nymphal stages were the first, second and fifth instars, where a higher mortality was also observed.*

In R. robustus *a progressive increase of the mortality from the first to fifth instars, was observed. The pathogenicity of* T. rangeli *as measured by overall mortality was the same in* R. prolixus *and* R. robustus. *The possible pathogenic mechanism of* T. rangeli *in triatomine-bugs and its epidemiological implications, are discussed.*

Grewal (1957) first demonstrated the pathological effect of *Trypanosoma rangeli* on triatomine bugs. He observed that nymphs of *Rhodnius prolixus* heavily infected in the haemolymph frequently died or failed to moult, while those that reached the adult stage showed various abnormalities. Tobie (1965) reported that when the first instar nymphs of *R. prolixus* were exposed to an infective meal, survival during the nymphal stages decreased to 62%, while 84% of the uninfected control bugs survived to become adults. She also observed that the infection by *T. rangeli* interfered with the moulting process principally during the first two and the last moults. Later, Gomez (1967) and Marinkelle (1968) confirmed the pathogenicity of *T. rangeli* to triatomine vectors under laboratory and field conditions. They showed that infected bugs had a significantly higher mortality rate, ranging from 64 to 87%.

Watkins (1971) carried out histological studies on uninfected and *T. rangeli*-infected bugs, showing that lesions were present in bugs with different degrees of infection. She observed intracellular stages of *T. rangeli* in most tissues and reported that heavy haemolymph infections often result in severe nerve damage and hypertrophy of tracheal cells. She concludes that a possible auto-intoxication, caused by damaged muscular, nervous and tracheal systems, would be a prime cause of mortality in bugs infected with *T. rangeli*.

Regarding time of moulting in *T. rangeli*-infected *R. prolixus*, Tobie (1965) observed longer intervals between feeding and moulting in infected nymphs than in uninfected bugs, particularly in the older stages. D'Alessandro (1976) reported that in heavier gut infections, 20% of the bugs showed a delay in moulting of up to 3 weeks.

The present paper describes a comparative study carried out on *R. prolixus* and *R. robustus*, experimentally infected with a fresh Venezuelan isolate of *T. rangeli*. Frequent observations on infected and uninfected first instar nymphs were made until they reached the adult stage.

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Details are given of the mortality rates at different stages of development and about the proportion of the infection in the gut, haemolymph and salivary glands both in bugs which died during the process of infection and in the survivors.

MATERIAL AND METHODS

Triatomine bugs – Only 4-8 days-old first instar nymphs of *Rhodnius prolixus* and *R. robustus*, were used. Their origin and maintenance have been described in previous papers (Añez, 1981, Añez & East, 1983).

Stock of *Trypanosoma rangeli* – The Betijoque/78 isolate of *T. rangeli*, was used. Details of the isolation and maintenance of the parasites were given by Añez (1981).

Mice – White mice infected with Betijoque/78 isolate of *T. rangeli*, were used to infect the experimental groups of triatomine-bugs. The mice were infected 48 hours previously by the bite of bugs with salivary glands infections, and their parasitaemia estimated minutes before they were offered to the experimental bugs. Uninfected mice of the same origin were used to feed the groups of uninfected control-bugs. Each mouse was held inside a plastic net restrainer placed in a beaker with the group of bugs to be fed.

Infection of triatomine-bugs – 170 first instar nymphs of *Rhodnius prolixus* and 105 of *R. robustus*, were infected with the Betijoque/78 isolate of *T. rangeli* on mice with a parasitaemia of 400 Tryps/mm^3. 20 nymphs of *R. prolixus* and 25 of *R. robustus* were fed on clean mice and kept as uninfected controls.

After engorging, bugs were place individually in 3.5cm x 1.5cm 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 number which was used to follow the life history of each bug.

The bugs were kept in a constant temperature room 25°C and 75% relative humidity. They were fed every 15 to 21 days on clean mice until they became adults.

Observation of infected bugs, record of death and time of moulting – Without handling the bugs, they were observed daily through the plastic vial wall, from the day of infection until they moulted to adults. In this way, it was possible to record accurately the time of death and moulting of each bug.

Detection of infection in dead bugs – After death occurred, the bugs were dissected and the gut, haemolymph and salivary glands examined for parasites. The same observation was made in the adult survivors.

Statistical analysis – To determine the statistical significance of the mortality between uninfected and *T. rangeli*-infected bugs, and between the different stages of development, a CHi square (x^2) test for two independent samples, was used.

RESULTS

Distribution of mortality during the nymphal stage in *R. prolixus* infected with *T. rangeli* – When 170 first instar nymphs of *R. prolixus* were fed with a *T. rangeli*-infected meal, 66 (39%) of them died during the nymphal stages and 104 (61%) survived and succeeded in reaching the adult stage. 95% of the uninfected control bugs survived and became adults. Details about the number of deaths incurred in each instar of the infected bugs and that observed in the controls, are given in Table I. Statistical comparison of the number of deaths of infected and uninfected bugs, revealed a significant difference

between the two groups ($x^2 = 7.5$; $P < 0.01$). The mortality which occurred between the different nymphal stages of the infected bugs, was also compared statistically, revealing significant differences between first and third instar nymphs ($x^2 = 5.13$; $P < 0.025$) first and fourth ($x^2 = 4.72$; $P < 0.05$) second and third ($x^2 = 5.92$; $P < 0.025$); second and fourth ($x^2 = 5.47$; $P < 0.025$); third and fifth ($x^2 = 7.03$; $P < 0.01$) and fourth and fifth ($x^2 = 6.5$; $P < 0.025$). No differences were observed in the number of deaths incurred among first, second and fifth instar nymphs. This indicates a greater mortality in the two first and the last nymphal stages than in intermediate instars.

TABLE I

Mortality distribution during nymphal stages in two species of triatomine bugs infected with *Trypanosoma rangeli*

Species of bug	No. of fed 1st instar	No. of deaths at different instar					Total dead	Survivors
		I	II	III	IV	V		
R. prolixus	170	20	19	5	5	17	66	104
Uninfected control	20	0	0	0	0	1	1	19
R. robustus	105	6	8	9	10	15	48	57
Uninfected control	25	0	0	2	0	1	3	22

Distribution of mortality during the nymphal stages in *R. robustus* infected with *T. rangeli* – Frequent observations made on 105 first instar nymphs of *R. robustus* infected with *T. rangeli*, revealed 46% mortality during the period of transformation from first instar to adult stage. The uninfected control-bugs showed 88% survival and became adults. Table I, shows the details of the mortality observed in each instar, both infected and control-bugs. The number of deaths recorded for the infected and uninfected groups were compared statistically, revealing significant differences between them ($x^2 = 8.26$, $p < 0.005$).

The statistical comparison of the mortality occurred among the different instar, revealed significance only between the first and fifth ($x^2 = 7.9$; $P < 0.005$) and the second and fifth ($x^2 = 4.7$; $P < 0.05$), indicating a higher mortality in the last nymphal stage than in the two first instars.

Comparison of mortality caused by *T. rangeli* in *R. prolixus* and in *R. robustus* – When the mortality caused by *T. rangeli* in *R. prolixus* and *R. robustus* was compared statistically, no significant differences were observed either between the total number of deaths incurred in the whole period of development or between the mortality which occurred in each of the 5 particular instars. Fig. 1, shows comparatively the distribution of real mortality (see Southwood, 1966) in nymphal stages of *R. prolixus* and *R. robustus*.

In both species it was observed that a considerable number of bugs was unable to moult, dying during the process of moulting or shortly afterwards. The bugs showed a bent body, being unable to escape from the old cuticle, which remained stuck to the new one until they died (Fig. 2).

Relation of mortality to infection in triatomine bugs experimentally infected with *T. rangeli* – Observations carried out in the gut, haemolymph and salivary glands of the infected bugs immediately after death had occurred, showed the presence of parasites in the 3 regions. Examination of 40 infected *R. prolixus* which had died between the first and fifth nymphal stage, revealed 48% with infection in the intestinal contents, 43% with haemolymph infection and 8% with parasites in the salivary gland. The same observations

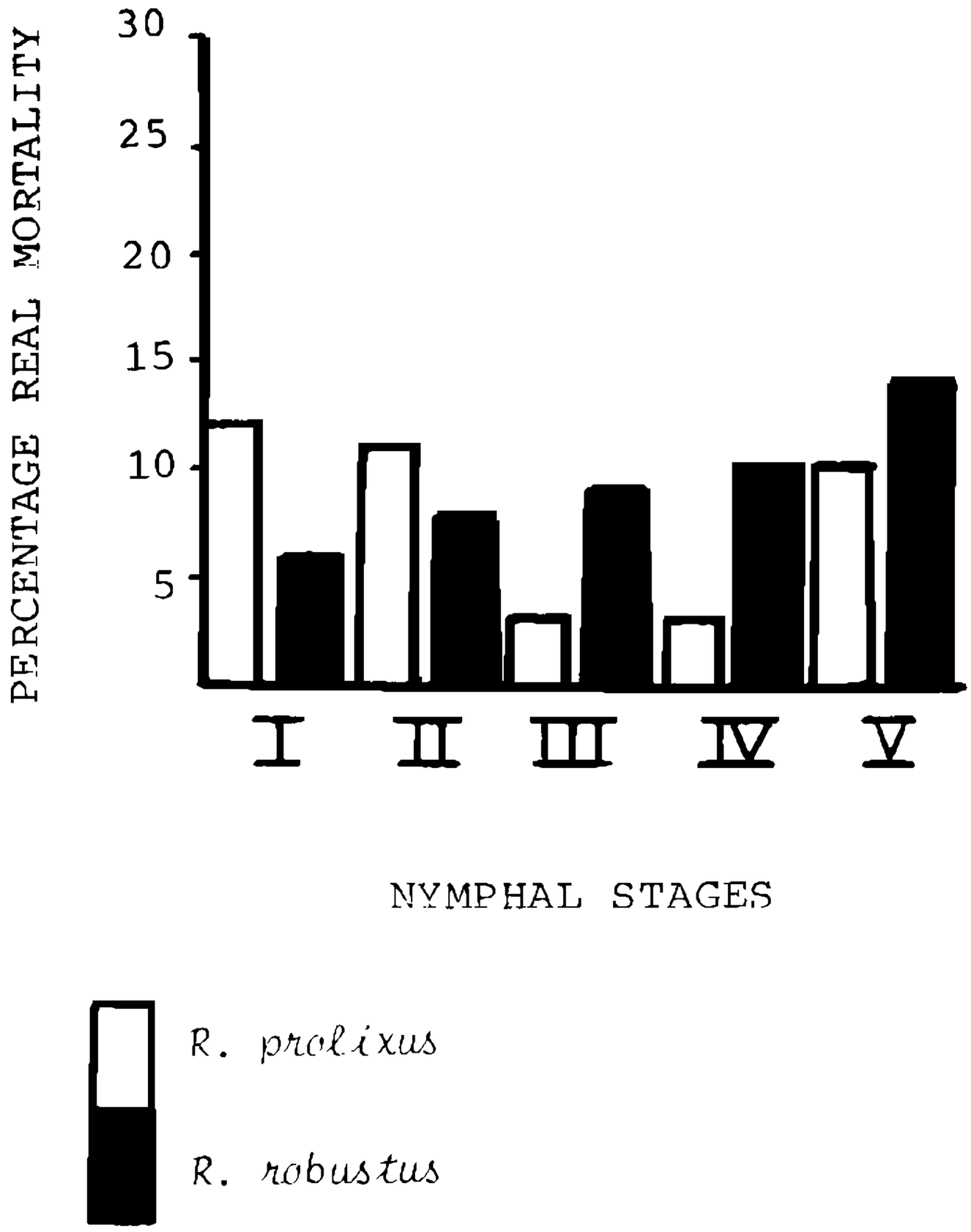

Fig. 1 – Percentage real mortality in nymphal stages of triatomine bugs infected with *T. rangeli*.

made on 48 *R. robustus* dead during the process of moulting from first to fifth instar, revealed 69%, 38% and 25% with gut, haemolymph and salivary gland infections respectively.

DISCUSSION

In the present study 170 specimens of *R. prolixus* and 105 of *R. robustus* infected with *T. rangeli* during the first nymphal stage were frequently observed. A higher mortality in the infected bugs than in the uninfected controls was found. In infected *R. prolixus,* 39% mortality was recorded during the whole process of development, from the first instar to adult stage, while the uninfected bugs showed only a 5% mortality during the same period and under the same conditions. When these figures were compared statistically, a significant difference between the two groups was observed, indicating that the infection by *T. rangeli* was responsible for the reduction of survival to 61%. These results agree with those observed by Tobie (1965), who reported that in *T. rangeli*-infected *R. prolixus* survival decreased to 62% following infection during the first nymphal stage, while controls survived in a 84%.

Regarding *R. robustus,* infected with the same isolate of *T. rangeli* and maintained under the same conditions as *R. prolixus,* the survival decreased to 54%, while 88% of the uninfected control survived to become adult. This difference was statistically significant.

Fig. 2 – Pathogenic effect of *T. rangeli* on Triatomine-bugs. Note the bent body of a recently moulted II instar nymph, showing inability to escape from the old cuticle.

No statistical difference was observed between the mortality caused by *T. rangeli* in *R. robustus* and that observed in *R. prolixus*. It can be assumed, therefore, that the mechanism used by the parasite to retard moulting and kill *R. prolixus*, the species responsible for the transmission in the domestic cycle of *T. rangeli*, also operates for *R. robustus*, a suspected vector of the sylvatic cycle of the parasite.

The analysis of the mortality rate at different stages of development in *R. prolixus*, revealed that from the 66 bugs which died during the infection with *T. rangeli*, 20 (30%) were first instar; 19 (29%) were second; 5 (7.5%) third; 5 (7.5%) fourth and 17 (25%) were fifth instar nymphs. These results indicate that the stages more prone to pathogenic activity of the parasite are the first two and the last instar nymphs, with the intermediate instar being dramatically less affected. Indeed, the fact that almost a 60% of the deaths in infected bugs occurred during the first and second instars and that the last instar nymphs have a higher mortality rate than the two middle instars, seems to indicate that there are at least two ways in which *T. rangeli* produces its pathogenic effect on *R. prolixus*. One immediate mechanism which would operate causing pathological effects among the less resistant individuals in the infected population. A further mechanism would act much more slowly than the former, causing some or no deaths in the intermediate stages and increased mortality in the last instar nymph. In this way, it is possible to divide the entire pathological mechanism of *T. rangeli* in *R. prolixus* into two phases:

1. An early or "acute" phase, characterized by a successful establishment of the parasite in the bugs. This is followed by a rapid multiplication and an early invasion of the haemolymph which carries the parasite to all the body tissues where the continous development would cause severe damage in the principal systems i.e. muscular, nervous and tracheal systems, with the consequent pathological manifestations and/or death.

In this case, the infected bugs would succumb to the action of the parasite in a few days after the infection. Moreover, due to the great number of parasites present in the body cavity, the salient features during the early phase of the infection are: i. – the rupture of the abdominal midgut (stomach), as demonstrated by the presence of blood cells when the end of a leg is cut off for diagnosis of haemolymph infections (Añez, unpublished information), ii. – the lack of digestion of the blood meal, first instar nymphs have been observed engorged until 30 days after ingesting the infective meal, with no signs of digestion and with increasing inactivity until they die and iii. – occurrence of bent bodies and an incapacity to escape from the old cuticle, which remains stuck to the abdomen or legs until the bug dies.

2. A later or "chronic" phase, characterized by an establishment of the parasite in the gut of infected bugs during at least the 3 first nymphal stages. In this case, there is a later invasion to the haemolymph responsible for the few deaths in the third and fourth instars and the higher mortality in the fifth instar nymphs. In some cases, there is no invasion to the haemolymph with the flagellates remaining in the gut lumen for long periods without pathological manifestations. Obviously there is also a group of bugs in which the invasion of the parasites to the haemolymph and the rest of body tissues, including salivary glands, causes little or no pathological effects. This is the group of infected survivors largely responsible for the transmission of *T. rangeli* to the vertebrate host and, in some cases, even to other bugs (Añez, 1982).

In relation to *R. robustus*, from the 105 specimens infected with *T. rangeli*, 48 died during the process of development from the first instar to adult stage. In this case, a progressive increase of the proportion of deaths was observed from the first to the last nymphal stage, indicating that the stages more affected were the last two instar, which showed 21% and 30% of mortality respectively. Contrary to that observed in *R. prolixus*, the first and second instar of *R. robustus* were less affected by the parasite, showing 13% and 17% mortality respectively. It can be assumed that in *R. robustus*, although affected during the early phase of the infection by *T. rangeli*, in which some losses are produced, the major mortality is detected during the later phase of the infection. This is demonstrated by the number of deaths produced between the third and fifth instar and also by the time in which most of deaths occurred, being from 60 to 204 days when 70% of bugs died.

Whatever the reason for the high mortality rate observed in triatomine-bugs infected by *T. rangeli*, what must be considered is that the parasite actually produces a great number of deaths in an experimentally infected population of bugs, being in our case, 39% for *R. prolixus* and 46% for *R. robustus*. This interesting fact may be investigated under natural conditions, in order to see if it may be considered as a biological control-mecanism against triatomine-bugs, which also act as vectors of *T. cruzi*, the aethiological agent of Chagas' disease, which affects a great proportion of the rural human population in the neotropical region.

Regarding the relationship of mortality to infection in triatomine infected with *T. rangeli*, it was observed that most of the bugs which died during the period of the infection, showed parasites in the gut, haemolymph and salivary glands both in *R. prolixus* and in *R. robustus*. In relation to the infection detected in bugs which survived to become adults, in *R. prolixus* 44% of them showed parasites in the intestinal contents; 15% in haemolymph and 11% in the salivary glands. In *R. robustus* 2% showed parasites in the haemolymph and 5% in the gut and the salivary glands. These figures suggest that *T. rangeli* survives better in *R. prolixus* than in *R. robustus* and that despite the mortality produced during the stage of development, the proportion of the infection in the remaining survivors is enough to ensure the transmission of the parasite to the vertebrate host. The proportion of infection in the survivors although low, has epidemiological importance considering the very efficient mechanism of transmission of *T. rangeli*, carried out by the bite of the infected bugs. More epidemiological implications can be added, considering the modification of the feeding behaviour of the infected bugs, when the number of

probings to take the blood meal is significantly increased (Añez & East, 1983), and the direct transmission of *T. rangeli* from bug to but (Añez, 1982).

RESUMO

Estuda-se, em condições experimentais, os efeitos patológicos do *Trypanosoma rangeli* em *Rhodnius prolixus* e *R. robustus* e a relação da mortalidade com a infecção.

Quando as ninfas de primeiro estádio de ambas as espécies de triatomíneos são infectadas com *T. rangeli,* a taxa de sobrevivência nos diferentes estádios diminui significativamente em relação aos controles. Isso indica que o *T. rangeli* é patogênico para *R. prolixus* e *R. robustus.*

Os estádios mais afetados de *R. prolixus* são o primeiro, o segundo e o quinto, observando-se nestes maior mortalidade, enquanto em *R. robustus* observa-se um incremento progressivo da mortalidade do primeiro ao quinto estádios.

A patogenicidade do *T. rangeli,* estimada pela mortalidade total foi a mesma para as duas espécies de triatomíneos.

Discute-se o possível mecanismo patogênico do *T. rangeli* para triatomíneos vetores e suas implicações epidemiológicas.

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