

ARE DEAD *TRITATOMA INFESTANS* A COMPETENT VECTOR OF *TRYPANOSOMA CRUZI*?

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After Triatoma infestans death, Trypanosoma cruzi survived several days, maintaining the ability to infect a vertebrate host. Dead bugs from an endemic area collected during an official spraying campaign showed mobile rectal tripanosomes up to 14 days after vector death. Two days after vector death 2,760 tripomastigotes were found alive in its rectal material. However, the number of mobile tripomastigotes decreased significantly from the 5th day after death.

Laboratory proofs with third and fifth nymphal stage showed similar results. Living tripanosomes were found in their rectal material at 10 days in third stage and even at 30 days in fifth nymphal stage. The mean number of tripomastigotes had no changes up to 10 days in third nymphal stage and increased significantly from 1 to 10 days in the fifth stage.

Conjunctival instillation as well as intraperitoneal inoculation to mice, of metacyclic forms from dead T. infestans produced infection in the vertebrate host.

Present results show that human contact with dead vector is highly probable in summer and living and infective T. cruzi are available for transmission in the vector.

Key words: vectorial transmission - *Trypanosoma cruzi* - *Triatoma infestans* - Chagas' disease

Trypanosoma cruzi, the etiologic agent of Chagas' disease, is mainly transmitted to man through contamination of the skin or mucosae with bug faeces containing the parasite metacyclic form. Another mechanism of vectorial transmission, is direct contact with fresh faeces which contaminate boxes, clothes or domestic tools (Pinto Dias, 1979). On the other hand, possible transmission through dead vectors has been mentioned (Wood, 1976). Lucena (cited by Brener, 1973) was the first to note that flagellates may survive in the bug intestine for as long as 9 days after vector death.

Our preliminary observations revealed that, during the hot season, frequent use of domestic insecticides in houses in a chagasic endemic area increases the contact possibility with dead bugs.

The aim of this work was to investigate the survival of *T. cruzi* in dead triatomids, to obtain some evidence on the possibility of man and domestic animal having contact with them and to test the virulence and infectivity of metacyclic tripomastigotes present in the rectal material of dead *T. infestans*.

MATERIALS AND METHODS

Field dead bugs observations — The field work was carried out in an endemic area at Rio Seco Department (NW of Córdoba, Argentina). *T. infestans* were collected from one infested house in the selected area, during the official campaign realized by Chagas National Service in May, 1987. Dead bugs were collected 24 h after having been sprayed (152 adults and 99 nymphs from second to fifth stage).

Captured triatomines were distributed into six experimental groups with the same age and sex

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distribution. Bugs on each group were kept at room temperature and 60-70% relative humidity, and examined at 2, 5, 6, 7, 9, and 14 days after death. The observation days were selected taking into account that contact possibility increases during first days after spraying. Later, the insects were swept up and thrown to the rubbish pit.

In order to estimate the contact possibility, between dead vectors and hosts, some observations on the inhabitant's behaviour were made. Twenty infested houses were investigated by means of a questionnaire.

Laboratory experiments with dead *T. infestans* — Second and fourth stage nymphs from Chagas National Service (First laboratory generation origin Santiago del Estero) were selected. Insects were kept at room temperature (25-26°C) and fed on mice (C3H strain) infected with X1 strain of *T. cruzi* (Cano & Rubiolo, 1985).

After moulting to following stage and previous checking for infection, insects were fed on an uninfected mouse and then killed with insecticide (HCH).

Dead bugs were distributed in 3 experimental groups which were analyzed at the following different times: Group 1: at the moment of death; Group 2: 10 days after death; Group 3: 30 days after death.

For insects collected in the field the following variables were estimated: percentage of dead infective bugs, with mobile tripomastigotes in their rectal material; percentage of dead infected bugs, with mobile tripomastigotes and/or epimastigotes; mean mobile tripomastigotes/ μl of rectal material.

From experimental laboratory bugs the following was analyzed: percentage of dead infective third and fifth stage nymphs; mean mobile tripomastigotes/ μl of rectal material.

In order to test infective capacity of metacyclic tripomastigotes from the rectal material of dead *T. infestans*, fifth stage nymphs infected with X1 strain of *T. cruzi*, were fed on an uninfected mouse (Bal-C strain) and killed with HCH 30 min after feeding. Two days after insects' death, 16 μl of rectal material from one nymph containing 24×10^3 tripomastigotes were instilled on a 35 days old mouse via conjunctival. Another 42 days old mouse was intraperitoneally inoculated with 107 μl of rectal material (from 3 insects), contain-

ing 249×10^4 metacyclic forms. In both cases parasitic infection was checked periodically by blood examination.

The number of flagellates present in both, individual triatomine rectum and mice's blood, was determined using a Neubauer chamber.

The data were analyzed through the variance analysis.

RESULTS

Field observations — The percentage of infective dead bugs has a significant diminution from the 6th to the 14th day ($p < 0.005$). The same behaviour was observed for percentage of infected dead bugs ($p < 0.005$) (Fig. 1).

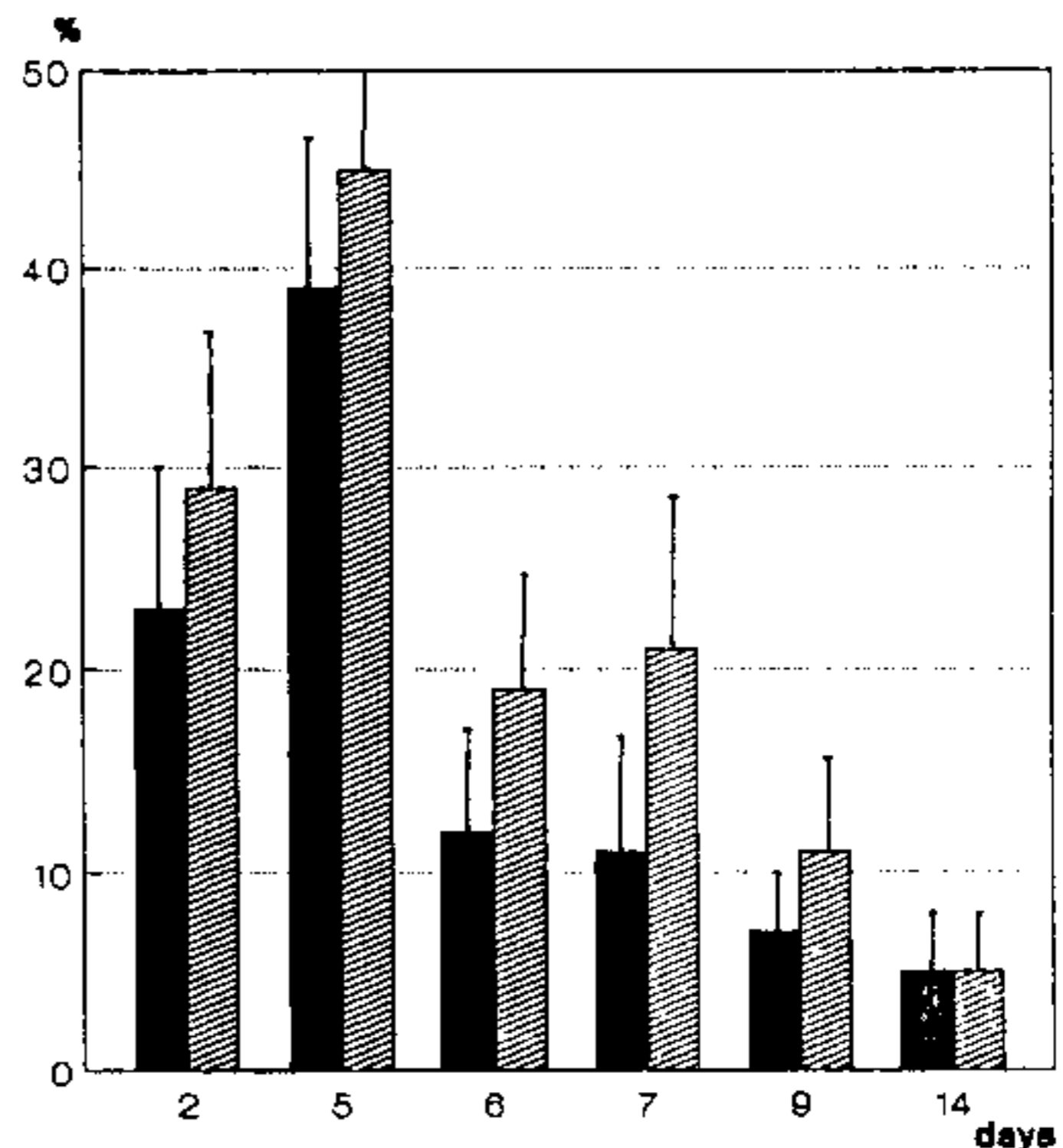


Fig. 1: percentage of dead infective ■ and infected ▨ bugs at different days after death (field bugs).

Parasitological features of rectal material — The mean number of mobile tripomastigotes per μl of rectal material lowered significantly from the 5th day after vector death ($p < 0.01$) (Fig. 2). The first check made 2 days after vector death showed 2,764 mobile tripomastigotes/ μl of rectal material.

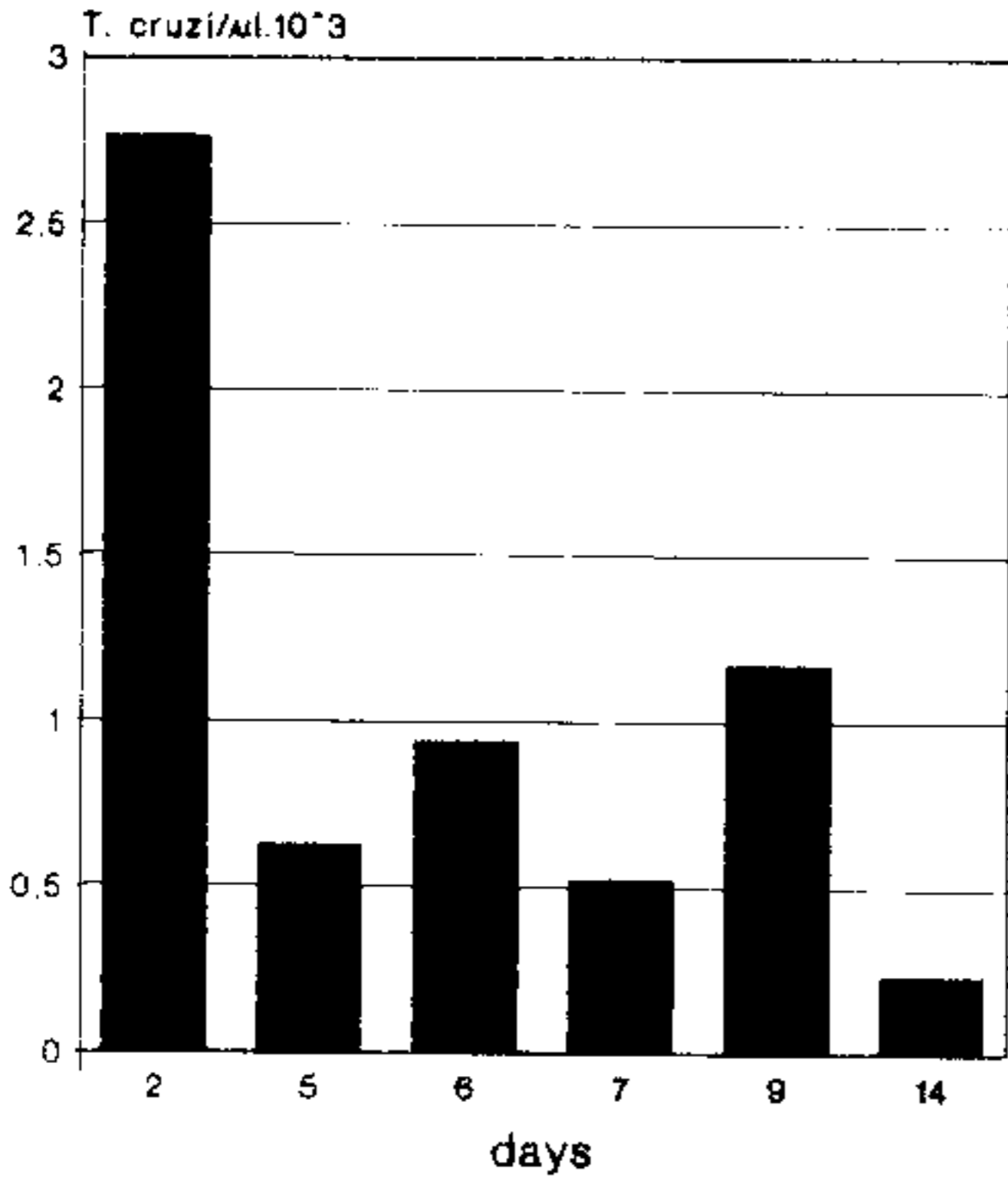


Fig. 2: number of *Trypanosoma cruzi* mobile trypomastigotes/μl of rectal material, at different days after death (field bugs).

Inhabitant customs in infested houses —

The inquiry revealed that: (1) It is frequent to observe dead bugs immediately after insecticide application; (2) During the hot season spontaneous domestic insecticide applications are carried out in 93% of infested houses; (3) After domestic or official spraying dead bugs can be found on the floor, clothes, beds and foods; (4) During the first 24 or 48 h after spraying dead bugs are swept or thrown to the rubbish pit; (5) To avoid domestic animals being poisoned with insecticides they are not allowed to eat dead bugs; (6) The majority of families do not permit children to play with dead bugs.

From the observations carried out at the site of spray we could notice that after sweeping, a lot of dead insects still remain inside the house, especially on floors or beds. Deficient cleaning is favoured by bugs colour and house's penumbra.

Laboratory experiments — The percentage of infective dead third stage nymphs was not modified during the first 10 days after insects death ($p > 0.05$). No bug exhibited infection on the 30th day after death. The percentage of infective dead fifth stage nymphs lowered significantly on the 10th day after death ($p = 0.05$). This variable had no significant change from the 10th to the 30th day after death ($p > 0.05$) (Fig. 3).

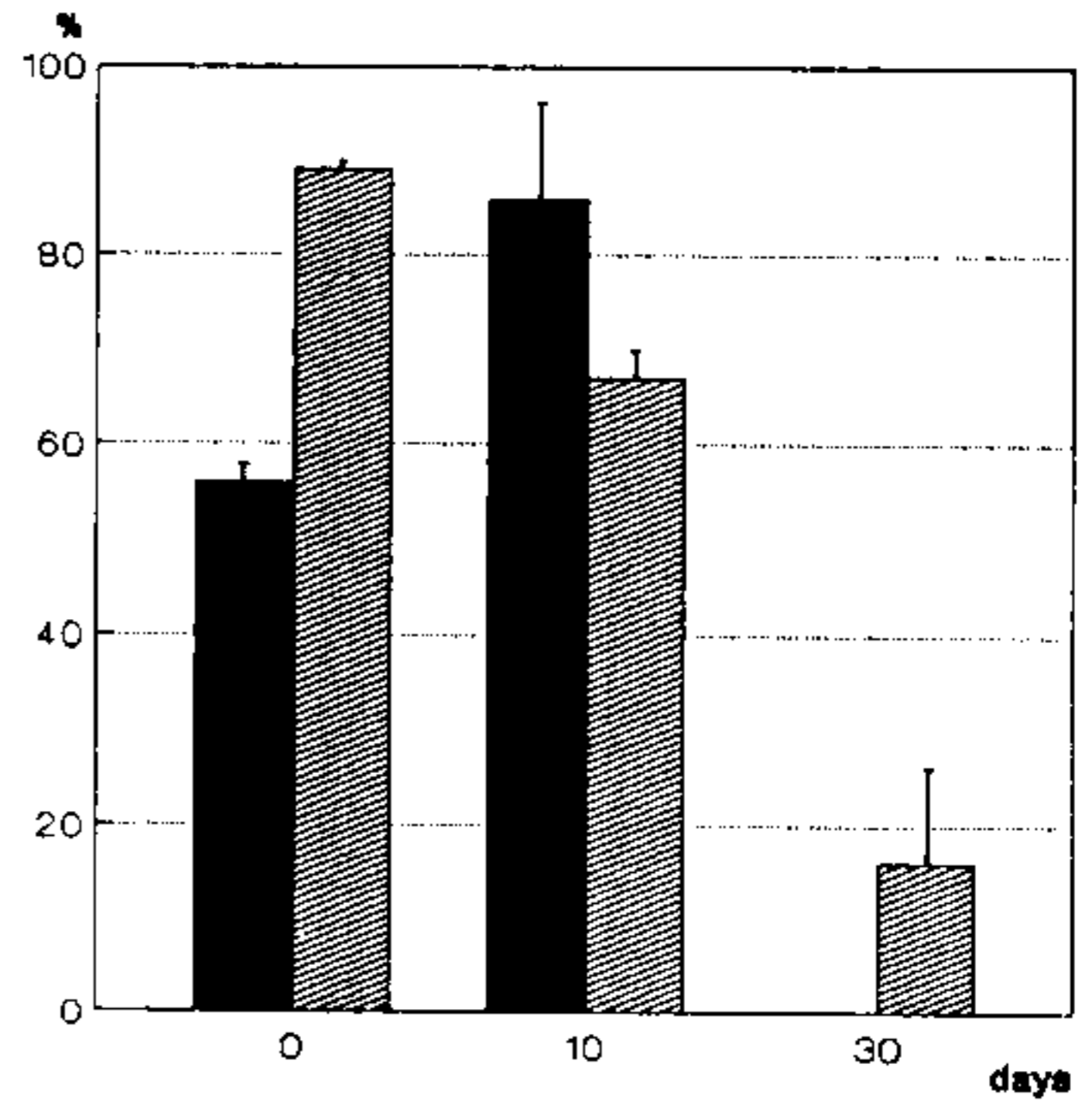


Fig. 3: proportion of infective dead third ■ and fifth ▨ stage nymphs, at different days after death (laboratory bugs).

In third stage nymphs the mean number of tripomastigotes/μl of rectal material had no changes during the first 10 days ($p = 0.32$) (Fig. 4). In fifth stage nymphs the mean number of mobile tripomastigotes per μl of rectal material increased from 24 h to 10 days ($p = 0.03$) and had no changes between the 10th and the 30th day after death ($p = 0.15$) (Fig. 4).

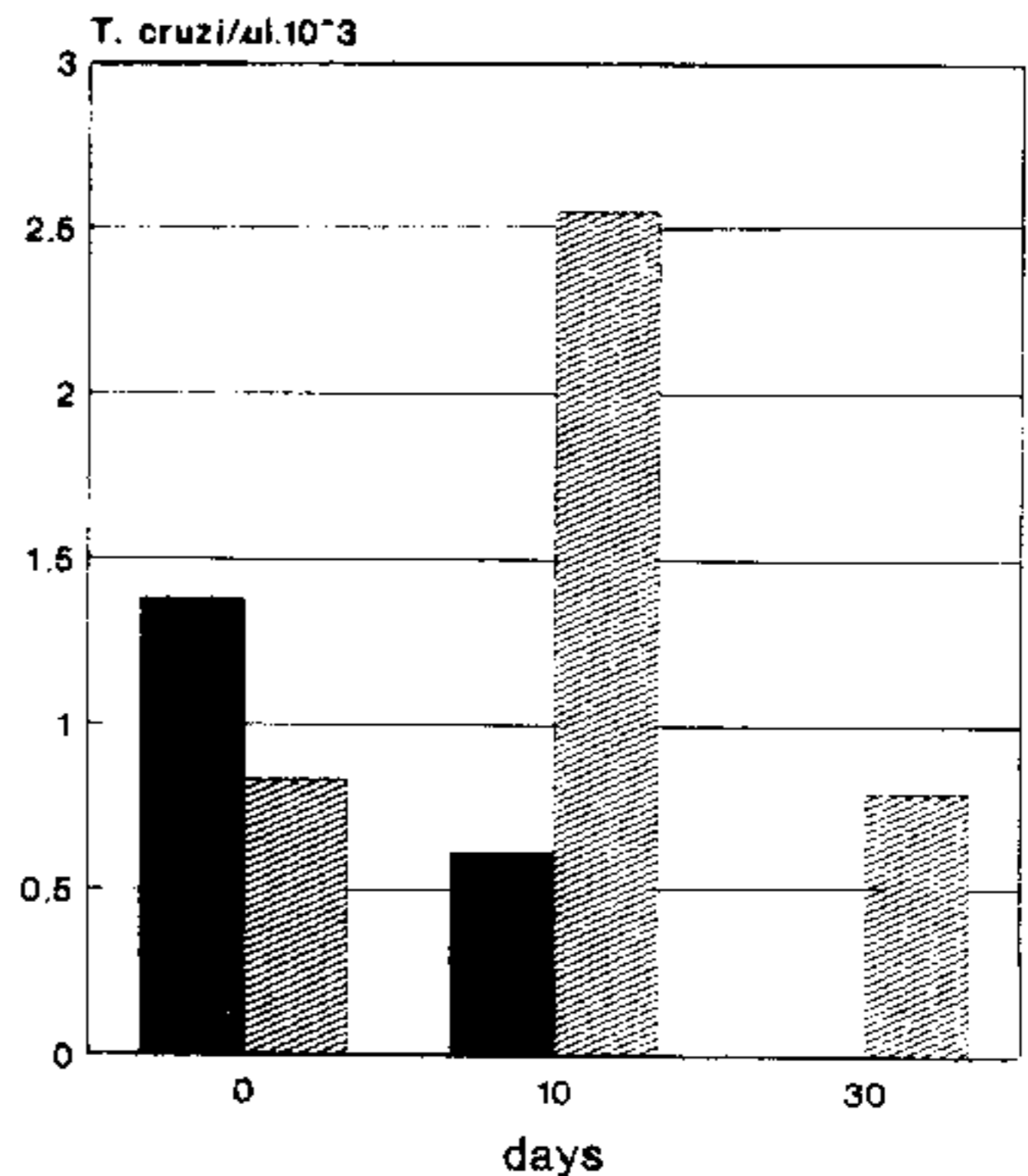


Fig. 4: number of *Trypanosoma cruzi* mobile trypomastigotes/μl of rectal material in third ■ and fifth ▨ stage nymphs (laboratory bugs).

Infective capacity of *T. cruzi* from dead *T. infestans* — Sixty days after conjunctival instillation 178,125 parasites/ml were present in the mouse's blood. On the other hand 38 days after intraperitoneally inoculation, 82,500 parasites/ml, were counted in the other mouse.

These results show that *T. cruzi* from dead *T. infestans* is able to penetrate and reproduce in the vertebrate host.

DISCUSSION

Present results demonstrate that *T. cruzi* remained alive until the 14th day after death of *T. infestans* collected in the field, as long as in those sacrificed in the laboratory. In laboratory assays on dead bugs, *T. cruzi* survival was observed even until 30 days after death. The inquiry carried out and the observations of Chagas National Service experts indicated that the highest probability of contact between dead vectors and people of infested houses occurs during the first 2 days after spraying. At this moment the main fall of dead bugs occurs and they are deficiently swept inside the house. Our results showed that two days after death there is a high percentage of infective bugs (29%), with a high number of mobile tripomastigotes in their rectal material (2,764/ μ l). On the other hand, *T. cruzi* from *T. infestans* with 2 days of dead was infective and virulente to the vertebrate host.

Wood (1976), was the first to mention possible transmission through dead vectors. Lucena (cited by Brener, 1973), proved that flagellates could survive in the bug intestine for as long as 9 days after vector death. Our results demonstrate the epidemiologic importance of dead *T. infestans* in *T. cruzi* transmission, especially during the first 2 days after domestic spraying and/or Chagas National Service spraying campaign. At this moment the metacyclic forms contained in vector rectal material conserve their infective capacity and exist a high probability of contact between dead vectors and inhabitants of infested houses. This proves that vectors remain effective transmitters of Chagas' disease even after their death. This fact could be reinforced by a facilitated emission of rectal material probably as a result of hind gut muscle relaxation. This mechanism of transmission could affect domestic animals and man. Wood (1976) pointed out that dead triatomines can be ingested by different animals, increasing the reservoir infection risk. People questioned by us expressed that only chickens were observed ingesting these insects.

It is important to remark that dead domestic *T. infestans* showed the same proportion of vector infection (28%) and the same number of tripomastigotes/ μ l as domestic *T. infestans* alive (Giojalas et al., 1990). It's know that for the same season the domestic population of *T. infestans* shows a marked increase (Schofield, 1980; Gorla & Schofield, 1989; Giojalas et al., 1990) which induces frequent use of insecticides and then a high number of dead triatomines can be found inside houses. The human contact with them is well favoured by the exposure of the skin because of high temperatures.

According to our laboratory results the nymphal stage could influence both survival and *T. cruzi* differentiation. In third stage nymphs the parasitic population remained the same during the first 10 days after death. In fifth stage nymphs the mean number of mobile tripomastigotes experienced a significant increase. This observations could be suggesting that after death, the vector not only conserves but increases its infectivity potential on permitting epimastigotes differentiation.

This work concludes that dead *T. infestans* do not lose their vectorial efficiency. This important fact could be noticed for inhabitants of endemic areas, especially during spraying in the hot season. Furthermore, it is clear that more information is necessary to understand the key factors that play an important role in *T. cruzi* survival on rectal material of the vector.

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