Rev. Inst. Med. trop. São Paulo
34(4):295-301, julho-agosto, 1992

LETHAL EFFECT OF A BAIT FOR *RHODNIUS PROLIXUS* (HEMIPTERA: REDUVIIDAE), THE VECTOR OF CHAGAS' DISEASE, CONTAINING HEXACHLOROCYCLOHEXANE (HCH), UNDER LABORATORY CONDITIONS

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SUMMARY

The lethal effect of a bait containing an aqueous hexachlorocyclohexane (HCH) suspension at the concentration of 1g/l and maintained at room temperature was studied in the laboratory over a period of 12 weeks. The suspension was placed in a latex bag hanging inside a 1000-ml beaker tightly covered with nylon netting, and left there with no changes for 85 days. Sixteen groups of *R. prolixus* bugs, consisting on average of 30 specimens each, were successively exposed to the bait and observed at different intervals for one week each.

The mortality rate was 100% for all groups, except for the 16th, whose mortality rate was 96.7%. As the groups succeeded one another, mortality started to occur more rapidly and was more marked at the 6- and 24-h intervals. Later tests respectively started at 6:00 a.m. and 6:00 p.m. showed that diurnal and nocturnal periodicity in the offer of food had no effect on mortality. First- and 2nd- instar nymphs and adults male were more sensitive and 5th- instar nymphs were more resistant to the active principle of the bait.

KEY WORDS: Trypanosomiasis; Triatomines; *Rhodnius prolixus;* Insecticide; Hexachlorocyclohexane; Lethality rate; Insect control.

INTRODUCTION

The methods utilized thus far for the control of triatomines have been based on the application of insecticides by aspersion, which favors insect contact with the sprinkled wall surface. The success of this methodology, that will probably eliminate, in the next future, **Triatoma infestans,** an exotic but very important vector of trypanosomiasis in Brazil, will not stop **T. cruzi** transmition completely because, other local triatomids will try to occupy the same ecological niche, in the human dwellings. When no more reasons exist, for the extensive and expensive campaigns of the vector control, alternative methods will be needed that could be addapted to local situations, at a low cost-benefit rate and easely utilized, without the risks of environmental contamination. In a search for more simple and economic alternatives, attempts are being made to improve triatomine control using baits containing insecticides that act by oral route. There are some pioneer papers about xenointoxication of these triatomines[7,13]. In early paper, initial laboratory tests using blood containing toxic products as food source and carried out at room temperature have shown satisfactory results[9].

Parallel to the tests based on blood, we have used a bait consisting of distilled water

Supported by Fundação Banco do Brasil and CNPq.
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containing HCH at the concentration of 1g/l. The initial hypothesis was based on the statement by LENT[6] that in the laboratory, triatomines need water in addition to a blood meal. We also investigated how long the bait would be able to exert a toxic effect on different groups of *Rhodnius prolixus*.

The experiment is a part of a series in which alternative techniques of insect intoxication through the digestive route will be applied using different types of baits and insecticides.

After having demonstrated, in the laboratory, that such a simple and inexpensive bait can kill the triatomids by oral route, we intend to start a new and logical step of this study, organizing experiments that will evaluation their efficacy in field conditions, and compare it with other control methods.

MATERIAL AND METHODS

Triatomines: The tests were performed on *R. prolixus* bugs from the colony of the National and International Triatomine Taxonomy Laboratory (Instituto Oswaldo Cruz, Brazil). This is one of the most important vectors of Chagas' disease in several countries of Latin America, and easily maintained in laboratory.

The experimental groups (EG) consisted of 493 specimens at all stages of evolution tested in 16 groups containing on average 30 specimens each (range 21 to 41) and distributed as follows: 42 1st- instar nymphs, 54 2nd- instar nymphs, 77 3rd- instar nymphs, 168 4th- instar nymphs, 117 5th- instar nymphs, and 30 adults (18 males and 12 females).

The control group (CG) consisted of 30 **R. prolixus** specimens maintained throughout the experiments, with possible deaths recorded. After the 8th EG had been exposed to the bait, 6 nymphs were added to CG (two 3rd- instar, three 4th- instar, and one 5th- instar nymph) to replace dead specimens. At that time, the CG specimens received warmed blood for 30 min[8] and were then allowed to be in touch with a latex bag identical to the experimental one, but containing only distilled water. All insects were submitted to food deprivation for 15 to 20 days prior to the experiment.

Bait: The bait was prepared with distilled water and HCH (Hexaclhorocyclohexane, 1, 2, 3, 4, 5, 6) at 1g/l, wettable powder with 30% of gamma isomer, at 75% purity. Considering the purity of the product the real weighed mass was 1.334g, with the insecticide, thus providing 1g of pure product. Although HCH is no more in routine use, it was more efficient than other insecticides when used orally[9]. This insecticide was dissolved in 30-ml distilled water and kept at room temperature in a latex bag. Rigorous care was taken with the latex bag, whose opening was perfectly sealed to avoid any leakage. The bag was left hanging inside a 1000-ml beaker lined with filter paper and covered with nylon netting which was fixed with a rubber band throughout the experiment. The 16 experimental groups (EG) of *R. prolixus* were successively exposed for one week each from December 5, 1989 to February 27, 1990. The tests were started in the morning (9:00 to 10:00 h) and observations were made after 5 and 30 min, after 4, 6, 24, 48, and 72 h, and after one week. At each observation time, we recorded the deaths that had occurred in each stage, and the intoxication symptoms, which were mainly characterized by leg tremors and paralysis, distension of the proboscis and formation of gas in the abdominal cavity. Temperature ranged from a maximum on $\bar{X}$=30.7±0,7°C and minimum of $\bar{X}$=29.6±1.8°C. Relative humidity was $\bar{X}$=75.8±5.9%.

When we finished these tests and, in view of the fact these insects have a nocturnal habits in nature, we performed two tests which only differed in terms of starting time: one started at 6:00 a.m. and the other at 6:00 p.m. They envolved 121 and 103 *R. prolixus* specimens respectively for the experimental groups and 119 and 123 specimens respectively for the control groups, at all phases of the developmental cycle.

RESULTS

When they contacted the latex bag, some specimens of both EG and CG started to suck. At times they spent several minutes feeding, whereas at others they interrupted feeding, making or not new immediate attempts to feed. Engorgement was rare.

Although the observation was started after 5 and 30 min, the first deaths were recorded only after 4h (Fig. 1). In groups 2 and 4, death only started to occur after 24h, although some nymphs already presented symptoms of intoxication after 4h.

As the groups succeeded one another, mortality started to occur more rapidly and was more marked between 6:00 a.m. and 12:00 p.m. (Fig. 2), but the mortality rate between groups started at 6:00 a.m. and 6:00 p.m. was found not to differ markedly (Fig. 3).

Table 1 compares overall mortality for the treated and control groups. Although most of the control insects received only one blood meal over the 85 days of the experiment, the number of dead insects was very small, with practically no deaths occurring until the 8th week.

Figure 4 shows the mortality rates of all insects studied according to the respective developmental stages. First-instar nymphs were the most sensitive to the insecticide, followed by 2nd-instar nymphs and adult males. Fifth-instar nymphs were the most resistant, this being the only instar in which the number of dead insects did not reach 100% at the end of one week, although the only remaining nymph presented very strong symptoms of intoxication and died on the day after the end of the experiment.

Among the general symptoms preceding death, the most frequent was leg tremor followed by paralysis, especially of the last pair, which impaired or fully prevented locomotion. Other symptoms were gas formation in the abdominal cavity and distension of the proboscis in a position similar to that observed when the insect feeds.

Gas formation occurred in 20.3% of the insects and was more frequent in adult females (33.3%) and 5th-instar nymphs (23.1%). The lowest frequency was observed in 1st- and 2nd-instar nymphs (11.9% and 14.8%, respectively).

Distension of the proboscis occurred in 15.2% of the insects and was more frequent in adult females (25%), in males (22.2%) and in 3rd-instar nymphs (19.5%). The phenomenon was less frequent in 1st-instar (2.4%), 2nd-instar (13%) and 4th-instar (13.9%) nymphs, and occurred among 17.9% of 5th instar nymphs.

DISCUSSION

The attempt to replace blood with water was made on the basis of the statement of LENT[6] that triatomines need water to such an extent that they even practice coprophagy in the laboratory. In a previous paper[9], we demonstrated that HCH was more effective than other insecticides when applied orally, and for this reason it was used in the present tests with water.

In the presence of baits, some *R. prolixus* specimens from both the experimental and the control groups rapidly approached the bag and started to suck. These insects are known to suck blood at all phases of the reproductive cycle[12] and to be highly resistant to food deprivation[2,5,14] two characteristics that are very important in the epidemiology of Chagas's disease.

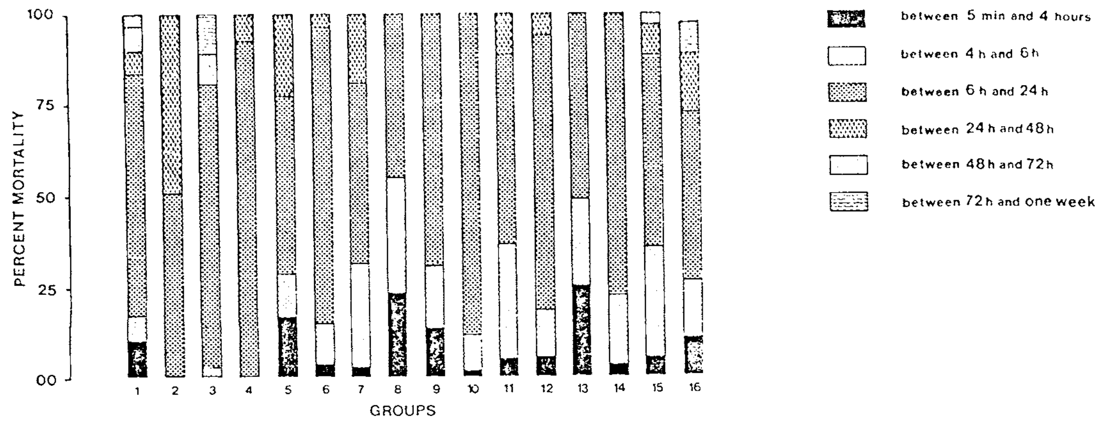

Fig. 1 – Percent mortality of 16 groups of *R. prolixus*, during 85 days. A single food bait placed in a latex bag, containing an aqueous solution of HCH (1g/l) was successively given to every group.

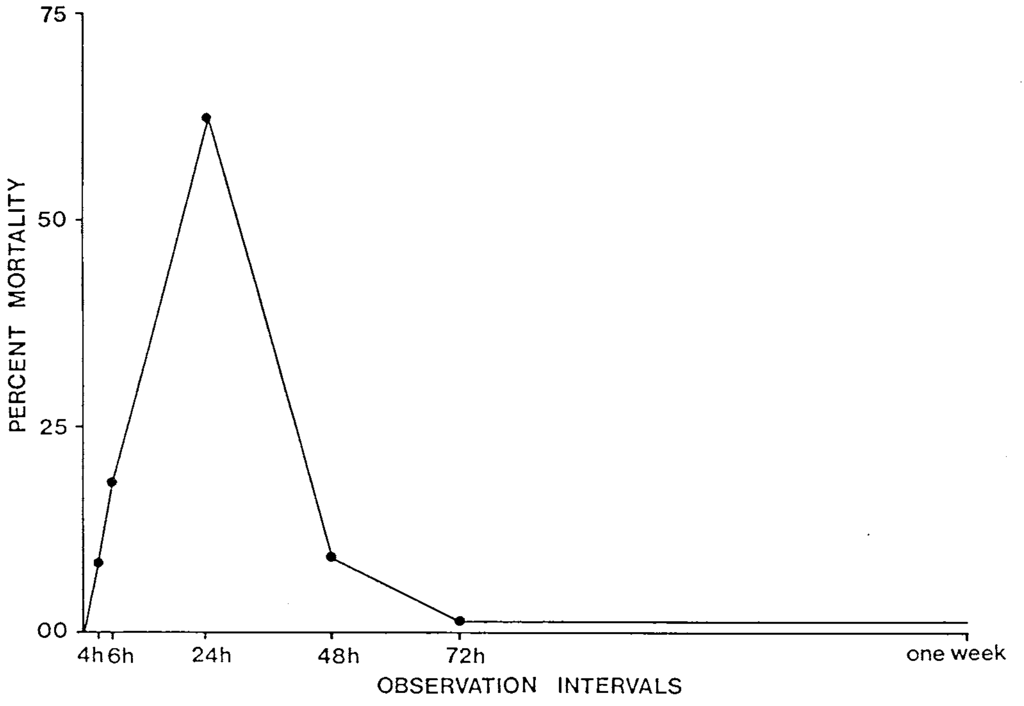

Fig. 2 – Variation of toxicity of a food bait containing an aqueous solution of HCH (1g/l), on 16 groups of *R. prolixus* (n=493 insects), tested along time. The figure shows percent mortality at different intervals.

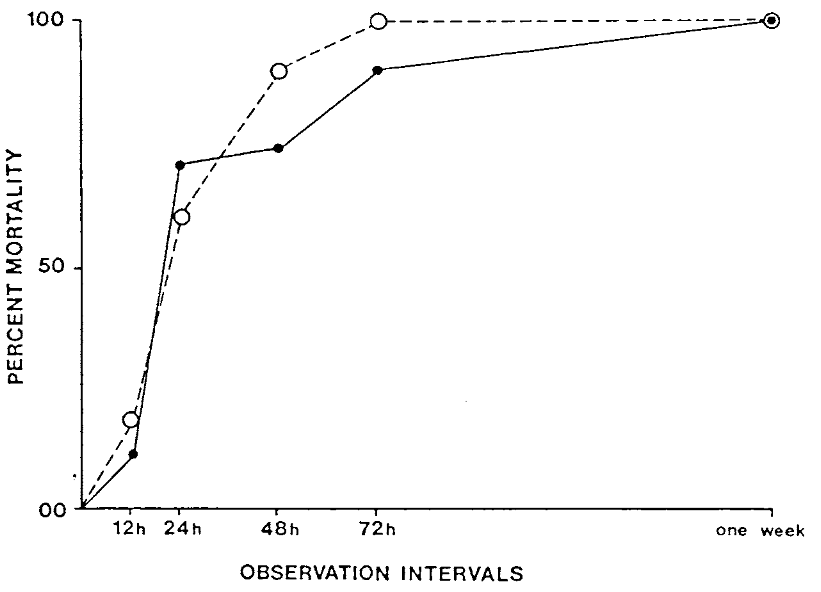

Fig. 3 – Percent mortality of two groups of *R. prolixus* after treatment with an aqueous solution of HCH (1g/l). One group was observed from 6 a.m. onwards (-O-) and the other from 6 p.m. onwards (-●-).

LIMA, M.M.; REY, L. & MELLO, R.P. de – Lethal effect of a bait for **Rhodnius prolixus** (Hemiptera: Reduviidae), the vector of Chagas' disease, containing hexachlorocyclohexane (HCH), under laboratory conditions. **Rev. Inst. Med. trop. S. Paulo, 34**(4):295-301, 1992.

TABLE 1

Percent mortality of 16 groups of *R. prolixus* treated with a single food bait containing an aqueous solution of HCH (1g/l) successively given to every group during 85 days. The data are compared with those for the control group maintained under the same conditions for one week of observation.

GROUPS	TREATMENT GROUP			CONTROL GROUP		
		MORTALITY			MORTALITY	
	SAMPLE	Nº	%	SAMPLE	Nº	%
1	30	30	100.0	30	0	0.0
2	23	23	100.0	30	0	0.0
3	21	21	100.0	30	0	0.0
4	21	21	100.0	30	0	0.0
5	31	31	100.0	30	0	0.0
6	25	25	100.0	30	0	0.0
7	32	32	100.0	30	1	3.3
8	41	41	100.0	29	0	0.0
9	29	29	100.0	29	4	13.8
10	34	34	100.0	31	0	0.0
11	38	38	100.0	31	0	0.0
12	35	35	100.0	31	0	0.0
13	31	31	100.0	31	0	0.0
14	30	30	100.0	31	5	16.1
15	33	33	100.0	26	3	12.0
16	39	38	97.4	23	4	18.2

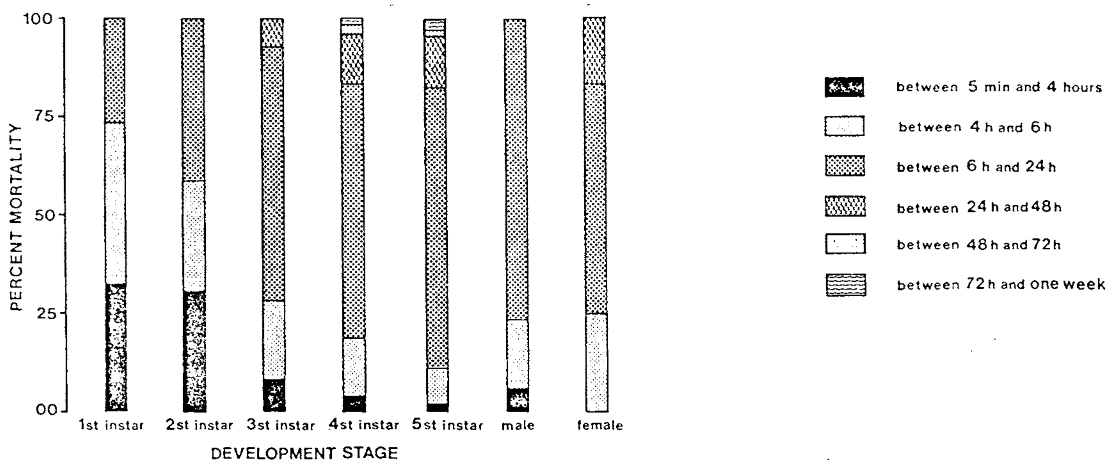

Fig. 4 – Percent mortality of 16 groups of *R. prolixus* during 85 days. A food bait placed in a latex bag, containing an aqueous solution of HCH (1g/l) was successively given to every group. Each column shows the total of dead insects, according to the development stage at different intervals.

According to LWOLF & NICOLLE[10], hematophagy is due to the insect's need for hematin as an exogenous source, since these insects cannot synthesize the porphyrin group.

GARCIA et al.[4] demonstrated that the blood volume ingested by *R. prolixus* depends on the viscosity of blood which, among other factors, modifies the efficiency of the physiological feeding mechanism of blood-sucking insects. These investigators also reported observing that triatomines can easily ingest milk in the laboratory at any phase of the developmental cycle.

From the present results we may deduce that, even though feeding on blood is of fundamental importance for the development of triatomines, and for the maturation of their eggs in particular[1,3,11] information about their food needs is still scarce.

By replacing blood with water, we made an attempt not only to evaluate the ability of these insects to suck fluids other than blood, but also to find simpler and less expensive solutions for a bait to be used for their control with minimum environmental contamination.

The duration of the lethal effect for several months is also important in economical terms if the results obtained in the laboratory should be confirmed in the field.

Figure 2 suggests that the mortality rate was not influenced by the circadian rhythm. Thus, we concluded that the higher mortality rate observed between 6:00 p.m. and 12:00 p.m. was due to the time needed by the insecticide to produce lethal effects and not to a search for food predominantly during the night. Furthermore, the **R. prolixus** specimens utilized in the tests were from a colony that was been maintained in the laboratory for several decades on a schedule in which food is always supplied during daytime, a fact that may have altered their tendency to feed at night.

RESUMO

Efeito letal de uma isca para *Rhodnius prolixus* (Hemiptera: Reduviidae) vetor da doença de Chagas, contendo Hexaclorociclohexano (HCH), em condições de laboratório.

O efeito letal de uma isca para *R. prolixus,* contendo suspensão aquosa de Hexaclorociclohexano (HCH), na concentração de 1g/l e mantida em temperatura ambiente, foi estudado no laboratório, por um período de 12 semanas. A suspensão foi colocada em uma bolsa de latex, que ficou sempre com o mesmo conteúdo durante 85 dias, pendurada na parede de um becker de 1000ml. Dezesseis grupos de *R. prolixus,* com média de 30 exemplares cada, sucederam-se um após o outro, sendo cada grupo observado em diferentes intervalos de tempo, durante uma semana.

A taxa de mortalidade foi de 100% em todos os grupos, exceto no 16º, que apresentou 96,7% de mortes nesse intervalo de tempo. À medida que os grupos se sucediam, a mortalidade passou a ocorrer mais rapidamente, acentuando-se no intervalo de 6h a 24h. Testes posteriores, um com início às 6h e outro às 18h, mostraram que a periodicidade (diurna e noturna) na oferta do alimento não interferiu na mortalidade. As ninfas de 1º e 2º instares e adultos foram mais sensíveis e as de 5º instar mais resistentes ao inseticida.

ACKNOWLEDGEMENTS

We are grateful to Dr. Lúcia Rotenberg for helpful discussion of the chronological aspects of the study.

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Recebido para publicação em 19/08/1991
Aceito para publicação em 18/05/1992