Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 38 (1981)
Heft: 2

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DOI: https://doi.org/10.5169/seals-312811

Acta Tropica *38*, 107–114 (1981)

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Trypanosoma cruzi: comparative studies of infectivity of parasites ingested by *Triatoma infestans* and those present in their feces

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Summary

Artificial feeding of the insects with whole blood containing trypomastigotes resulted in triatome infection; the parasites were present in the host feces after 15–30 days and, when inoculated into mice, elicited parasitemia in 100% of the cases and deaths in 20–70%. This mortality indicates a reduction of the original infective capacity of the bloodstream form, which kills 100% of the mice even when inoculated with a single trypomastigote.

When triatomes were fed whole normal mouse blood containing culture forms of low infective capacity (Tul_L), mice inoculated with feces containing the progeny of the culture forms failed to develop a parasitemia. The absence of any infective capacity of these parasites was proven when the mice challenged with lethal doses of trypomastigotes 30 days after the fecal inoculations died at the same time as the controls. Mice injected with feces from triatomes fed culture forms with high infectivity for mice (Tul_B) developed patent parasitemia, indicating that triatomes may be infected by parasite forms other than bloodstream trypomastigotes. Experiments on infectivity of parasites present in the feces of triatomes fed Tul_L and Tul_B culture forms, also showed that a single passage through the digestive tract of the insects did not significantly modify the infective capacity of the parasite population.

When stomachs and intestines from insects fed Tul_B were processed separately, parasites obtained from the latter showed higher infectivity for mice than those obtained from the former.

Key words: Triatoma infestans; T. cruzi; artificial infection; epimastigotes; trypomastigotes.

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Introduction

The most usual mode of transmission of *Trypanosoma cruzi* in an endemic area is by means of haematophagus insect intermediate hosts (Hoare, 1972). These insects become parasitized after feeding on infected mammals. Bloodstream trypomastigotes differentiate in the digestive tract of the triatome bugs and metacyclic forms eliminated in the insect feces are considered responsible from the vertebrate hosts' infection (Brack, 1968; Alvarenga, 1974; Zeledón, 1974).

When bloodstream trypomastigotes are transferred to a culture medium they differentiate primarily into epimastigotes, but some metacyclic forms can also be detected in the cultures (Pizzi, 1957). The degree of infectivity of culture forms is usually lower than that of trypomastigotes; in some instances infectivity is so low that is very hard to measure by in vivo tests (Pizzi, 1957; Katzin et al., 1979).

As far as we know, in studies dealing with the life-cycle of *T.cruzi* no experiments have been conducted to demonstrate whether stages other than the bloodstream form are able to infect the intermediate host or if the infective capacity of ingested parasites may vary. We carried out the following studies in an attempt to answer these questions.

Materials and methods

Third-stage nymphs of *Triatoma infestans* reared in our laboratory were used. The triatomes were reared naturally by feeding on live chicken weekly before they were infected and on live mouse, weekly, after infection, unless otherwise stated.

Trypomastigotes of the Tulahuén strain of *T.cruzi* were collected from infected Rockland mice at the peak of parasitemia. Two lines of culture forms of the same strain were used: one had decreased in infectivity significantly (Tul_L) and the other (Tul_B) possessed good infectivity for mice. Both were used at the end of the exponential growth period and at least 98% of the parasites were epimastigotes.

A modification of the Pipkin and Connor feeding apparatus was used to infect the triatomes (Pipkin and Connor, 1968; Isola et al., 1980). The container for artificial feeding was filled with normal mouse blood (NMB) from which the red blood cells had been previously separated and washed three times with 0.15 M NaCl; the plasma was inactivated for 30 min at 56° C and then remixed with the washed red blood cells. Plasma inactivation was performed to ensure the integrity of the parasites even though normal mouse serum has practically no lytic activity against epimastigotes (Rubio, 1956). Parasites, either as trypomastigotes or culture forms, were added in a concentration of 1×10^6/ml, except in experiment 3. Temperature of the feeding apparatus was kept at 37° C. Triatomes ingested on average of 0.7–0.8 ml blood/insect.

Fecal pools of the triatomes were prepared by squeezing each lot of insects and mixing them with 2 ml of 0.15 M NaCl. The parasites were counted in a Neubauer type chamber and their concentration adjusted to $1.0–1.5 \times 10^5$/ml. Each mouse was inoculated with 0.1 ml by subcutaneous route (SCR), and the infective capacity of the parasites evaluated by: a) positive parasitemia and b) resistance to lethal challenge with blood trypomastigotes. Groups of mice were also injected with triatome feces in which parasites had not been detected by direct microscopic observation; in these cases none of the mice was injected with more than $1–4 \times 10^2$ parasites.

Experiment 1: Two lots (A and B) of 50 insects each, reared naturally, were used. Lot A was

Table 1. Detection of parasites in the feces of *Triatoma infestans* after feeding with culture forms or trypomastigotes of *Trypanosoma cruzi* (Tulahuén strain)

Triatomes	Fed with	Parasites found in feces at day*			
		15	30	45	60
Starved	Epi. (Tul_L)[a]	neg.	neg.	neg.	neg.
Fed weekly	Epi. (Tul_L)	neg.	neg.	neg.	neg.
Starved	Tryp.[b]	neg.	*pos.*[1]	neg.	neg.
Fed weekly	Tryp.	neg.	*pos.*[2]	*pos.*[1]	neg.

* days after ingestion of the parasites
[a] 98% epimastigotes
[b] bloodstream trypomastigotes
[1] 100% epimastigotes
[2] 10% metacyclic forms

infected with trypomastigotes and Lot B with Tul_L-culture forms (low infectivity). Half of each group was fed weekly after infection; the rest were not fed (starved insects). Feces of the four subgroups were observed under phase microscopy and were injected into 25-day old Rockland mice (groups of 10 each) by SCR on days 15, 30, 45 and 60 after the parasites' ingestion. Parasitemias in the mice were checked by weekly examination of tail blood for one month (Pizzi, 1957); those without patent parasitemia were challenged with lethal doses of trypomastigotes of the Tulahuén strain. A control group of normal mice was similarly challenged.

Experiment 2: 50 artificially reared insects (Isola et al., 1980) were separated into two lots: one was fed with NMB plus trypomastigotes and the other with NMB plus Tul_L-culture forms; their feces were checked on days 30 and 60 by microscopic observation and by mouse inoculation. Mice without patent parasitemia were challenged as indicated in experiment 1.

Experiment 3: 60 triatomes were separated into 3 lots and fed with NMB plus 10^6, 10^7 or 10^8 Tul_L-culture forms/ml. The presence of parasites in the triatome feces was ascertained on day 30 by microscopic examination and infectivity was assayed by mouse inoculation. The follow-up of the animals was as in experiment 1.

Experiment 4: Tul_B-culture forms were fed to 60 triatomes. Feces of 20 triatomes were obtained after 45 days. The remaining insects were pooled into two lots of 20 each: the stomach was separated from the intestine by dissection (hindgut was included with intestine) and the stomach and intestinal contents were recovered by washing the organs in 0.15 M NaCl. The presence of parasites was ascertained by microscopic examination, the parasite concentration was adjusted as in the feces experiments, and infectivity assayed in mice; parasitemias and deaths were checked and registered for 30 days post infection.

Results

Experiment 1: No parasites were observed in the feces of triatomes fed with Tul_L-culture forms, whether or not they were starved (Table 1). Mice inoculated with these feces never developed patent parasitemia, and all survived at least 30 days except 20% of the group injected with the feces from fed bugs (Table 2). All the surviving mice died as well as the controls after challenge with lethal doses of trypomastigotes.

Table 2. Parasitemia and death of mice injected with feces of triatomes fed Tul_L-culture forms or trypomastigotes of *Trypanosoma cruzi* (Tulahuén strain)

Feces of triatomes			Observation of mice (days)			
Triatomes	infected with	days after infection	parasitemia			death
			15	20	30	30
Starved	Epi.*	15	0/10[a]	0/10	0/10	0/10[b]
		30	0/10	ND***	0/10	0/10
		45	0/10	ND	0/10	0/10
		60	0/10	ND	0/10	0/10
Fed	Epi.	15	0/10	0/10	0/10	0/10
		30	0/10	ND	0/8	2/10
		45	0/10	ND	0/10	0/10
		60	0/10	ND	0/10	0/10
Starved	Tryp.**	15	3/10	3/10	5/10	0/10[c]
		30	9/10	ND	9/10	6/10
		45	1/10	ND	3/10	1/10
		60	1/10	ND	1/10	1/10
Fed	Tryp.	15	6/10	10/10	3/3	7/10
		30	10/10	ND	ND	5/10
		45	10/10	ND	ND	3/10
		60	7/10	ND	10/10	3/10

* 98% epimastigotes
** Bloodstream trypomastigotes
*** Not done
[a] Number of mice with positive parasitemia/total number of mice
[b] Number of deaths/total number of mice
[c] 3 mice of this group died between days 32 and 35 after inoculation

When NMB with trypomastigotes was used to feed the triatomes, parasites were detected in the feces of starved and fed groups on the 30th day after ingestion; the numbers of parasites detected in the feces of the fed triatomes was at least 5 times higher than those observed in the feces of the starved insects. On day 45, parasites were detected only in feces from fed triatomes (Table 1). Microscopic observation revealed mainly epimastigotes but on day 30, 10% metacyclic forms were observed in the feces of the fed group. Mice injected with feces of triatomes previously fed blood trypomastigotes revealed positive parasitemias and a number died during the first month after inoculation (Table 2). Whether or not their feces revealed parasites all mice injected with feces of weekly fed triatomes developed patent parasitemias; less than 50% of those injected with feces from starved triatomes became positive (Table 2). In the former group mortality was 45% and 20% in the latter.

Experiment 2: Parasites were seen in the feces of artificially reared insects

Table 3. Parasites observed in the feces of artificially-reared triatomes after ingestion of culture forms or trypomastigotes of *Trypanosoma cruzi* (Tulahuén strain)

Triatomes fed with	Feces observed on days	
	30	60
Epi. (Tul_L)*	*pos.*[1]	*pos.*[2]
Tryp.**	*pos.*[1]	*pos.*[3]

* 98% epimastigotes
** Bloodstream trypomastigotes
[1] Only epimastigotes
[2] 10% metacyclic forms
[3] 25% metacyclic forms

Table 4. Infectivity of parasites from the feces of artificially-reared triatomes fed Tul_L-culture forms or trypomastigotes of *Trypanosoma cruzi* (Tulahuén strain)

Feces of triatomes		Observation of mice (days)			
fed with	days after infection	parasitemia			death
		15	20	30	30
Epi.*	30	0/10[a]	0/10	0/10	0/10[b]
	60	0/10	0/10	0/10	0/10
Tryp.**	30	9/10	10/10	ND***	2/10
	60	9/10	10/10	ND	4/10

* 98% epimastigotes
** Bloodstream trypomastigotes
*** Not done
[a] Number of mice with positive parasitemia/total number of mice
[b] Number of death mice/total number of mice

fed 30 or 60 days before with either NMB plus Tul_L-culture forms or NMB plus trypomastigotes; metacyclic forms were observed in both groups on the 60th day (10 and 25%, respectively) (Table 3). However, whereas mice injected with feces of insects fed culture forms neither developed patent parasitemias nor was death noted during the first 30 days, mice inoculated with feces of insects fed trypomastigotes (either 30 or 60 days before) developed patent parasitemias with 20 and 40% mortality, respectively (Tables 3 and 4). When survival mice were challenged with lethal doses of trypomastigotes those receiving feces from insects fed Tul_L-culture forms died at the same time as the controls but those

Table 5. Infectivity of parasites from the digestive tract of triatomes fed Tul_B-culture forms of *T.cruzi* (Tulahuén strain)

Mice inoculated with*	Parasitemia		Death rate at 30 days
	15 days	30 days	
Feces	30%**	45%	10%
Stomach			
lot 1[a]	25%	50%	0%
lot 2[a]	20%	30%	0%
Intestine			
lot 1[b]	95%	100%	5%
lot 2[b]	60%	90%	0%

* 20 mice in each group
** % of mice with positive parasitemia
[a] epimastigotes and transition forms
[b] 20% (lot 1) and 25% (lot 2) metacyclic forms

injected with feces from triatomes fed with bloodstream trypomastigotes survived.

Experiment 3: When triatomes were fed 10^6, 10^7 or 10^8 Tul_L-culture forms, parasites were present in the feces on the 30th day but none of the mice inoculated with these feces developed patent parasitemia. The absence of infection in these mice was shown because they died at the same time as the controls after being challenged.

Experiment 4: Parasites were seen in the feces and in digestive tract contents of insects fed Tul_B-culture forms (good infective capacity). In the two groups where the stomach was separated from the intestine by dissection, the number of parasites seen in the intestinal contents was 5 and 10 times higher than in the stomach. Only epimastigote-like or transition forms were seen in the stomach while at least 20% metacyclic forms were seen in the intestinal contents. Some of the mice inoculated with feces obtained by squeezing, with the stomach, or with the intestinal contents developed patent parasitemias but the death rate was extremely low. The number of mice with patent parasitemias was higher in those groups inoculated with intestinal contents (Table 5).

Discussion

Successful infection of the triatomes by artificial feeding confirms the results of Alvarenga and Brener (1978). This method is useful for studying how the different stages of *T.cruzi* progress in the triatomes' digestive tract, as well as the influence that frequency of feeding and type of food have on the life cycle of the parasite.

It was reported that bloodstream trypomastigotes are able to develop in the vector in complete absence of blood by feeding the insects with trypomastigotes suspended in saline, Hanks solution or 199 medium (Alvarenga and Brener, 1978). Our results with starved and weekly-fed triatomes showed a better degree of infectivity and longer persistance of parasites in the insect feces of the fed triatomes. These in vivo results are in accordance with in vitro studies where differentiation was reached using a culture medium for insect cells enriched with extracts of digestive tract of *Triatoma infestans* fed recently (Isola et al., 1980), wich suggests that the frequency of the feeding rather than the type of food is related to the process of differentiation of the parasite. The ingested food might stimulate the production and/or the secretion of specific components. Lanar (1979) had suggested that factor(s) secreted by the *T.infestans*-embryo-cell-line might be responsible for differentiation to metacyclic forms.

The artificial rearing of the vectors by means of the feeding apparatus, despite the modifications it introduced in the natural flora of the digestive tract (Rondinone et al., 1978), failed to modify the capacity of the insects to become infected.

If the trypomastigotes used to feed the insects were injected directly into mice, 100% died during the first 30 days, even if they only received a single parasite; therefore we can assume that infectivity was decreased after the bloodstream form had passed through the digestive tract of the vector. A similar decrease in the degree of infectivity of bloodstream forms was communicated for the metacycles differentiated in *T.infestans*-embryo-cell-line (Lanar, 1979).

The infectivity of parasites present in the feces of triatomes fed Tul_B-culture forms indicates that the cycle in the insect could be initiated not only by the ingestion of the bloodstream trypomastigotes but also with cultures containing more than 98% epimastigotes. With these experiments it was not possible to elucidate whether the successful infection of these triatomes was dependent on the epimastigote population or on the 1–2% metacyclic forms present in the cultures; but as the metacycles were also present in Tul_L-cultures it may be affirmed that their presence does not assure successful infection in the vector. Accordingly, the parasites found in the feces of the triatomes fed Tul_L-culture forms failed to infect mice whether metacyclic forms were present or not; therefore, at least in some cases, differentiation to metacycles detected by optical microscopy might not correlate with the degree of infectivity of the sample considered. It was reported that Tul_L-culture forms, which differentiate to the metacyclic type form (60–80%) in a culture medium enriched with extracts of triatomes, increased their infectivity after sequential transfers in this medium (Isola et al., 1980); therefore, one passage through the digestive tract of the insect may not be enough to detect a real increase in infectivity of the culture forms.

Parasites recovered from the intestine are more infective than those recovered from the stomach of the vector. This may be explained by the obser-

vation that differentiation to metacyclic forms is induced at the hindgut level (Zeledón et al., 1977). On the other hand, successful infection of mice with parasites recovered from the stomach may be caused by transition forms, similar to those obtained in cultures and reported by Nogueira and Cohn (1976) as resistant to lysis of normal mammalian sera, capable of infecting and reproducing in macrophages.

Acknowledgments: The authors wish to thank Dr. Teresa Mercado for editorial assistance. – This investigation received financial support from the UNDP/World Bank/WHO (Special Programme for Research and Training in Tropical Diseases) and from Secretaría de Estado de Ciencia y Technología of Argentina.

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