Rev. Inst. Med. trop. São Paulo
33 (6): 427-433, novembro-dezembro, 1991

LETHALITY OF TRIATOMINES (HEMIPTERA: REDUVIIDAE), VECTORS OF CHAGAS' DISEASE, FEEDING ON BLOOD BAITS CONTAINING SYNTHETIC INSECTICIDES, UNDER LABORATORY CONDITIONS.

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SUMMARY

A laboratory study was conducted to test the toxicity of synthetic insecticides added to defibrinated sheep blood kept at room temperature and offered as food to the following triatomine species: **Triatoma infestans, Panstrongylus megistus, Triatoma vitticeps, Triatoma pseudomaculata, Triatoma brasiliensis** and **Rhodnius prolixus.** The insecticides used, at a concentration of 1g/l, were: HCH, DDT, Malathion and Trichlorfon, and the lethalithy observed at the end of a 7-day period varied according to the active principle of each.

HCH was the most effective by the oral route, killing 100% of the insects, except **P. megistus** (95.7%) and **T. pseudomaculata** (94.1%). Trichlorfon killed the insects at rates ranging from 71.8% (**T. vitticeps**) to 98% (**R. prolixus**). Malathion was slightly less efficient, killing the insects at rates from 56.8% (**T. vitticeps**) to 97% (**T.brasiliensis**). DDT was the least effective, with a killing rate of 10% (**T. vitticeps**) to 75% (**T.brasiliensis**).

Since the tests were performed at room temperature, we suggest that baits of this type should be tried for the control of triatomines in the field.

KEY WORDS: Trypanosomiasis; Triatomines; Vector control; Blood feeding; Insecticides; Lethality rate.

INTRODUCTION

In Brazil, the principal method to reduce triatomine populations infesting human dwellings is spraying with chemical insecticides of prolonged residual action (SHERLOCK, 1975; MARSDEN, 1980; DIAS, 1986; OLIVEIRA-FILHO, 1984). These are costly products which are almost always imported and which require a complex operational schedule of application.

On the basis of the proven effect of these insecticides and of the observation that triatomines can feed on artificial devices (latex bags) containing blood even when blood is at room temperature (LIMA et al., 1989; PINTO et al., 1990), in the present study we tested the toxicity of defibrinated sheep blood containing insecticides and offered as a food source to the triatomines.

The objective of the study was to contribute to the development of alternative methods for domiciliary control of the vectors of Chagas' disease in risk areas when home spraying is not an available solution. The experiment is part of a series in which alternative techniques of insect in-

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toxication through the digestive route will be applied using different types of baits and insecticides, such as carbamates and pyrethroids.

MATERIAL AND METHODS

Triatomines

The tests were carried out on six of the main species that transmit **Trypanosoma cruzi**: **Triatoma infestans** (KLUG, 1834), **Panstrongylus megistus** (BURMEISTER, 1835); **Rhodnius prolixus** (STÅL, 1834), **Triatoma brasiliensis** NEIVA, 1911, **Triatoma pseudomaculata** CORREIA & ESPINOLA, 1964, and **Triatoma vitticeps** (STAL, 1859).

These triatomines originated from the "Laboratório Nacional e Internacional em Taxonomia de Triatomíneos" (Instituto Oswaldo Cruz, Brazil), where they have been mantained for many years. A total of 1736 specimens were tested, distributed as follows: 241 specimens of **T. infestans**, 206 of **T. brasiliensis**, 283 of **T. vitticeps,** 293 of **T. pseudomaculata**, 342 of **R. prolixus** and 371 of **P. megistus**, at all phases of the evolutionary cycle except adult females. All insects were starved for 15 to 30 days prior to the experiment.

Feeding

Defibrinated sheep blood was provided by the Central Animal House of Fundação Oswaldo Cruz, Brazil. Before the tests, the flasks were removed from the refrigerator and heated on a water-bath until they reached a temperature approximately equal to that of the laboratory (25-30ºC). The flasks were then left to stand for approximately 30 minutes at room temperature before the tests were initiated.

Insecticides

For the first stage of the research the following insecticides were selected:

HCH (Provided by SUCAM) (Hexacloro-cyclo-hexane, 1,2,3,4,5,6) (30% gamma isomer) at 75% purity. This is the earliest and most widely used insecticide in the campaigns in Latin America (BUSTAMANTE & GUSMÃO, 1954; FLORES, 1972; SHERLOCK & MUNIZ, 1973; GONZALES, 1978).

DDT (Provided by SUCAM) (1,1,1,-trichloro-2-2, bis p-chlorophenyl-ethane) at 75% purity. It was preliminarly studied by the oral route (LENT & OLIVEIRA, 1944).

TRICHLORFON (Provided by "Instituto Nacional de Controle de Qualidade em Saúde" (INCQS), FIOCRUZ, Brazil) (dimethyl 2,2,2,-trichloro-1-hydroxyethylphosphonate), at 99.8% purity. It is a contact and stmomach insecticide with penetrant action (NAKANO et al. 1977; WORTHING, 1979).

MALATHION (Provided by "Instituto Nacional de Controle de Qualidade em Saúde" (INCQS), FIOCRUZ, Brazil) (S-1, 2-bis (ethoxycarbonyl) ethyl O,O-dimethylphosphorodithioate) at 93% purity. This insecticide has been tested in Brazilian campaigns (SHERLOCK et MUNIZ, 1975; MARTINEZ et al., 1975; SHERLOCK et al., 1976; PICOLLO et al., 1976; OLIVEIRA-FILHO, 1986).

Considering the purity of the products, the real weighed mass was 1.334g for HCH and DDT, 1.075g for Malathion, and 1.002g for Trichlorfon, with each insecticide thus providing 1g of pure product per 30-ml blood bait. The same mass of active product (1g) was chosen because the oral LD_{50} for triatomines of each insecticide tested is not known.

Each insecticide was dissolved in 30ml blood and placed in latex bags (non-lubrificated condoms) that had been previously washed with running water and dried in an oven at 45°C. After, the bags were filled with blood, the opening was tied with string to prevent any leakage.

At testing, each lot of aproximately 30 insects per species was placed in a 1000-ml glass lined with filter paper and covered with nylon netting, and the latex bag was hung on the inner wall of the flasks.

A control group was submitted to the same procedure except that insecticides were added to blood.

The flasks were manipulated only at the times scheduled for observation, avoiding the presence of human observers and the consequent stimulation of the insects with CO_2 or thermal currents.

The observations were scheduled at 5 minutes, 30 minutes, 4h, 6h, 24h, 48h, 72h and one week after the beginning of the experiment. At each observation time, mortality of the treated and control groups, as well environmental temperature and humidity, was recorded. Temperature ranged on average from a maximum 27.7 ± 4.3°C to a minimum of 26.8 ± 4.2°C. Relative humidity was X = 79.5 ± 5.7%.

RESULTS

I. Observation of feeding behaviour of the control groups:

Although the insects had been starved for 15 to 30 days prior to the experiment, their behaviour with respect to the food offered was highly irregular. While some fed during the first hours, as **T. brasiliensis** and **P. megistus** in particular, others waited as long as one week before looking for a meal, as was the case for **T. vitticeps** and **R. prolixus**. The insects were considered to have gorged only when their abdomen appeared to be visibly dilated. Thus, whereas, some insects showed high levels of repletion, as was the case of **T. pseudomaculata** (97.8%) and **T. brasiliensis** (90%), the other species had lower ingestion levels, as was the case of **R. prolixus** (64.6%), **T. infestans** (63.9%), **P. megistus** (60%) and **T. vitticeps** (38.2%).

Since the insects had not been weighed before or after the meal it has not been possible to detect any feeding when the amount of blood sucked by the insects was reduced.

II. Observation of the experimental groups:

Tables 1 through 4 show the results of the behaviour of the six triatomines species of the treatment and control groups.

a) Action of HCH:

Table 1 presents the percentage of dead insects observed up to one week after treatment with HCH at the concentration of 1g/l. At the end of this period, all insects were dead, except for two **T. pseudomaculata** specimens that died on the 8th day, and two of **P. megistus** that died at the 10th and 16th days, although all of them presented very strong symptoms of intoxication one week after beginning of the experiment. The two **T. pseudomaculata** specimens, for exemple, only presented slight leg tremors when lightly touched.

Of the six species tested, **T. vitticeps** was the only one that did not reach 50% mortality after 48h, whereas the mortality of all other species was above this level. Even after 24h, the effect of the insecticide was already close to, or even higher than, 50% for all species except **T. vitticeps**.

During the first five minutes, only **T. infestans** and **P. megistus** presented sensitivity, although this was very low.

TABLE I

Percent insect mortality observed up to one week after the beginning of oral treatment with HCH (1g/l), added to defibrinated sheep blood offered **ad libitum**.

Species	Treatment group								Control group*		
	Sample	Observation times							Sample	Mortality	
		5min	30min	4h	6h	24h	48h	72h	1 week		N	%
T. infestans	27	18.4	22.2	44.1	48.1	63.0	74.1	81.5	100.0	30	04	13.3
T. brasiliensis	30	0.0	3.2	25.8	29.0	48.4	74.2	83.2	100.0	30	00	0.0
T. pseudomaculata	34	0.0	20.6	26.5	29.4	44.1	64.7	76.5	94.1	36	01	7.7
T. vitticeps	31	0.0	3.2	3.2	3.2	32.3	61.3	61.3	100.0	34	00	0.0
P. megistus	46	6.5	26.1	41.3	43.5	45.7	80.4	88.4	95.7	38	04	10.5
R. prolixus	34	0.0	11.8	20.6	26.5	52.9	73.5	88.2	100.0	38	02	5.3

* Observation only after one week.

b) **Action of DDT:**

Table 2 lists the data recorded for oral DDT applications. Only **T. brasiliensis** and **T. pseudomaculata** showed susceptibility at the 50% mortality level after 48h of observations, an effect that continued up to the end of one week without reaching 80% mortality. In the other species, the effect was less than 50% mortality.

c) **Action of Malathion:**

Table 3 reports the results of oral application of Malathion. After 30 minutes, **T. brasiliensis** and **T.pseudomaculata** reached a mortality rate of more than 50% (63.5% and 60%, respectively), which continued to increase up to the end of one week, with 97% and 92.5% being the highest indices reached, respectively, among all the species treated with the insecticide. The least sensitive species were **T. vitticeps** and **R. prolixus**, which showed respective mortality rates of 56.8% and 47.7 after one week.

d) **Action of Trichlorfon:**

Table 4 reports the mortality rates for the insects treated orally with Trichlorfon. **T. brasiliensis** was the most sensitive species with a mortality rate of 81.8% as early as 30 minutes after feeding, followed by **T. infestans** (54.1%) and **T. pseudomaculata** (50%). From 24h on, all species showed more than 50% sensitivity, **T. vitticeps** being the most resistant with a maximum mortality of 71.5% after one week.

TABLE II

Percent insect mortality observed up to one week after the beginning of oral treatment with DDT (1g/l), added to defibrinated sheep blood offered **ad libitum**.

Species	Treatment group								Control group*		
	Sample	Observation times							Sample	Mortality	
		5min	30min	4h	6h	24h	48h	72h	1 week		N	%
T. infestans	30	0.0	0.0	0.0	6.7	33.3	36.7	40.0	43.3	30	04	13.3
T. brasiliensis	32	0.0	0.0	21.9	25.0	34.4	53.1	59.4	75.0	22	01	4.5
T. pseudomaculata	26	2.8	2.8	2.8	8.3	47.2	55.6	58.3	61.1	36	03	3.3
T. vitticeps	30	0.0	0.0	0.0	0.0	0.0	6.7	6.7	10.0	34	00	0.0
P. megistus	66	0.0	1.5	10.6	13.6	25.8	36.4	39.4	40.9	58	04	6.9
R. prolixus	33	0.0	0.0	0.0	0.0	3.0	6.1	6.1	15.5	34	03	8.8

* Observation only after one week.

TABLE III

Percent insect mortality observed up to one week after the beginning of oral treatment with Malathion (1g/l), added to defibrinated sheep blood offered **ad libitum**.

Species	Treatment group								Control group*		
	Sample	Observation times							Sample	Mortality	
		5min	30min	4h	6h	24h	48h	72h	1 week		N	%
T. infestans	28	0.0	17.9	28.6	28.6	35.7	53.6	60.7	71.4	23	01	4.3
T. brasiliensis	33	0.0	63.6	69.7	69.7	81.8	81.8	84.8	97.0	17	02	11.8
T. pseudomaculata	40	12.5	60.0	70.0	70.0	77.5	85.0	87.5	92.5	33	01	3.0
T. vitticeps	37	2.7	18.9	18.9	21.6	21.6	32.4	37.8	56.8	36	07	19.4
P. megistus	43	7.0	37.2	41.9	41.9	51.2	55.8	55.8	65.1	40	02	5.0
R. prolixus	44	0.0	27.3	29.5	29.5	31.8	31.8	31.8	47.7	58	02	3.4

* Observation only after one week.

TABLE IV

Percent insect mortality observed up to one week after the beginning of treatment with Trichlorfon (1g/l), added to defibrinated sheep blood offered **ad libitum**.

Species	Treatment group								Control group*		
	Sample	Observation times							Sample	Mortality	
		5min	30min	4h	6h	24h	48h	72h	1 week		N	%
T. infestans	37	5.4	54.1	59.5	67.6	86.5	86.5	86.5	89.2	36	01	2.1
T. brasiliensis	22	4.5	81.8	81.8	90.9	90.9	90.9	90.9	90.9	20	00	0.0
T. pseudomaculata	42	2.4	50.0	64.3	71.4	92.9	92.9	92.9	92.9	36	03	8.3
T. vitticeps	39	10.3	12.8	15.4	33.3	53.8	64.1	66.7	71.8	42	12	28.6
P. megistus	40	17.5	27.5	32.5	35.0	60.0	75.0	75.0	77.5	40	01	2.5
R. prolixus	49	2.0	10.2	16.3	24.5	57.1	89.8	95.9	98.0	52	06	11.5

* Observation only after one week.

Among the general symptoms preceding death, leg tremor was the most frequent, followed by paralysis, especially of the last pair of legs, which caused difficult in locomotion.

DDT induced the lowest number of symptoms especially during the first 30 minutes when the observations were made at shorter intervals.

The other symptoms were gas formation in the abdominal cavity and partial or total forward projection of the proboscis similar to that performed by the insect at feeding time.

Gas formation in the abdominal cavity was more frequent in **T. brasiliensis** treated with HCH (33.3%) and **T. infestans** treated with Malathion (21.4%) and in all insects treated with Trichlorfon (18.9%). **T. brasiliensis** treated with HCH presented the highest frequency of proboscis distention (43.3%), followed by all insects treated with DDT (36.4%).

DISCUSSION

Of the four insecticides tested, HCH proved to be the most effective by the oral route, whereas DDT was the least effective. Trichlorfon and Malathion had more rapid effects, with the insects dying soon after started feeding. DDT had the slowest effect since mortality could occur as late as 24 to 48h after feeding. At times the insects were able to reach repletion with the blood containing DDT, whereas a small amount of blood containing Trichlorfon was sufficient to induce a lethal effect.

The mortality rates of up to 100% in the experimental groups suggest that all insects have fed on the baits, even when no visible dilation of the abdomen was detected. In those cases of the insecticides that did not reach 100% of mortality, it is possible that the ingested quantity was not enough to cause death or the insecticide was not efficient enough.

HCH is the most frequently insecticide used in spraying campaigns in almost all Latin American countries were Chagas'disease occurs. Although this is a good insecticide for vector control, it has some disadvantages when sprayed, the most serious being toxicity to humans and domestic animals and environmental pollution (LEAL et al., 1965; SHERLOCK, 1975; SILVA, 1979; MARICONI et al., 1988). Furthermore, HCH is not active for long periods of time in treated house, its action lasting two or three months at most on mud wall, and little longer during warmer months. This requires frequent reapplications involving expenditure of time and money (MARSDEN, 1980; MARICONI et al., 1988). Also, because of its strong odor, it is often rejected by home dwellers (SHERLOCK, 1979).

In Venezuela, **R. prolixus**, the main species transmitting chagasic infection, has presented cross-resistance to HCH and Dieldrin and, according to various investigators (SHERLOCK, 1975; MARSDEN, 1980) this resistance will continue to increase. Fortunately, this problem has not yet arisen in Brazil (DIAS, 1986).

The above considerations, taken together with the fact that the use of chlorated active principles - HCH, DDT, Lindane, etc - is legally restricted due

to their polluting and stablished cancerigenous properties, with consequent scarcity of these products on market, require the elaboration of alternative control mesures.

In the present investigation, on attempt was made to study the possibility of finding a simpler solution - less expensive and easy to apply - using the insecticides in small quantities and in a manner that would not pollute the environment, if the results obtained in the laboratory should be confirmed in the field. Furthermore, date from this laboratory (LIMA et al., 1991) showed that lethal effects of baits containing blood with HCH can be maintained for several months.

Vector control with baits to be placed in the most appropriate locations in human dwellings would be mainly indicated in: a) places where systematic spraying control is not being done; b) during the intervals between applications or c) to avoid reinfestations when spraying is interrupted.

An attempt to control triatomines with insecticides application by oral route was previously made by LENT & OLIVEIRA (1944). These investigators administered varying amounts of pure DDT in the form of gelatin capsules to pigeons, which were used to provide blood meals to **R. prolixus**. The authors obtained positive results but did not clarify how many specimens they used or the mortality rate they obtained. However, they stated that mortality was slow during all experimental phases, a fact observed in our study: DDT did not prove to be effective for oral control in any of the species studied, since more than 50% mortality only occurred 48 hours after the meal and only two species (**T. brasiliensis** and **T. pseudomaculata**) (Table 2). At times the insects gorged until repletion and only died 24 to 48 hours later.

Some investigators have stated that, in Brazil, DDT is not effective against triatomines when sprayed (SILVA JR, 1947; DIAS & PELLEGRINO, 1948; FREITAS, 1950; PINTO & BICALHO, 1952; SHERLOCK, 1979; SILVA, 1979).

Gas formation in the abdominal cavity of the insects occurred to varying extents with all fou. insecticides. According to LENT & OLIVEIRA (1944), gas formation was more frequent in **R. prolixus** when the insects fed on blood of pigeons that had received DDT that when DDT was applied topically.

RESUMO

Letalidade de triatomíneos (Hemiptera: Reduviidae), vetores da doença de Chagas, alimentados sobre iscas de sangue contendo inseticidas sintéticos, em condições de laboratório.

Ensaiou-se, em laboratório, o efeito tóxico de inseticidas sintéticos, adicionados ao sangue de carneiro desfibrinado, mantidos em temperatura ambiente, oferecidos como fonte alimentar às seguintes espécies de triatomíneos: **Triatoma infestans, Panstrongylus megistus, Triatoma vitticeps, Triatoma pseudomaculata, Triatoma brasiliensis** e **Rhodnius prolixus**. Os inseticidas usados, na concentração de 1g/l, foram: HCH, DDT, Malathion e Triclorfon e a letalidade observada, ao fim de 7 dias variou, de acordo com o princípio ativo.

HCH foi o mais efetivo por via oral, matando 100% dos insetos, exceto **P. megistus** (95,7%) e **T. pseudomaculata** (94,1%). Triclorfon eliminou entre 71,8% (**T. vitticeps**) e 98% (**R. prolixus**). Malathion ficou ligeiramente abaixo, matando entre 56,8% (**T. vitticeps**) e 97% (**T. brasiliensis**). DDT foi o menos efetivo, matando entre 10% (**T. vitticeps**) e 75% (**T. brasiliensis**).

Como os testes foram feitos em temperatura ambiente, espera-se que iscas desse tipo possam ser usadas no controle de triatomíneos no campo.

ACKNOWLEDGEMENTS

We are greatful to Dr. José Jurberg, for the triatomines utilized, Dr. Wanderley Mascarenhas Passos, from the "Universidade Federal Rural do Rio de Janeiro", for helpful discussions on chemical aspects and José Augusto Albuquerque dos Santos, for technical assistance.

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Recebido para publicação em 08/02/1991.
Aceito para publicação em 30/09/1991.