VE

VECTORS

VE-001
PRELIMINARY STUDIES ON RHODNIUS PROLIXUS PROTEASES: CROP, HEMOLYMPH AND FAT BODY ENZYMES

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It has demonstrated that proteolytic enzymes play an important role in digestion of food, cell metabolism, blood coagulation and prophenoloxidase system in insects. In the hemipteran, *Rhodnius prolixus*, digestion of the blood meal is fully dependent on proteases found in the intestine. However, the functions of proteases in other tissues of this hematophagous insect are poorly understood. Herein, we have studied the proteolytic activities in the crop, hemolymph and fat body of larvae and adults of *R. prolixus* at different intervals after feeding.

Hemolymph was taken through a micropipette and cell-free hemolymph obtained by centrifugation was prepared just before analysis. Crop content and lysed abdominal fat body were centrifuged and the supernatants stored until used. Samples of these materials were analyzed by Laemmli SDS-10% PAGE using gelatin, casein and fibrin as substrates for proteases. Also, *in vitro* studies using different protease inhibitors and azocoll, as substrate, were performed.

The main results obtained in these experiments were: (i) detection in the crop of two alkaline proteases having activity on gelatin, casein and fibrin and molecular weights of 45 and 66 KDA; (ii) presence in the hemolymph and fat body only of the protease with 66 KDA; (iii) crop protease of 45 KDA was inhibited by PMFS, a serine protease inhibitor; (iv) crop, hemolymph and fat body protease of 66 KDA were inhibited by ortho-phenanthroline, a metalloprotease inhibitor; and (v) both proteases were not inhibited by pepstatin, STI and elastatinol.

In conclusion, two proteases were identified in the crop (trypsin-like and metalloprotease) and one protease was detected in the hemolymph and fat body (metalloprotease). Further biochemical investigations on these enzymes and their functions for the insect are being now developed.

This work was supported by grants from CNPq, FINEP and PAPES.
VE-002
TRYPANOSOMA CRUZI AND RHODNIUS PROLIXUS INTERACTIONS: CROP LYtic AC-TIVITY

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Our laboratory has demonstrated an inducible lytic factor released in the crop of Rhodnius prolixus after feeding. The lytic activity may cause lysis of the erythrocyte membrane with consequent release of hemoglobin and may affect the infection of triatomine by parasites. In the present paper, we investigated, in vivo and in vitro experiments, the dynamic of development of three strain clones of Trypanosoma cruzi within the crop, and/or with crop content of fifth-instar larvae of Rhodnius prolixus at different intervals after feeding / infection. The main results obtained in these experiments were: (i) in vivo experiments demonstrated that Y strain was rapidly lysed in the crop in 3 days after feeding and Dm 28 and CL strain clone were more resistant that Y strain to the crop lysis; (ii) in vitro experiments (with only Y strain of T. cruzi) demonstrated that (a) 2 days crop content reduced significantly the number of parasites after 24 h incubation at 27°C when compared with controls and completely destroyed the parasites after 48 h incubation; (b) 4 days crop content slightly diminished the number of parasites after 24 h incubation and infection confirmed after 48 h incubation; and (c) 12 days crop content significantly increase the number of parasites 24 h and 48 h incubation.

These results clearly demonstrates that the crop lytic activity can provide a selective advantage for the growth or elimination of certain strains of T. cruzi over other strains in the triatomine vector. More widely investigations are been done in our laboratory to better understand the influence of the parasites lysis in the interaction T. cruzi and vector.

This work was supported by grants from the CNPq, Finap and Papes.

VE-003
A COMPARATIVE STUDY OF THE GUT AND HEMOLYMPH AGGLUTININS IN RHODNIUS PROLIXUS

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The activity of agglutinins found in the gut tissues and hemolymph of Rhodnius prolixus was tested using rabbit erythrocytes, Trypanosoma cruzi and Trypanosoma rangeli as test particles. In addition, investigations were made on the influence of parasitic infection and insect diet on the agglutination titers. A range of physico-chemical tests and carbohydrate binding studies were performed and inhibitors were subsequently found for both the crop agglutinin (p-nitrophenol derived sugars) and the hemolymph agglutinin (galactose-type sugars). As a result, affinity chromatography was utilized for an attempted purification of these agglutinins, and a one-step purification protocol for the R. prolixus hemolymph agglutinin has been developed. The crop agglutinin appears to be of a more complex nature, exhibiting, as yet, unresolved peculiarities, such as the inability to detected the agglutinin in presence of Trizma-based buffers, and the inhibition of the agglutinating activity by the non-carbohydrate compound p-nitrophenol. These results represent a starting point for future studies of lectin/parasite interaction in this reduviid-trypanosome model.

The work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação Oswaldo Cruz (PAPES project), Commission of the European Communities (grant n TS3-CT93-0226 to E.S.G and N.A.R.), The British Council (N.A.R.) and the Welcome Trust (N.A.R. and E.S.G.). ESG, NAR and PA are CNPq Senior Research Fellow.
VE-004
BIOACTIVITY OF LIGNANS AND NEOLIGNANS IN RHODNIUS PROLIXUS.

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Lignans and neolignans are widely distributed metabolites of angiosperms. Some representatives of this category of natural products were shown to possess antitumoral, antimutic and antiviral properties, and are known to inhibit the activity of enzymes and to show physiological effects in bacteria, fungi and insects. Structure-activity relationships were disclosed for some lignoids (MacRae & Towers, 1984).

We tested three lignans (pinoresinol, sesamoline, podophyllotoxin) and one neolignan (burchellin) analysed with respect to toxicity, phagoinhibition and growth disruption in 4th-instar nymphs of Rhodnius prolixus. Interference in the excretion and immune systems of the insects were also observed.

The compounds were tested in concentrations of 1, 10 and 100 ug/ml in blood. Anti-moulting activity for podophyllotoxin at 10 ug/ml and for burchellin at 100 ug/ml, corresponded respectively to 50% and to 82% of ecdyse inhibition.

Administration of podophyllotoxin and burchellin at 100 ug/ml in blood for 72 h resulted in only 3-4% loss of corporal weight and a production of 2-3 mg of urine, while the control group lost 17% of corporal weight and 18 mg of urine.

The insect lost 50% of haemocytes upon treatment with podophyllotoxin against the same proportion upon treatment with burchellin, respectively 5 and 7 days after feeding.

The results suggest the dependence of bioactivity of lignoid structure and confirm their powerful interference in the physiology of Rhodnius prolixus. (CAPES).

VE-005
PURIFICATION, CHARACTERIZATION AND LOCALIZATION OF A SIALIDASE IN TRIATOMA INFESTANS.

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We have previously shown that Triatoma infestans, the main vector of Trypanosoma cruzi in Brazil, has a sialidase in the midgut. The sialidase is responsible for the low levels of sialylation of epimastigote growth in the insect vector. To better understand the role of this enzyme and its importance for the development of T. cruzi, we have purified and characterized the enzyme of T. infestans and investigated whether the same activity is present in other T. cruzi vectors. T. infestans midguts were homogenized, the soluble proteins recovered after ultracentrifugation and sequential chromatographed in DEAE Sephacell and Mono S columns. The resulting fractions appeared as a single band of 15 kDa on SDS-PAGE after silver nitrate staining. The sialidase activity co-eluted with this 15 kDa component on Superdex-75 chromatography in a volume corresponding to a 29 kDa protein, suggesting that it is a dimer. The sialidase is probably synthesized by the salivary glands since high activity was found in the insect’s D-1 and D-2 glands. Moreover, the insect secretes sialidase as seen when force-feed with an agar plate containing a fluorescent substrate, methyl-umbelilferyl-N-acetyl-neuraminic acid. Differently from T. infestans, Rhodnius prolirxus does not have sialidase activity and there is no free sialic acid in the gut. As R. prolirxus is less infected by several strains of T. cruzi, it is possible that the immediate removal of sialic acid from the blood meal might be important for the parasite growth and differentiation in the vector. The absence of the sialidase in any other insect genera besides Triatoma raises questions about the enzyme origin and evolution in these organisms, and the establishment of T. cruzi parasitism.

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VE-006
INTERACTION BETWEEN HEMOCYTES AND CHRONIC CHAGASIC SERA SEEMS RESTRICTED TO VECTOR SPECIES


*T. infestans* hemocytes can be recognized by chagasic sera (CChS) (Mem Inst. Oswaldo Cruz, 89, suppl I, 1994). In order to know, if this recognition is related to vector species, hemocytes from different insect genus were studied: *Triatoma* (*T. pallidipennis* 5th instar nymphs); *Dipetalogaster* (*D. maximus* 5th instar nymphs) and *Diactor* (*D. bilineatus* nymphs and adults), a phytophagous bug commonly found in plants of genus *Passiflora* (maracocock).

Under optical microscopy, the main cell types were: prohemocytes (PR), plasmatocytes (PL) and granulocytes (GR). In the hemolymph from *D. maximus* and *D. bilineatus* few PR were observed, whereas PL were found in great number in the *D. maximus* hemolymph. Fixed hemocytes were allowed to react with CChS (n=14) and the interaction evaluated by indirect immunofluorescence using fluorescein labelled anti human globulin (H+L). Sera (n=7) from healthy individuals were included as controls. High reactivity has been observed, when CChS interacted with hemocytes from insects involved in the epidemiological chain of Chagas' disease. Cells from genus *Triatoma* were the most reactive, while cells from *D. maximus* were less reactive. In contrast, the phytophagous hemocytes gave very low or no reactivity with the same sera. Also, hemocytes from different genera showed very low or no reactivity with nonchagasic sera. Titters of CChS expressed in geometric mean (log 10) were as follows:

<table>
<thead>
<tr>
<th>species</th>
<th>PR</th>
<th>PL</th>
<th>GR</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. infestans</em></td>
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<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td><em>T. pallidipennis</em></td>
<td>1.3</td>
<td>1.9</td>
<td>2.0</td>
</tr>
<tr>
<td><em>D. maximus</em></td>
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<td>1.5</td>
<td>1.7</td>
</tr>
<tr>
<td><em>D. bilineatus</em></td>
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<td>0.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

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VE-007
CARBOHYDRATES EPITOPE FROM HEMOCYTES OF UNINFECTED *T. INFESTANS* ARE RECOGNIZED BY CHAGASIC SERA

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Hemocytes from uninfected *T. infestans* were recognized by chronic chagasic sera[CChS] (Mem Inst.Oswaldo Cruz, 89,suppl I,1994). In order to characterize epitopes implicated in this recognition, hemocytes from uninfected 5th instar nymphs of *T. infestans* were treated with 10mM sodium metaperiodate and allowed to react with 17 CChS in the indirect immunofluorescence (IFA) using fluorescein labelled anti-human gamma globulin(H+L). Reduction process diminished intensity and reactivity of cells fluorescent with serum antibodies compared with those of non treated hemocytes using the same sera. The geometric mean titters (log10)[GMT] against treated cells were: 0.7 for prohemocytes(PR), 0.9 for plasmatocytes (PL) and 1.5 for granulocytes (GR), whereas the GMT against non treated hemocytes were: 1.2 for PR, 1.7 for PL and 1.9 for GR. These data show that carbohydrates are one of the components recognized by CChS. Assaying hemocytes with fluorescein labelled WGA lectin, a strong positive reaction was seen demonstrating the presence of N-acetyl-d-glucosamine in the surface of hemocytes. In fact, when hemocytes were submitted to treatment with a 1:1000 neuraminidase from *V. cholerae* and tested against the same sera, the fluorescence intensity diminished, particularly the PR and PL. More studies are needed to verify if other carbohydrates components of hemocytes are recognized by CChS.

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VE-008
THE INFLUENCE OF THE ALIMENTARY DIET ON THE DEVELOPMENT OF RHODNIUS PICTIPES STAL, 1876 (HEMIPTERA, REDUVIIDAE, TRIATOMINAE)

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The biology of Triatomines in laboratory has been studied by several authors, but we have seen that the silvatic species were less studied than those which are found in the dwellings. All the species which nowadays lives in endemic area are the responsible for the transmission of Chagas' disease, but in the past they were considered as a silvatic one. Although R. picipes has silvatic habits, it was found in artificialbiotypes, showing a tendency of domiciliation (Silva et al. 1992, An Soc Ent Brasil 21: 139-154). This species has been found naturally infected with Trypanosoma cruzi, with T. rangeli or by both (Otero et al.1976, Bol Dir Malarial y San Amb 16: 163-168). The alimentary diet (quantity and quality) has an important influence on the biology of the species under laboratory conditions. Several authors have shown the epidemiological importance of R. picipes because of its vectorial capacity. According to Rocha et al., 1994 (Mem. Inst. Oswaldo Cruz, 89: 265-270) during all the life cycle R. picipes realized 35% of defecation immediately after the blood meal, being 52.5% on the blood source and sometimes nearly the place of the bite increasing the possibilities of infection when the contact with the susceptible way happen. Sixty nymphs of 5th instar were isolated and after the imaginal moult 3 groups with 3 couples were kept separately. From its oviposition 3 colonies were set up, and to each one was stablished a source of blood- meal: group 1, pigeon (Columba livia); group 2, mice (Mus musculus) and group 3 both alternatively. The insects are being kept in flasks with 20 cm of high and 20 cm of diameter, in a BOD chamber (28°C +/- 1°C and 80 +/- 5% of U.R.). Up to this time only the results concerning to the 1st and 2nd instars of the 3 groups are concluded. In the group 1, the 1st instar showed a high number of moult: 78.2% and less mortality (21.8%), the same was not observed to the 2nd instar. In the group 2, the 2nd instar has less mortality (13.5%) and a high number of moult: 86.5%. The results of group 3 were intermediary. All groups wen started with 110 eggs.

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VE-009
RESISTANCE TO STARVATION OF TRIATOMA NITIDA USINGER, 1939 IN LABORATORY (HEMIPTERA, REDUVIIDAE, TRIATOMINAE)

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Triatoma nitida Usinger, 1939 is a silvatic species found occasionally in dwellings at Costa Rica, Guatemala, Honduras and Mexico. The taxonomic and morphological aspects were studied by Lent & Wygodzinsky, 1979 (Bull Mus Nat Hist.163:123,520) and Lent & Jurberg, 1992 (Mem Inst Oswaldo Cruz, 87:123-130), however the biology, natural hosts and habitats are poorly know. Some aspects of its biology were studied by Galvão et al.,1995(Mem Inst Oswaldo Cruz, 90(5)). In this paper it was observed the resistance to starvation in laboratory conditions (28 +/- 1°C, 80 +/- 5% of U.R. and photophase of 12 hours) A group of 50 eggs and 50 nymphs unfed of each instar (except on the 5th instar which were selected 60 specimens) were observed. The insects were grouped by the instars and submitted to starvation for 15 days. At the end of this period they were fed in pigeon (Columba livia) for 5 hours. The aim of this conduct was standardize the amount of blood swallowed and the ecdisis. After the hatching or moultling, 30 specimens of each instar, 15 males and 15 females were kept, separately, in Borrel flasks properly listed. After this, they were kept in starvation and observed daily until death. The results showed that 1st and 2nd instars resisted for approximately 2 months (X=56,3 and 63 days respectively); the 3rd instar resisted for more than 3 months (X=102,5 days) and the 4th instar were the most resistant (X=158 days). This period decreased on the 5th instar (X=114,3 days). The adults survived for a period similar to the one observed to 1st and 2nd instars and the females were a little bit more resistant than the males (X=58,6 and 66 days for males and females, respectively).

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VE-010

ARTIFICIAL FEEDING OF RHODNIUS PICTIPES STAL,1872 THROUGH SILICONE MEMBRANE (HEMIPTERA, REDUVIDAE, TRIATOMINAE)

Rocha,D.S. & Fonseca,A.H.*

The laboratory maintenance of a nucleus of haematoxagus insects is of great biological importance especially in areas of insecticide testing, vector/agent interaction and methods of rearing. It has incited many authors to search for artificial methods for such insects as an alternative to the utilization of laboratory animals. Artificial feeding has the objective of supplying the nutritional needs of these insects with physical space economy, low maintenance cost and personal usage. Rhodnius pictipes Stal,1872 is a wild species with ample distribution in South America. It is found in the Brazilian states of Acre, Amazonas, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará and Tocantins (Castro-Filho & Silveira,1979; Lent & Wygodzinsky,1979; Silveira et al.,1984; Brazil et al, 1985 e Silva & Silva,1990). According to Rocha et al. (1994), this species demonstrates great avidity in its search for blood source, low mortality rate, and an elevated defecation frequency during or soon after feeding. These attributes demonstrate its great potential vectorial. Fifty nymphs were randomly selected from each instar along with one hundred and fifty adults that were segregated in the following manner: a) 50 nymphs of each instar; b) 50 males; c) 50 females in each group. Each group was placed in a glass flask with a height of 8 cm and a diameter of 3 cm. The insects were maintained in an incubator (B.O.D. at 28+/-1oC and 80+/-5% R.U., while being fed artificially with defibrinated sheep blood, employing a silicone membrane, in accordance with that described by Wetzel (1979) and adapted by Butler et al. (1984). The same methodology was employed for the control group with the exception of blood feeding source (conventional way, using pigeons). Up to date, the following results were obtained: a) 16 nymphs (3rd instar) moultng after 2 feedings; b) 6 nymphs (3rd instar) moultng after 3 feedings; c) 3 nymphs (3rd instar) moultng after 4 feedings. In relation to the volume of blood ingested by these third-instar nymphs the following results were observed: 1st feeding X= 0,0293g N= 25; 2nd feeding X= 0,0034g N= 25; 3rd feeding X= 0,0010g N= 09; 4th feeding X= 0,0026g N= 03. These results clearly demonstrate the ready acceptancy of defibrinated sheep blood by this species and engangement with the employment of the silicone membrane.

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VE-011

TRIATOMA INFESTANS FEEDING FREQUENCY IN NATURE: COMPARISON BETWEEN DOMESTIC AND PERIDOMESTIC HABITATS.

Catalá, Silvia; Crocco,Liliana, Lopez, Ana y Morales, Gustavo.

The number of bites produced by populations of Triatoma infestans in nature had not been determined. A simple method to estimate the biting rate of triatomine was recently used to demonstrate seasonal changes in the host-contact rate of Triatoma infestans living in experimental chicken and guinea pig houses. The same method was used in this work to compare the feeding frequency of this species in human houses and chicken nests. The project was carried out in Atamisqui (Province of Santiago del Estero) during the hot season. Domestic populations from 25 houses were collected after knock down with fumigant canisters. Eight chicken nest placed on the peridomestic area were dismantled and the triatomines collected. Bugs from both habitats were immediately frozen at 4°C until their analysis in the laboratory. The insects were counted, weighed and the colour of their rectal material was recorded in order to identify the recently feeding individuals.

Results: Estimation of the biting frequency using the colourless urine as indicator, revealed that 30 percent of Triatoma infestans from human houses took a meal every night. The chicken nests populations showed 20% of insects fed the last night. These differences were significant (p<0.05). Nymphs were responsible for 16.28 % of the bites in domiciles and 18.37 % in chicken nests. On the other hand the adults were responsible for 13.9 % of the bites in domiciles and 2.1% in chicken houses.

Triatomine population density is closely associated with the number of fed insects. Correlation analysis between these variables showed a linear coefficient r= 0.91 with a probability of 0.00001. Residuals are bigger at high densities.

The analysis of the weights of recently fed bugs showed significant differences between the insects from human houses and chicken nests. Nymphs and females from chicken houses were heavier than those in human houses. Males did not differ among habitats.

Conclusions: These results suggest that Triatoma infestans take larger meals in chicken nests than in human houses. Differences on host irritability could be responsible for this differences.

This project is carried out with grants from WHO/TDR/AFR (940213), CONICOR and SECYT UNC.
VE-012
NUTRITIONAL AND GONADOTROPHIC STATUS OF DOMESTIC AND PERIDOMESTIC POPULATIONS OF TRIATOMA INFESTANS.

Catalá, Silvia; Crocco, Liliana, Lopez, Ana y Morales, Gustavo.

Temperature and host irritability are the main factors that could influence the nutritional and gonadotrophic status of Triatominae populations. Scarcely research has been carried out on this topic using populations in nature. The objective of this work was to compare the nutritional and gonadotrophic status of T. infestans populations developed in two different habitats: the human domiciles and the chicken nests from the peridomestic area.

Materials and methods: The project was carried out on Atamasquí (Prov. Santiago del Estero) during the month of October, December (1994) and February (1995). Twenty five houses were treated with fumigant canisters and the knocked down insects were collected and kept at 4°C. Seven chicken houses located at different peridomiciles were dismantled and the triatomines collected and kept at 4°C. The fourth and fifth instar nymphs and adults from houses (H) and chicken nests (C) were weighed in the laboratory and a sample of the females were dissected (H: n=27 y C: n=59) in order to analyze the ovaries and the spermatheca with a light microscope. The number of chorionated eggs within each female were recorded.

Results: 1. Nutritional status. Fourth and fifth instar nymphs displayed a bimodal distribution of weights showing poor and well fed insects. The proportion of poorly fed nymphs was significantly larger in domestic populations. The mean weight of females, males and nymphs was larger in chicken nests populations.

2. Gonadotrophic status. Fifty to sixty percent of the females on both habitats were mated. The number of chorionated eggs in the reproductive tract differs in domestic and peridomestic females. A mean of 16 eggs/female were found in females collected within chicken nests. Females from human houses showed only 9 eggs/female.

Conclusions: Triatoma infestans populations from chicken nests showed a better nutritional status than those from human houses. According with these results, the female gonadotrophic status were also higher in chicken houses. These findings suggest that chicken houses, being very frequent near the human domicile, could act as a productive breeding site for triatomines and a risky focus to human houses.

This project is carried out with grants from WHO/TDR/AFR (940213), CONICOR and SECYT UNC.

VE-013
INFLUENCE OF THE NOURISH DIET IN THE RESISTANCE TO STARVATION OF CIMEX HEMIPTERUS (FABRICIUS, 1803) (HEMIPTERA:CIMICIDAЕ:CIMICINAE)

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The cimicids causes discomfort when present in the dwelling. Recently, the increase in the incidence of Cimex hemipterus in the suburb of Rio de Janeiro city is correlated with the poor conditions of population hygiene. However, rare foci have been found in area with good conditions of hygiene. The susceptibility of this species to different parasites has been carefully studied in laboratory conditions. Zeledón e col (1965) have observed the experimental infection with Trypanosoma cruzi and Trypanosoma rangeli. A review of the cimicids was done by Forattini (1990), and it was observed that there are few studies about the biology if this species. A study was carried out with five hundred eggs obtained from a colony maintained during two years in the laboratory. After the eclosion, the nymphs were kept in gelatine capsule properly listed. The blood-meal of 250 nymphs was performed in bird, and the other 250 nymphs in mice. The observation for each stage (2nd -adult) was done with 50 nymphs, and as soon as they reached the intend stage they were not feed anymore. The starvation was evaluated in two different ways: the time-lapse in days between the last meal/death (lm/d) and between moult/death (m/d). Both source of nourishment and parameters observed presents similar results to 2nd - 5th instars. No difference was observed in the 2nd instar. The 3rd, 4th and 5th instars were more resistant when fed in bird (X lm/d= 25, 32 and 38 days; X m/d= 19, 28 and 34 days), instead of mice (X lm/d= 22, 30 and 34; X m/d= 16, 24 and 28). The adult stage was more resistant when fed in mice (X lm/d= 25; X m/d= 18), instead of bird (X lm/d= 22 days; X m/d= 16 days).

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VE-014
MAINTENANCE OF FECUNDITY IN TRIATOMA INFESTANS AFTER DECADES OF BREEDING UNDER LABORATORY CONDITIONS

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The aim of this study was to evaluate the possibility of maintenance of fecundity in reduviid bugs after decades of breeding under laboratory conditions. We observed the performance of eleven strains of *Triatoma infestans* from the Laboratory of Triatomines of Adolfo Lutz Institute. Insects were mated in different ratio of males and females. Two rhythms of almentation were used. In the first, triatomines were fed weekly, and in the second rhythm the meal were offered every fifteen of twenty one days. During all the study teals were used as blood source, and temperature and humidity were constant, at 27-28°C and 70-80%, respectively. The results confirm the importance of the rhythm of almentation for oviposition. Insects feed weekly showed the best rates of oviposition, represented by the number of eggs by female per day (efd), between 0.39 to 4.40 efd, being more frequent up to 1.30 efd. Colonies feed every fifteen or twenty one days showed inexpressives rates of oviposition, between 0.05 to 1.07 efd, being more frequent below to 0.30 efd.

We concluded that after decades of breeding under laboratory conditions it is possible to maintain the fecundity, into normal patterns, in the *T. infestans* if the rhythm of almentation of adults don’t exceed seven days. Rhythm of almentation don’t affected the survival rates of adults, but it is critical for the fecundity and oviposition.

VE-015
INFECTION BY THE TRYPANOSOMA CRUZI AND FEEDING SOURCES OF TRIATOMA SORDIDA IN THE COUNTY OF PORTEIRINHA, MG.

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As *T.infestans*’ control can be accomplished by the application of insecticides, *T.sordida* appears as one of the species of greater epidemiologic importance due to its wide dispersion and high peridomiciliary infestation, being the triatomine species more captured in Brazil nowadays. Two searches for triatomine infestation in the peridomicile and intradomicile were made in 12 localities of the county of Porteirinha, MG, one the first in October 1993 and the second in April 1994. The infestation indexes in the first and in the second searches were, respectively, 35.0% and 32.7%. The total of triatomines captured was 1178 *T.sordida* with an index of infection by *T.cruzi* of 3.1%. The majority (97.5%) of the triatomines was found in the peridomicile and only 2.5% in the intradomicile. The stomach content was collected from 612 triatomines in order to study their feeding source by the Precipitin Reaction (PR). Rabbit anti-serum against serum proteins from man, cat, dog, opossum, bird, rodent, goat and pig were used. The triatomines presented one (93.6%), two (6.2%) and three (0.2%) feeding sources. Twenty one percent of them didn’t have enough material for the PR. In 422 stomach contents tested the feeding sources were 87.4% bird, 4.6% dog, 3.9% rodent, 3.7% pig, 0.6% opossum, 0.4% man, 0.2% cat. None of the triatomines had goat as feeding source. Sixteen samples of *T.cruzi* isolated from *T.sordida* captured in the peridomicile (chicken yard and pigsty) and two samples isolated from dogs were characterized by the enzymes ALAT, ASAT, PGM, GPI, ME and G6PD. Two variants of the Z1 zymodeme were observed. Twelve *T.cruzi* samples isolated from triatomines and one from dog presented a Z1 pattern for the enzymes ALAT, ASAT, GPI, ME and PGM, and a ZC pattern for G6PD, named Z1-1. *Four T.cruzi* samples isolated from triatomines and one from dog presented a Z1 pattern for the enzymes ASAT, GPI, ME and PGM; ZC pattern for G6PD and ZB pattern for ALAT, named Z1-2. The feeding sources of these 16 triatomines were bird, pig and rodent with 6 (37.5%) presenting not enough material for the PR. The results show two isoenzymatically distinct populations of *T.cruzi* Z1 zymodeme circulating in the peridomiciome between *T.sordida* and dogs. It should be emphasized that in Brazil the populations of *T.cruzi* Z1 zymodeme have been found almost exclusively in sylvatic and peridomestic environments. An important fact is the very low participation of man as feeding source of *T.sordida*, reducing the possibility of vectorial transmission of the human Chagas disease in the region. However, we should consider its capacity of maintaining the transmission of *T.cruzi* in the peridomiciome strengthening the importance of continuing the Chagas Disease Control Program to avoid the intradomiciome infestation by those triatomines.

Supported by: FNS/CNPq/CPqRR-FIOCRUZ/FAPEMIG
VE-016

KINETICS OF BLOOD MEAL INTAKE BY TRIATOMA INFESTANS AND RHODNIUS PROLIXUS FROM NON ANAESTHETIZED MICE

PEREIRA, M.H.1,2, PENIDO, C.M.3, MARTINS, M.S.2 & DIOTAIUTI, L.2,3
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The life cycle and population dynamics of bugs depend critically on their interactions with vertebrate hosts. Experiments with laboratory animals and human volunteers, demonstrate a direct correlation between irritation of the host and increase in number of bugs that feed. Consequently, the average amount of blood ingested by the bugs in non anesthetized hosts is inversely proportional to bug density. The density-dependent regulation of blood intake size seems to play a major role in regulating the size of bug population. However, the rate of reduction on the size of the blood meal intake with increase in density depends on triatominae species, which is probably related with intensity of irritation that each species provokes in the host during the process of feeding. The aim of the present work is to attempt a better understanding of the interactions of T. infestans and R. prolixus with vertebrate hosts, using non anaesthetized mice, attempting to simulate natural conditions.

For the experiments third-stage nymphs obtained from second-stage nymphs fed on Chickens and stained for 21 days were used. Each group of 15 nymphs of T. infestans or R. prolixus were placed separately in cages (25.0 x 17.5 x 9.5 cm) to feed on non anaesthetized mice (NAM) (for ) 2, 4 and 8 hours, at 26-27°C, light) or on anaesthetized mice (control group for 1 hour, 26-27°C, light). Each cage contained 3 swiss mice (20-25g) laid on a perforated wooden platform (24.0 X 17.5 X 3.5cm) fitted over each cage so that triatomines were able to pass freely through the holes. Insect weight was recorded before, immediately after and 24 hours after each experiment done in triplicate.

The average increase in body weight of third-stage nymphs of T. infestans versus time of contact with NAM showed a significant increase at the intervals of time studied (t Test, p<0,05), at 4 hours time interval it was similar in experimental and control groups. At 8 hours interval the average weight increase with NAM was significantly higher than in control group (t Test, p<0,05). The average increase in body weight of third-stage nymphs of R. prolixus was progressive, but no significant difference between 4 and 8 hours intervals was observed (t Test, p>0,05) and even after 8 hours of contact with NAM the average weight increase of R. prolixus nymphs was significantly lower than that recorded for control group (t Test, p<0,05). The average weight increase in experiments with NAM in comparison with control group after 8 hours of contact was 142,0 % for T. infestans and 64,2 % for R. prolixus. The weight increase for both species versus time of contact with NAM was due to successive small blood meals. The number of nymphs deaths and of nymphs that did not feed after 2 hours of contact with NAM was higher for R. prolixus. Such results demonstrate the better exploitation of blood source of T. infestans probably because it caused less irritation of the mice during the process of feeding.

Support by: CNPq, FAPEMIG and CPRR(FioCruz).

VE-017

THE EFFECTS OF STARVATION ON THE FAT BODY AND HEMOLYMPHATIC COMPONENTS IN TRIATOMA INFESTANS AND DIPETALOGASTER MAXIMUS (HEMIPTERA: REDUVIIDAE)

Canavoso, L.E.; Touz, M.C.; Cano, R.C. & Rubiolo, E.R.

Fat body in insects combines many functions and properties of vertebrate’s adipose tissue and liver, developing a fundamental function in the energetic metabolism being a storage site for glycogen and lipids. In vectors of Trypanosoma cruzi, the biochemical and physiological aspects associated with the flying are not well known (Canavoso et al. Mem.Inst.Oswaldo Cruz 89, 233, 1994), although the purpose of establishing efficient starving conditions for the high density lipophorin (HDLp) interconversion in other of low density (LDLp), it has been performed measuring variations of wet weight, total lipids, acylglycerols and glycogen in the fat body as well as the hemolymphatic levels of total proteins and HDLp in two starred species of triatomine bugs: Triatoma infestans and Dipetalogaster maximus. The two male adult insects were fed, weighted and then, separated in 8-10 insects groups and putted to starving for 10, 15, 20, 25 and 30 days post-feeding. The fat bodies were removed, washed, dried and weighted with 0.01 mg precision with the previous hemolymph collection. The lipids and glycogen were extracted determining total lipids, acylglycerols and glycogen in tissue. At the same time, in the hemolymph were determined total proteins and HDLp. In two species, the higher weight of fat bodies were obtained at 10-15 days post-feeding, being in between 1.5 and 3.2 times higher to the one showed on 30 day. The amount of total lipids represented at the 15 day in between 14 and 24% of the organ wet weight and decrease at the 30 day (p < 0.001 15 day vs 30 day). The acylglycerols reached up to the point of 85% the amount of total lipids from fat body the day 30. Additionally, the glycogen content reached to the top the day 20. During the starvation was observed a relative increase of HDLp levels with respect to the total protein levels. These results showed that the decrease of total lipids and glycogen observed in fat body as well as the relative increase of acylglycerols and HDLp amount could be one of the «trigger» of insect fly to look for food before they run out the energy and/or colonization of new ecotopes.

VE-018
HEMOLYMPHATIC MODIFICATIONS IN VECTORS OF CHAGAS DISEASE INFECTED WITH TRYPANOSOMA CRUZI STRAINS.

Cano, R.C.; Canavuso, L.E., Stariolo, R., Touz, M.C. & Rubiolo, E.R.

The interaction between the invertebrate host- *T. cruzi* has been scarcely studied. This may be due to the fact that the *T. cruzi* was considered as a non-pathogen organism for its vector, being recently reclassified as subpathogen (Parasitol. Today 10: 463, 1994). On the other hand, several macromolecules of the insect hemolymph among them, lectins and the pro-phenol oxidase system (PPO) has been cited as its defense mechanisms. With the aim to improve the known about the interaction vector- *T. cruzi*, we studied the proteic pattern and the activation of the PPO system in the hemolymph from 2 vectors (Flia. Reduviiidae) infected by digestive via with *T. cruzi* strains. For the experimental design were employed fifth instars (NV) and adult males (Ad o) of *Triatoma infestans* and *Rhodnius prolixus* as well as the X-1 and Tulahuén (Tu) *T. cruzi* strains. The infection of the insects was carried out by feeding on BALB/c mice with high levels of parasitemia. The control ones were fed on BALB/c mice without *T. cruzi* infection. The dose of parasites ingested was estimated by differential weighted of the insects pre and post feeding (p.f.). The hemolymph was obtained at different times and collected in appropriate conditions for evaluation of the proteic pattern and the PPO activity. The proteic pattern was evaluated on SDS-PAGE and the PPO activity measured in microplates using L-DOPA as substrate. The pattern of the hemolymphatic proteins of NV of *R. prolixus* infected with Tu showed quantitative modifications in the cathodic and anodic regions with the appearance of a band in the middle region more important at the 7 respect than the 14 and 21 days p.f. The proteic pattern corresponding to Ad o at 7 days p.f. showed quantitative changes in the cathodic region and mainly qualitative in the anodic one. The modifications observed at posterior times were only quantitatives. The changes observed in the pattern of proteins from NV and Ad o of *T. infestans* infected with X-1 at 7 days p.f., were characterized by quantitative modifications of the fractions in the middle and anodic regions, with appearance of new bands and quantitative modifications at posterior times. The PPO system showed a spontaneous activation in both stages of *T. infestans* infected with Tu from 5 days p.f.; whereas no changes were observed in *R. prolixus* at least at the studied times. * *The hemolymphatic modifications observed in the proteic pattern of NV and Ad o of both vectors as well as in the PPO system of both stages of *T. infestans*, suggest us that these modifications would be a consequence of the invertebrate host-parasite interaction. ** The differences in the activation of the PPO system in both vectors infected with Tu may be an expression of the metabolic characteristics of these species of vectors.

Supported by SECyT. U.N.Córdoba. Argentina.

VE-019
PERSISTENCE OF ENTEROBACTER CLOACAE IN RHODNIUS PROLIXUS


The present paper aims to present the study of the colonization of digestive tract of *Rhodnius prolixus* maintained in artificial conditions through the disinfection of eggs and feeding nymphs with a membrane feeder apparatus containing desfibrinated sheep blood and Enterobacter cloacae (Nal r, Sor +). Based on the results with the bacteria isolation from triatomines, phenotypes marks were searched in the most frequently bacteria. We had a mutant *E. cloacae* (Nal r, Sor +) isolated from *Triatoma vitriceps* collected in the State of Rio de Janeiro, Brasil. This attempt was not successful and the reisolation of bacteria was not obtained in neither of the nymphs instar. The digestive tract of *R. prolixus* showed to be inadequate to the colonization of this bacteria. However, when we compared the development of the triatomines feed with desfibrinated sheep blood, desfibrinated sheep blood plus BHI broth (Brain-Hearth-Infusion) and desfibrinated sheep blood with *E. cloacae* mutant, we observed that the second and the third groups developed faster.
VE-020
STUDIES OF RHODNIUS NEGLECTUS POPULATIONS FROM DIFFERENT REGIONS ON THE PARAMETERS ISOZYMENIC, MORMHOMETRY AND GENITALIA.

1,3 Barbosa, SE, 1,3 Soares, RPP, 2Santos, ARG, 4Dujardin, JP, 5Schofield, CI, 2 Siqueira, AM & 1,3 Diotauiuti, L.
1Dep. Parasitologia, ICB, UFMG, CP 486, CEP 31.270-901; 2Dep. Bioquimica e Imunologia, ICB, UFMG, Belo Horizonte, MG, Brazil; 3Centro Pesquisas René Rachou, Belo Horizonte, MG, Brazil; 4Instituto Boliviano de Biologia de Altura, La Paz, Bolivia; 5 London School of Tropical Medicine and Hygiene, London, United Kingdom.

Among the triatomines considered secondary in the epidemiology of Chagas disease, Rhodnius neglectus is widely spread in the natural ecotopes, being frequently captured in the artificial environments, especially peridomiciliary, rarely producing colonies indoors. Nevertheless, its intradomiciliation was unquestionably demonstrated in some areas of Goiás state, Brazil. In order to find possible variations that could justify these different behaviors, we have studied wild insects from Jaraguá, Goiás state, where the intradomiciliation has been observed. These insects were compared to wild insects from Uberaba, Minas Gerais state, Brazil, where they are often found in peridomiciliary areas. Since the specie was described from exemplaries captured in this region, we considered the electrophoresis of their isozenymes as “type”. The running electrophoresis were made in starch gel allowing the study of 14 loci concerning to the following enzymes: ALAT, ASAT, G6PD, GPI, ICD, HK, MDH, ME, MPI, PEP-1, PEP-2, PK, 6PGD and PGM. It was not possible to visualize the G6PD, 6PGD and PEP-1 profiles in any experiment. For the other enzymes it was not found any difference between the populations. Male genitalia and morphology were also analyzed. In the former were observed the following structures: parameres, median pygophore process, phallus, phallopsoma, phallopsoma support, phallopsoma process, endosoma process and vesica; in the latter were measured total length, pronotum length, pronotum+escutenum length, antecocular distance, wide across eyes, postocular distance and head length. Although male genitalia did not show any difference, morphology presented significant estatistical differences. Nevertheless, other parameters should be studied to access characteristics that should explain the different domiciliary potentials of Rhodnius neglectus.

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VE-021
COMPARATIVE STUDIES OF DISTINCT POPULATIONS OF TRIATOMA INFESTANS. IV. KARYOTYPE.

Pires, H.H.R.1, Pérez, R.2, Diotauiuti, L.1,2 & Panzera, F.1
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T. infestans has 10 pairs of autosomes and one pair of sexual chromosomes (2n=22), besides presenting a heteropycnose phenomenon in some of its autosomes (Ueshima, 1966; Vaiol et al., 1985). It presents heteropycnotic chromcenters formed by C heterochromatine blocks easily visualized in the Y chromosome and in three big pairs of autosomes (Solari, 1979). Panzera et al. (1992) and Pérez et al. (1991), while studying distinct populations of T. infestans, demonstrated that there are differences in the distribution patterns of the C block in the pairs of autosomes, observing 5 different karyotypes, characterized according to the presence, absence, quantity of blocks and distribution of C heterochromatine. With the purpose of better evaluating the effects of the isolation imposed to the colonies of triatomines maintained in insectary, two colonies of T. infestans were studied. One of them has been maintained in the insectary for more than 10 years and the other was recently introduced (F3), derived from Bolivia (Cochabamba).

The cytogenetic studies are summarized in the table:

<table>
<thead>
<tr>
<th></th>
<th>Brazilian Colony</th>
<th>Bolivian Colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomes with C block</td>
<td>Variable, of 2 to 3 pairs</td>
<td>Variable, of 5 to 8 pairs</td>
</tr>
<tr>
<td>Quantity of heterochromatine</td>
<td>23-30% of the total of chromatine</td>
<td>40-50% of the total of chromatine</td>
</tr>
<tr>
<td>Quantity of DNA</td>
<td>1.03 ± 0.13 pg</td>
<td>1.63 ± 0.2 pg</td>
</tr>
</tbody>
</table>

Comparing these results with those observed by other authors, which include populations from several other countries, we verified that Bolivia is the area where T. infestans presents the greatest number of autosomes with C heterochromatine blocks decreasing while the populations moved away from this region. The Brazilian population is the one that presents the smallest quantity of C heterochromatine blocks. Likewise, the quantity of DNA is also decreased. These results must be evaluated at the light of the experiments about the biology of these colonies, with the expectation of correlating them with possible behavioral variations.

SUPPORTED BY FAPEMIG
**VE-022**

**COMPARATIVE STUDIES OF DISTINCT POPULATIONS OF TRIATOMA INFESTANS. V. MORPHOLOGICAL PATTERNS OF THE MALE GENITALIA**

Pires, H.H.R.1, Barbosa, S.H.1,2, Jurberg, J.3 & Diotaianì, L.1,2
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The triatomine's biosystematic has a great importance in the determination of different populations and the possible correlation between phenotypical and behavioral differences. Variations in the morphology of the male genitalia of *Triatoma infestans* have already been described (Lent & Jurberg, 1985), without an evaluation of the frequency that they occur in different populations. In the present work, the results related to the study of both of the populations of this species, one constituted by insects from several regions of the state of Minas Gerais, Brazil and maintained in an insectary for more than 10 years and the other from Cochabamba, Bolivia, being in the third generation (30 genitalia for each population). Variations in the endosoma process and in the phallosoma support, compatible wider to the patterns described by Lent & Jurberg (1985), were observed in the structures analyzed. The population of the insectary presented 29 (96.7%) insects with thorns on the apex of the endosoma process and one insect without any thorn. The Bolivian population presented 16 (53.3%) insects with thorns in the endosoma process and 14 (44.7%) insects without thorns. According to the phallosoma support it was observed that 29 insects (96.7%) of the population of the insectary presented an open apex. From these, 2 (6.6%) insects presented a conjugation membrane between both of the extremities. The population which was recently introduced in the insectary also presented 29 (96.7%) insects with the an open apex of the phallosoma support, from which 7 (19.8%) presented conjugation membrane. In each one of the species, one insect presented a closed apex of the phallosoma support. While counting the thorns on the apex of the endosoma process, it was observed that the population maintained in the insectary presented 10 insects with more than 30 thorns, 17 presented from 11 to 30 thorns, two with less than 11 thorns and one with no thorn. The Bolivian colony did not present any insect with more than 30 thorns, four insects with 11 to 30 thorns, 12 with less than 11 thorns and 14 without thorns. A significant difference was observed between both species as for the morphological profiles of the endosoma process, and which was not verified for the phallosoma support. No association was observed between the profiles of the phallosoma support and the presence or absence variable, and neither with the number of thorns. The crossing among insects from the two colonies was successful, showing that these differences do not represent a reproductive isolation.

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**VE-023**

**RELATIONSHIP BETWEEN NUMBER OF NEURONES ASSOCIATED WITH ANTENNAL CHEMORECEPTORS AND HABITAT SELECTION IN TRIATOMINAE.**

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Antennal chemoreceptors are involved in the reception of stimuli that allow the insect to display the fitted behaviour to find meals, couple and habitat. It was postulated that the number of neurons associated to chemoreceptors (NACH) in haematophagous insects is larger in species with high capacity to detect the host or to use different habitats. The aim of this work was to determine the number of neurons associated to chemoreceptors in four species of Triatominae and to analyze this number as function of the ecologic characteristics of each species.

Fifth instar nymphs and adults of two species of the genus *Triatoma* (*T. sordida* and *T. infestans*) and two species of *Rhodnius* (*R. prolixus* and *R. neglectus*) were used. The antennae were fixed, dehydrated and included in araldite. Thin sections of 2 um stained with Giemsa or ATO, allowed counting the number of neurons associated with each of the three types of chemoreceptors: basicinoc(B), uniporous (U) and multiporous(M). It was determined that the four species have the same number of neurons associated to each type of sensillum. The basicinoc has 3-4 neurons, the multiporous 10-11 and the uniporous, 4 neurons.

The total number of each sensillum on the antennae, were counted on whole cleared mounts and then, multiplied by the number of neurons on each type of sensillum. The total number of neurons belonging to basicinoc, uniporous and multiporous were obtained. Nymphs and adults differ greatly on number of NACH. More no than 3000 neurons are present in the fifth instar antennae while in adults it ranges between 7900 and 16700 neurons. This means that the number of NACH increase 3 to 7.8 times when the insect moult from fifth stage nymph to adult. This raise is particularly produced by the augment of multiporous sensilla.

The adults of the two species of the genus *Rhodnius* have less NACH than the two species of the genus *Triatoma*. *Triatoma sordida* has the largest number of NACH: (16700). A regression analysis between the length of the antennae (all species) and the number of NACH demonstrated no correlation (r=0.41; p=0.128) among variables. The number of neurons associated to uniporous sensilla was larger than those associated to the others chemoreceptors. However, the number of neurons associated to multiporous sensillum of *Triatoma sordida* was as large as those on uniporous sensilla.

A literature review of the ecological characteristics of the four species showed that *T. sordida* has a wider range of habitats than *Rhodnius prolixus*, *R. neglectus* and *T. infestans*. Regression analysis between number of habitats visited by each species and number of NACH was significative (r=0.97; p=0.02) and the association among variables followed a reciprocal function. The larger the number of NACH the wider the range of habitats a species live. This results suggest a relationship between the ability to use several habitats and the sensorial pattern on Triatominae bugs.

This project was carried out with grants from CONICOR and SECYT UNC.
VE-024

OCCURRENCE OF RHODNIUS PROLIXUS STAL, 1859 (HEMIPTERA:REDUVIIDAE) IN ATLANTIC RAIN FOREST - TERESÓPOLIS DISTRICT - RIO DE JANEIRO STATE, BRASIL.

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Rhodnius prolixus is a very important vector of Trypanosoma cruzi in Colombia, Venezuela and Suriname where it is well adapted to human dwellings. This species was also reported in other 11 countries in the north of South America. In Brasil R. prolixus occurs basically in Amazonas and Pará but there are reports on the occurrence of this species also in Goiás, Ceará, Minas Gerais and São Paulo. It has been mainly associated with birds but this species can also be found in other sylvan ecotopes such as burrows of armadillos.

During a search for triatomids done in an atlantic rain forest area in Teresópolis, Rio de Janeiro we collected a total of 21 specimens (two adults and 19 nymphs) of Rhodnius prolixus. The homogeneity of 13 specimens (six females and seven males) was observed through the morphometrical analysis by the following aspects: length of the head, antecocular region, length of the eye, postocular region, synthelips and length and width of the pronotum. The total length of four females and three males was a little bit smaller than the standard established to this species by Lent and Wygodzinsky, 1979. The male external genitalia presented the dorsal phallobase plate and the median process of pygophore similar to that described by Lent & Jurberg, 1969. The insects were found on palm trees and in axils of leaves of Pteridophyta. Four insects were infected with Trypanosoma cruzi. Swiss mice inoculated with metacyclic forms obtained from the infected bugs displayed a low patent parasitemia and no mortality. Zymodeem profile corresponded to Z2. (Miles, 1977).

In Brasil R. prolixus is essentially found in the northern region and so we believe that our and other reports on the occurrence of this species far from this area, reflects an accidentally introduction of the bugs through birds and humans. It is worthwhile to remember that the eggs from R. prolixus adhere to almost any substratum. Our findings on adults and nymphs in different stages including eggs suggest that R. prolixus seems to be well adapted to this environment.

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VE-025

ISOLATION OF MICROORGANISMS OF TRIATOMINES MAINTAINED IN ARTIFICIAL AND SYLVATIC CONDITIONS

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Considering the exiguous available data on the studies of microorganisms of insects specially of triatomines maintained in artificial and sylvatic conditions, we developed a project aiming to isolate and characterize bacteria in eight species of triatomines bred in laboratory, in nymphs used in xenodiagnosis and sylvatic bugs.

From the results obtained we observed a high incidence of Enterobacter cloacae, Serratia marcescens and Enterococcus faecalis in adults of most species studied. With respect to sylvatic triatomines the family Enterobacteriaceae was only represented by Enterobacter cloacae.

In nymphs used in xenodiagnosis we observed the prevalence of Enterococcus faecalis in all species analyzed even in those infected with Trypanosoma cruzi. The most frequent Gram negative bacteria was Pseudomonas aeruginosa found in bugs with negative and positive infections for T. cruzi. Such bacteria was only observed when the insects were fed in dogs.

The Gram positive bacteria was represented for Enterococcus, faecalis, Strptococcus sp., Bacillus sp. and catalase positive cocci. This diversity microorganisms was observed in nymphs used in xenodiagnosis in man and dogs.
VE-026
PRELIMINARY STUDIES ON THE INTRA-SPECIFIC VARIATION OF TRIATOMA BRASILIENSIS NEIVA, 1911 (HEMIPTERA, REDUVIIDAE, TRIATOMINAE). II. NATURAL INFECTIVITY.

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Coleção Entomológica, Departamento de Biologia e Bioquímica Molecular* - Instituto Oswaldo Cruz & Departamento de Ciências Biológicas* - Escola Nacional de Saúde Pública. Av. Brasil, 4365, Manguinhos, R.J. Brasil - CEP 21.045-900

_T. brasiensis_ is considered one of the most important Chagas Disease vectors in the semi-arid zone of Northeast region of Brazil and has been frequently found infected by the _T. cruzi_.

Melanic forms have been described as sub-species, according to their different chromatic patterns on pronotum, hemelytron and legs: _T. brasiensis brasiensis_ Neiva, 1911; _T. brasiensis melanica_ Neiva & Lent, 1941 and _T. brasiensis macromelasoma_ Galvão, 1956.

In order to improve morphological and biological knowledgements about the intra-specific variation of _T. brasiensis_, captures are being performed in their type localities: Caicó (Rio Grande do Norte), Espinosa (Minas Gerais) and Petrolina (Pernambuco), as well as in Juazeiro (Bahia).

Thirty four specimens of _T. brasiensis brasiensis_ were collected, seven of which (18.9%) were infected by _T. cruzi_ (microscope observation of faeces diluted in PBS).

Fifty five specimens of _T. brasiensis macromelasoma_ were captured, all being negative for _T. cruzi_; these specimens were fed in laboratory born mice and reexamined 10 days after bloodmeal, confirming the negativiness.

The gut contents of the positive specimens of _T. brasiensis brasiensis_ were diluted in PBS, inoculated in mice and in culture medium (BHI). The phenotypic and genotypic characterization of the strains will be carry out by isoenzymatic and K-DNA analysis. These strains will be used to evaluate the infectivity and the vector status of these melanic forms.

Supported by FNS

VE-027
STUDIES IN PROGRESS ON THE INTRA-SPECIFIC VARIATION OF TRIATOMA BRASILIENSIS NEIVA, 1911 (HEMIPTERA, REDUVIIDAE, TRIATOMINAE). I. FEEDING HABITS.

Costa, J.; Duarte, R.* & Marchon-Silva, V.

Aiming at bringing up bionomic comparative informations between different chromatic patterns that distinguish the melanic forms of _T. brasiensis_, captures have been performed at their type localities: Caicó, State of Rio Grande do Norte (_T. brasiensis brasiensis_ Neiva, 1911), Espinosa, Minas Gerais (_T. brasiensis melanica_ Neiva & Lent, 1941) and Petrolina, Pernambuco (_T. brasiensis macromelasoma_ Galvão, 1956).

Captures were carried out from October 18 to November 4 of 1994 (Caicó, Juazeiro and Petrolina) and 89 specimens were captured inhabiting the peridomestic environment.

The data obtained in these captures are presented by locality, considering that the specimens of each capture point, were packed in the same bottle containing filter paper, later submitted to a precipitin test. The test was assayed using pools of gut content, in average, of three triatominine bugs for the samples collected in Juazeiro and Petrolina (_T. brasiensis macromelasoma_) and pools of gut content, in average, of four triatominine bugs, for the samples from Caicó (_T. brasiensis brasiensis_). Positive results were obtained for more than one source of blood, which are compatible with the vertebrate fauna recorded in the area.

The results for _T. brasiensis brasiensis_ were: 20.5% for goat blood; 17.6% for human blood; 14.7% for opossum; 2.9% for dog; 8.8% for bird and 8.8% for armadillo.

The blood sources of _T. brasiensis macromelasoma_ were the following: 27.2% for goat blood; 25.4% for human blood; 12.7% for opossum; 7.2% for dog; 1.85% for rodent; 9.0% for bird and 9.0% for armadillo.

Both were negative for horse and pig's blood.

Supported by FNS
VE-028
EVOLUTIVE CYCLE OF RHODNIUS DOMESTICUS NEIVA & PINTO, 1923 (HEMIPTERA REDUVIIDAE) UNDER LABORATORY CONDITIONS.

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Rhodnius domesticus is a sylvatic triatomine associated with rodents and opossums nests in brome-lids clumps in the Atlantic forest. Its geographical distribution comprises an area between Santa Catarina and Bahia states in Brazil (Lent & Wygodzinski, 1979). In Santa Catarina, R. domesticus has been found naturally infected by Trypanosoma cruzi and it is possibly involved in the transmission of T. rangeli among wild mammals as well (Steindel et al., 1991 Mem. Inst. Oswaldo Cruz, 86: 73-79). With the aim to study the biological cycle of this triatomine under laboratory conditions, one hundred eggs of R. domesticus were randomly selected from a colony originated from the locality of Ribeirão da Ilha, Florianópolis, Santa Catarina and maintained in our laboratory since 1985. After hatching nymphs were individualized in plastic cups covered with cotton cloth, maintained at 281C and 70% of relative humidity and feed on mice daily. After the first blood meal, nymphs were feed on mice weekly and observed every day for moultng time and mortality until the cycle was completed. Hatchlng rate was 57% with a mean of 15.60.9 days and the entire evolutive cycle was completed by 80.7% of the insects. Total time from egg to adult was of 93.87.3 days. The time in days for each stage was of 12.63.5 NI, 9.81.4 NII, 14.32.0 NIII, 16.92.9 NIV and 25.05.0 NV. Only one complete blood meal was necessary for molting in each stage. Mortality rate was 12.3% for NI, 3.5% for NII, 1.7% for NIII, and NV. This is the first report on the biological cycle of R. domesticus.

Supported by CNPq.

VE-029
DESCRIPTION OF EIGHTH AND NINETH UROSTERNITE OF MALE AND FEMALE FIFTH INSTAR NYMPHS OF THREE SPECIES OF TRIATOMA (HEMIPTERA, REDUVIIDAE).

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Through scanning electron microscope the eighth and ninth urosternite of male and female fifth instar nymph of Triatoma lecticularia, T. sordida and T. vitticeps are described. The ninth urosternite of fifth instar male nymphs are broader than female ones in the three refered species. The ninth urosternite of male nymphs shows one longitudinal central depression, that is different in the three species. The female nymphs have the central portion of ninth urosternite united to eighth, probably like every Triatominae. T.lecticularia : In the ninth urosternite the female nymph shows four curved slit that delimit two upper portions. The chitinous plate of eighth urosternite of female nymph is triangular and locate in the half anterior longitudinal segment, the posterior half is occupied by one slit clearly delimited. T.sordida: The central portion of ninth urosternite of female nymphs are high and the lateral portions have a keel form with two transverse anterior granulous parts. T. vitticeps: The female nymph shows the ninth urosternite united to eighth by one broad and accentuated depression, whose end is clearly delimited in the limit with the tenth urosternite. In the central portion depression of ninth urosternite there is one little longitudinal slit. The eighth and ninth urosternite of this three species differ of the other six: T. brasiliensis, T. circummaculata, T. infestans, T. matogrosensis, T. rubrovaria and T. tibiamaculata described by Rosa et alii (1992) and Rosa (1995).
VE-030
FOUR CITRIC OILS INSECTICIDE VALUATION AGAINST RHODNIUS NEGLECTUS (HEMIPTERA, REDUVIDAE)


The insecticide activity of seeds vegetables oils of the Citrus sinensis, Citrus reticulata, Citrus reticulata X Citrus sinensis (Morcote), Citrus deliciosa peel essential oil and hexano (solvent used to extract seeds oil) was evaluated on 100 examples (nymphs of 2nd, 3rd, 4th, 5th instar and adults) of Rhodnius neglectus. Citrus deliciosa peel essential oil was extracted by steam distillation. The triatominos were sprinkled with determined volume of the each product and they were kept in glass container during one month, the number of triatominos dead were verified every day. The mortality rate is described right down.

<table>
<thead>
<tr>
<th>Substance (used volume)</th>
<th>Mortality Rate</th>
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<tr>
<td></td>
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<tr>
<td>C. sinensis</td>
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<tr>
<td>(3.8 ml)</td>
<td>70 %</td>
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<tr>
<td>C. reticulata</td>
<td>(5.0 ml)</td>
</tr>
<tr>
<td>Morcotte</td>
<td>(2.4 ml)</td>
</tr>
<tr>
<td>C. deliciosa</td>
<td>(5.0 ml)</td>
</tr>
<tr>
<td>Hexano</td>
<td>(5.0 ml)</td>
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<tr>
<td>Controle</td>
<td>2 %</td>
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Thanks: João Luiz Molina Gil and Bento Gregório de Jesus (Serviço Especial de Saúde de Araraquara)

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VE-031
A TRANSMISSION RISK INDEX TO ESTIMATE TRYPANOSOMA CRUZI VECTORIAL TRANSMISSION TO HUMAN, UNDER FIELD CONDITIONS.

Catalá, Silvia; Crocco, Liliana y Morales, Gustavo.

The transmission risk index for diseases transmitted by haematophagous insects has successfully been used to understand vectorial transmission dynamics and to implement appropriate programs of vector control. The transmission risk of Chagas disease, estimated as number of infective bites that a human being receives per time unit has not been yet determined. The objective of this project is to produce an index to evaluate and to compare the risk of vectorial transmission, for example, among zones and climatic seasons. This first part of the project was carried out on Atasmisqui (Prov.Santiago del Estero). The area is located within the phytogeographic region of «Chaco». It is a very poor zone, without Triatominae control since 1980.

Materials and methods: Twenty five houses were treated with fumigant canisters (AGUFOG). The knocked down insects were collected and kept at 4°C. A blood sample (Serokit) was obtained from people living in the houses. Simultaneously we carried out a questionnaire to the family head. The insects collected were analyzed in the laboratory and the following variables were recorded: Population density (IV and V stage nympha, and adults), urine colour, infectivity and blood meals from human. The Trypanosoma cruzi transmission risk index (TcTRI) was estimated as the number of risky bites that a human receive per night. Risky bites are defined as those bites produced by infective bugs. TcTRI is calculated as: Biting rate on human * Proportion of infective bugs/ number of humans. Estimation of the biting rate is based on the presence of colourless urine in the rectum, method proposed by Catalá (1991) complemented with a test to detect human blood within the bug’s promesenteron (gel immune diffusion). Percentage of infective bugs is estimated through microscopic analysis of the rectum contents.

Results: All the houses checked were infested. Half of them showed a low density of Triatoma infestans (under 40 insects). Only three houses displayed a high vector density of 200 to 350 insects (it is important to note that 5 of the 6 seropositive children belong to this houses). Thirty to 160 Triatoma infestans were captured in the remaining domiciles. Serology for Chagas disease revealed that no children under 5 years is positive. Thirty percent of humans between 5 and 50 years showed positive serology. Without the adults above 50 years the prevalence was more than 50 percent. Mean prevalence for Chagas on the total population was 36 percent.

TcTRI for this group of 25 houses on Santiago del Estero ranged from 0 to 5.85 infective bites per human per night.

We conclude that a human TcTRI is likely to obtain. Field techniques combine vector control procedures with T.infestans sampling, whereas laboratory techniques are relatively simple, fast and cheap.

This project is carried out with grants from WHO/TDR/AFR (940213), CONICOR and SECYT UNC.
VE-032
HIGH PREVALENCE OF TRIATOMA INFESTANS IN AN ENDEMIC AREA IN COCHABAMBA-BOLIVIA

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Chagas’ disease is one of the major health problems in Bolivia. It is estimated that 50% of the Bolivian population is at risk of getting infected. Originally, this zoonosis was restricted to the non-populated wild areas of the country. With the increase in agricultural practice, the domestic transmission and the human life cycle were established. One of the major contributors for the spread of the disease is the living conditions of the population, under extreme poverty in the rural areas and valleys of the subtropical zones of Bolivia and Latin America. The high levels of prevalence of the disease in Cochabamba is well known, with the main transmission for the Trypanosoma cruzi being the Triatoma infestans. The vector with active infection is easily found in the domiciliar area. In this study, our group evaluated the infection of 300 T. infestans found in the households of one of the endemic areas in Cochabamba. The vectors were collected in the various houses and submitted to parasitological evaluation in our laboratory. The percentage of infection was surprisingly high, 86% of the triatomines were found positive. Of the positive insects 6.7% were of the second stage, 5% of the third and 10% of the fifth stage. Of the dead triatomines collected, 3.33% were positive. The insects were found in various location such as clothes, under the beds, inside newspapers and in the food. These results clearly demonstrate the close contact of the individuals in their homes with the infected population of triatomines. Our results clearly explain why transmission and prevalence are also so high in Cochabamba. Furthermore, due to the close relationship of the individuals with the parasite and also due to our recent finding of a significant number of dead triatomines in various location including food, suggest that also transmission may also occur under non-bite condition, by ingestion of the parasite.

Supported by NIH, FAPEMIG and CNPq

VE-033
CURRENT STATUS OF THE NATIONAL DATABASE OF THE SERVICIO NACIONAL DE CHAGAS. DECISION SUPPORT TOOL FOR VECTORIAL CONTROL, FROM LOCAL TO NATIONAL LEVEL.

Spillmann, Cynthia (1); Gorla, David(2); Hurvitz, Abel(1).
(1) Servicio Nacional de Chagas Córdoba. 9 de Julio 356. 5000 Córdoba. (2) Fac. Cs Ex. Fis. y Nat. Universidad de Córdoba.

The Servicio Nacional de Chagas Córdoba (Argentina) created in 1992 a National Database containing information on the activities carried out by the National Programme for the Chagas Disease Vector Control, to improve its managerial activities through the use of the data routinely collected during the field activities of houses evaluation and vector control. In this communication, the current status of the data administration system is presented, on the basis of the instructions from the Dirección Nacional de Epidemiología, the National Programme of Chagas Disease and the suggestions of the Comisión Intergubernamental de la Iniciativa Cono Sur.

The database runs over the architecture of EPI-INFO, from the monthly report on standardized forms sent by the 19 provinces under the jurisdiction of the National Programme, to the Information Division of the Servicio Nacional de Chagas (Córdoba). The current advances in the information system are focused on three main aspects: 1) the system runs under a standardized and stable protocol since 1992, allowing the analysis of historical data that are comparable in time and space, and has the ability of making projections and evaluations on data continually updated and with complete coverage of the area where the Programme works on; 2) it uses the locality as the information unit, allowing a narrow-focus geographic analysis, and the possibility of data aggregation in geographically nested levels (i.e. localities within departments, within provinces, within the country); 3) the system helped to improve the efficiency of the evaluation of local and regional situations. Specifically, the information administration procedure allows a) the quick and precise detection of critical areas, in terms of the domestic and/or peridomestic infestation, b) to evaluate the field activities coverage (urban and rural areas), in terms of the number of sprayed and/or evaluated houses in relation with the total number of houses within a region; c) to evaluate the reinfestation potentiality in particular areas after an attack phase, etc. Although the system offers several advantages, its operation did not imply neither an increase of personnel nor important equipment expenditures.

As control and vigilance activities are dynamic processes, there is a continuous evaluation of a) the quality and appropriateness of the data entered to the system; b) the way to simplify data collection from the field; c) the construction of software for the automatic analysis, and d) the inclusion of a system to produce epidemiological maps linked to a geographic information system locally developed, to quickly visualize the geographic distribution of the information contained in the database.

(*) This work received the support of the Dirección Nacional de Epidemiología, Instituto Fatala Chaben and TDR(WHO). We acknowledge the support from the Information Division staff of the Serv. Nac. Chagas Córdoba.
VE-034
CHANGES IN LIPOPHOSPHOGLYCAN AND GENE EXPRESSION ASSOCIATED WITH THE DEVELOPMENT OF LEISHMANIA MAJOR IN PHLEBOTOMUS PAPATASI

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Stage-specific molecular and morphogenic markers were used to follow the kinetics of appearance, number, and position of metacyclic promastigotes developing during the course of L. major infection in a natural vector, Phlebotomus papatasi. Expression of surface lipophosphoglycan (LPG) on transformed promastigotes was delayed until the appearance of nectomonal forms on day 3, and continued to be abundantly expressed by all promastigotes thereafter. An epitope associate with arabinose substitution of LPG side-chain oligosaccharides, identified by its differential expression by metacyclics in vitro, was detected on the surface of a low proportion of midgut promastigotes beginning on day 5, and on up to 60% of promastigotes on day 10 and 15. In contrast 100% of the parasites egested from the mouthparts during forced feeding of 15 days infected flies stained strongly for this epitope. At each time-point, the surface expression of the modified LPG was restricted to morphologically distinguished metacyclic forms. Ultrastructural study of the metacyclic surface revealed an approximate 2-fold increase in the thickness of the surface coat compared to nectomonal forms, suggesting elongation of LPG as occurs during metacyclogenesis in vitro. A metacyclic-associated transcript (MAT-1), another marker identified by its differential expression in vitro, also showed selective expression by promastigotes in the fly, and was used in in situ hybridization studies to demonstrate the positioning of metacyclics in the anterior gut.

Supported by CNPq and WHO.

VE-035
LABORATORY ASSAY OF MUD BRICKS TREATED WITH INSECTICIDES AGAINST PHLEBOTOMINE SAND FLIES.

Oliveira Filho, Alfredo M.*; Santos, Celso E.*; Melo, Marli T.V.* & Souza, Nataly A.**

The use of insecticides is the most common approach for the control of transmission of endemic tropical diseases like Chagas disease, malaria, dengue, yellow-fever, etc. For the control of leishmaniasis in Brazil it has been seldom recommended because of lack of data showing which insecticides and formulations could better fit this situation. Recently FNS (Brazilian Ministry of Health) has delivered recommendations on visceral leishmaniasis control in Brazil mentioning the need for use of insecticides when autogenous cases of the disease are found in humans or dogs in an area where Lutzomyia longipalpis is present. This laboratory experiment was planned to provide means to understand activity and persistence of selected insecticides and formulations when sprayed over the most common surface found in the endemic areas, i.e., mud. The insects used were bred at the Dept. of Entomology, FIOCRUZ, Rio de Janeiro. We used the F1 generation of insects collected in the Sãu Luís Island, Maranhão, Brazil. Ten females Lu. longipalpis, 3 days old, sugar fed, were exposed during one hour to treated mud bricks inside WHO plexiglas test kit cones at 25+10C and 85+10%HR. After that, they were transferred to acrylic cylinders kept inside a glass camera at 27+10C and 95+5%HR until the 24 hour post-treatment reading. Three repetitions were performed per treatment group. The mud bricks were treated in a semi-automatic device providing the same conditions found in the field, i.e., pressure of 55 psi, Tejet 8002 nozzles, spraying rate of 760 ml/minute, 45cm distance, providing 40ml/m2 of final formulation. The insecticides were malathion 8.3% slow-release emulsifiable suspension developed by NPPN (DURATION M at 2 g a.i./m2), lambdacyhalothrin (ICON 10%WP and ICON 10%CS, both 30mg a.i./m2) and DDT (75%WP at 2 g a.i./m2). Preliminary results of initial activity showed that mud bricks aged 6-10 days (sprayed at 28/5/95), treated with slow-release malathion or wettable powder DDT can provide an excellent level of control, showing 97 to 100% mortality. Lambdacyhalothrin provided only 40-43% mortality on both formulations probably needing a higher dose to increase the level of control when sprayed over the chemically hostile surface of a mud brick. New assays are planned for 3, 6 and 12 months post-treatment to evaluate the residual effect. (WHO/UNDP-WORLD BANK/TDR, FNS, CNPq, CEPEG-UFRJ).
VE-036
FIELD ASSAY ON CHEMICAL CONTROL OF VECTORS OF VISCERAL LEISHMANIASIS IN MARANHÃO - BRAZIL.

Oliveira Filho, Alfredo M.; Melo, Marli T.V. & Santos, Celso E.

DDT and a few other insecticides have shown the capacity of reducing visceral leishmaniasis transmission in Brazil when used to control vectors of malaria or Chagas' disease in overlapping endemic areas. These empirical observations however were never confirmed by scientifically sound field experiments. This assay began on August 1994 and is being performed to show if there is any meaning of using insecticides to control Lutzomyia longipalpis and if formulations that protect the active ingredients against fast degradation can play a long-lasting role in reducing intra and peridomesticary sand flies catches. The assay was held in Vila Coronel Riod, municipality of São José do Ribamar and Vila Pavao Filho and Santa Clara, both in the municipality of São Luís (440 15'W, 20 33'S). The assay area, comprising 1899 houses, was divided in six groups, two of them taken as controls. The four treatments were DDT (75%WP at 2ga.i./m2), lambdacyhalothrin (Icon 10%WP and another group with 10% microencapsulated, named ICON 10CS, both at 0.03ga.i./m2) and malathion (DURATION M 8.3% slow-release at 2g a.i./m2). The spraying was performed by FNS personnel using Hudson-X-Pert pumps, fitted with Teejet 8002 nozzles releasing 40ml of final formulation/m2. The evaluation consisted in manual captures and also catches using CDC light traps kept overnight in the intra and peridomiciûm. Preliminary results showed a strong reduction in the number of insects captured mainly for the six months reading in the groups treated with formulations designed for longer residual effect (40 insects captured in the slow-release malathion developed by the NPPN, 145 in the lambdacyhalothrin microencapsulated and 3266 in the control). Among the preliminary conclusions that can be draw by now the most important is that the insecticide treatment of internal houses walls and peridomiciûmal animal shelters and other phlebotomine resting places like tree trunks and fences is efficient in promoting reduction of insect population. The second one is that slow-release malathion and microencapsulated lambdacyhalothrin performed better than conventional formulations. Results also show that CDC light traps captured much more insects than the manual captures, which were only effective when capturing over domestic animals such as dogs, pigs, cows and chickens, between 19-24hs. The number of sand flies captured in the peridomiciûm was significantly higher than in the intradomiciciûm. The nine months results were inconclusive due to reduced catches in control groups, probably due to the heavy rains observed during May 95 in the São Luís Island. Next reading is planned for one year post-treatment. (WHO/UNDP-WORLD BANK/TDR, FNS, CNPq, CEPEG-UFRJ).

VE-037
BIOLOGICAL FIELD ASSAY OF INSECTICIDE TREATED HOUSES AGAINST LUTZOMYIA LONGIPALPIS.

Oliveira Filho, Alfredo M.*; Melo, Marli T.V.* & Souza, Nataly A.**
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In public health usually the evaluation of the performance of an insecticide in field conditions is done by the measurement of reduction of the vector population and, when possible, through the epidemiological approach measuring the reduction of the number of news cases of the disease itself. However, biological assays of treated house walls can give a direct measure of the insecticide activity and persistence under real conditions, becoming an important tool for comparisons among active ingredients, formulations and dosages. This field assay was performed in accordance to the recommendations of WHO, willing to provide a parallel measure of the activity of a group of insecticides and formulations used to treat houses in a village scale field assay being performed by the NPPN and FNS in Maranhão State, Brazil. Part of three villages in the municipalities of São Luís and São José do Ribamar in the São Luís Island were chosen as the experimental area (44015'W, 2033'S). The whole assay involved around 1900 houses from which four treatment and two control groups were established. The four treatments were DDT (75%WP at 2ga.i./m2), lambdacyhalothrin (Icon 10%WP and another group with 10% microencapsulated, named ICON 10CS, both at 0.03ga.i./m2) and malathion (DURATION M 8.3% slow-release at 2g a.i./m2). The spraying was performed by FNS accordingly to the usual technique recommended by WHO for residual treatment inside houses and also in the peridomiciûm.

The bioassays were performed at 6 and 9 months post-treatment, always inside the houses, over plastered non-painted walls. Ten female Lu. longipalpis, two days old, sugar fed, from the colony of Depto. Entomologia, FIOCRUZ, Rio de Janeiro, were kept in contact with the treated surface during one hour under the Plexiglas cones of WHO test kit. The hole was sealed with a cotton plug and the cone was covered with a black cloth. After that, they were transferred to non-treated cylinders until 24 hour post-treatment when the final reading was performed. Local conditions varied between 29-31°C and 76-82%HR. The bioassays were performed between 18-24hs. One to three houses with 2 to 6 cones per treatment were used.

Results of 6 and 9 months showed no significant mortality in any treatment group. As the manual and trap sand flies captures performed at the same period were significantly reduced, we believe that this time of exposition of sandflies to the treated walls was not enough to cause intoxication. Laboratory experiments are on the run to provide these answers. (WHO/UNDP-WORLD BANK/TDR, FNS, CNPq, CEPEG-UFRJ).
VE-038
OBSERVATIONS ABOUT SYMPTOMS AND SIGNS ON LUTZOMYIA LONGIPALPIS LARVAE (LUTZ & NEIVA, 1912) (DIPTERA; PSYCHODIDAE, PHLEBOTOMINAE) UNDER BIO-ASSAYS WITH BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS STRAINS LFB/FIOCRUZ 584 AND LFB/FIOCRUZ 710.

Wermelinger, I.E.D.; Zanuncio, J.C.; Rangel, I.E.F. & Rabinovitch, L.J.

Leishmaniasis has a large distribution in the Americas and Lutzomyia longipalpis is considered as vector of visceral leishmaniasis. The spore forming bacteria Bacillus thuringiensis spbs. israelensis has been successful in the control of some important diptera vector transmitted diseases, in particular cuticidae and simulidae. In order to investigate the potentiality of B. thuringiensis spbs. israelensis in the control of phlebotomine vectors, bioassays were made with 3rd instar larvae of L. longipalpis bred in laboratory. Symptoms and signals showed by the larvae of the L. longipalpis of tested lots were studied. The bioassays were conducted at 25°C with the strain B. thuringiensis spbs. israelensis LFB/FIOCRUZ 584 (received from Institut Pasteur de Paris, strain belonging to standard powder IPS-82) and LFB/FIOCRUZ 710 isolated from brazilian soil. These strains were supplied by the Coleção de Cultura do Gênero Bacillus (CCGH) of the Departamento de Bacteriologia, FIOCRUZ. The larvae of L. longipalpis was supplied from a closed colony of the Departamento de Entomologia, FIOCRUZ. Concentrations used were 10 and 20 mg of B. thuringiensis spbs. israelensis biomass bearing delta-endotoxin crystals and spores, respectively mixed and homogenized with 1 gram of previously autoclaved larvae food. The sample unit were 20 larvae. The symptom more clearly observed was the delayed life cycle. On tested lots, the larvae achieved the adult phase eight days later (in average) than the compared control lots. The majority of dead larvae presented a dusty body, sometimes with darker regions. However, this symptom was also observed in some dead larvae of control lots yet much less frequently. By slide preparation of smears from dead larvae, chains of vegetative cells of B. thuringiensis spbs. israelensis were detected, sugestking a bacterial multiplication inside the larvae body.

Work supported by CNPq and FAPEMIG / Brasil.

VE-039
A STUDY ON LUTZOMYIA INTERMEDIA LUTZ & NEIVA, 1912 COMPLEX: TWO SPECIES CAN BE DISTINGUISHED (L. INTERMEDIA S. ST. AND L. MARIAE SP. N.)
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Lutzomyia intermedia was summarily described and, after the demonstration of the importance of the spermathecae and cibarium (Theodor, 1932), it was possible to distinguish it from L. whitmani and other similar species. A study of about 700 specimens from Bolivia, Paraguay, two provinces of Argentina and 12 states of Brazil indicated that two species can be distinguished. In L. intermedia s. st., the spermathecae are long (54±6.55m), have a greater number of rings (11-17), their "heads" are frequently bilobated and are very wide, compared to the "bodies" (0.75+0.16), and the length of individual ducts is usually less than thrice (2.41±0.99) that of common duct, and the cibarium has only eighth horizontal teeth. Compared to L. intermedia s. st., spermatheca of L. mariae sp. n. (dedicated to illustrious Dr. Maria P. Deane) are smaller (40.49+4.68 m), have a smaller number of rings (6-10), their "heads" are narrower (0.47±0.1), individual ducts much longer than common duct (7.55+3.33), and there are 10-12 cibarial horizontal teeth. Significative differences in size of several other structures help the identification of females. The distinction of males is very difficult, although L. intermedia has several structures greater than L. mariae. A more complete formal description is being publised.

L. intermedia was found in localities of Pernambuco, Bahia, Espirito Santo, Rio de Janeiro, east of Minas Gerais, and, in São Paulo, in the east of "Serra do Mar", L. mariae was found in localities of west of Minas Gerais, in São Paulo (in localities of "Serra do Mar" and in the west of it), in Goiás, and in Paraguay, Argentina and Bolivia. Both species were found in Pariquera Águ and Dourado Municipalities (SP). Studies on cuticle hydrocarbons, isoenzimes and DNA of these species are being developed. It is important to distinguish the species on each locality and situation and situation and to evaluate their importance in the epidemiology of leishmaniasis.

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VE-040
LUTZOMYIA WHITMANI AND AMERICAN CUTANEOUS LEISHMANIASIS DUE TO LEISHMANIA BRAZILIENSIS IN PERNAMBUCO, BRAZIL.

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American cutaneous leishmaniasis (ACL) has been an important health problem in the last years in the State of Pernambuco, Northeast of Brazil. Most cases occur in the Zona da Mata region (64.2% of the total). This region corresponds geographically to the primitive area of the Atlantic forest, one of the most important primary rain forest in Brazil. In order to characterize the eco-epidemiology of ACL in this region a study has been realized in two localities of the highest incidence. A new variant of Leishmania braziliensis was characterized and identified on the basis of enzyme profiles in stocks obtained of patients from this area (BRITO et al., Mem. Inst. Oswaldo Cruz, 88: 633-634, 1993). Monthly captures of sandflies are carried out in both localities by using CDC light traps as well as manual captures. Collections were made in domiciles, peridomestic and extradomicile areas. Extradomiciliary areas are defined as those located 100m from the house. Lutzomyia whitmani is predominant, representing 97.4% of the species captured and identified. Other species found were Lu. evandroi, Lu. quinquefei, Lu. lenti and Lu. aragaoi. The extra and peridomicle are the predominant places for capturing Lu. whitmani, mainly in locals corresponding to chicken pen and equine stable. The relation of the mean monthly variation density of Lu. whitmani to the number of cases reported showed that the highest density in the period were followed by increased number of human cases in both localities. These data provide evidence for the involvement of Lu. whitmani in the transmission of ACL in this region. Studies about host preference and natural infection are currently being undertaken.

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VE-041
ANTHROPIC ACTIVITIES AND PERPETUATION OF AMERICAN CUTANEOUS LEISHMANIASIS (ACL) IN THE STATE OF SÃO PAULO, BRASIL*

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The aim of this study was to evaluate the importance of man’s activities on the perpetuation of ACL, nowadays spread and with foci all over of the State of São Paulo. Anthropic activities resulted in a dramatic reduction of the forested regions of the State and molded the present landscape, which brought a gradual change in the epidemiology of ACL during this century. Changes were also observed in the phlebotomine fauna composition with Lutzomyia intermedia being predominant in endemic areas.

In Eldorado Paulista - Ribeira River Valley we defined three different environments: I. Inside of forest - primitive environment; II. Near of the edge of forest - environment with little modification by the presence of an experimental chicken coop; III modified environment by the presence of man’s activities (house, banana cultivation and domestic animals). Environment I and II showed great diversity of phlebotomine species with predominance of Lu fischeri (82.13 and 65.47%, respectively). Lu fischeri, Lu migonei, and Lu ayrosai added 97.28 and 87.02%, respectively in I and II. Lu intermedia was inexpressive in I (1.31%), frequent in II (12.22%) and absolute in the modified environment III, with 99.29%. We conclude from these data, that the anthropic activities upon natural environment were determinant pressure for the changes in the sandfly fauna composition that facilitated Lu intermedia to show its vectorial capability as consequence of its behavioral and ecological attributes.

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VE-042
COMPARATIVE STUDIES ON TWO POPULATIONS OF LUTZOMYIA LONGIPALPIS (LUTZ & NEIVA, 1912) (DIPTERA: PSYCHODIDAE: PHLEBOTOMINAE) FROM BRAZIL.

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_Lutzomyia longipalpis_ has been implicated as the vector of visceral leishmaniasis and has a wide geographic range in the Americas. Mangabeira (1969) noted that males from North Brazil bore a single pair of pale spots on the abdominal tergites, whilst those he examined from Northeast Brazil had two pairs. Based on these findings, Ward et al (1983) suggested _L. longipalpis_ as a species complex with two sibling species. In order to study this problem we decided to analyse several populations from different geographical regions in Brazil, using morphology, morphometry, DNA and isozymes. We initiated the study by comparing specimens from Marajó Island (Pará State) and São Luiz (Maranhão State). There was observed one pair of tergal spots in males from Marajó Island while those from São Luiz had two pairs of tergal spots. However, no morphological differences were found between these two populations using other 46 structural characters of the head, thorax and abdomen of males and females, as recommended by the Computer Aided Identification on Phlebotomine Sand flies of America (CIPA, 1991).

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VE-043
THE LABORATORY BIOLOGY OF SANDFLY LUTZOMYIA LENTI (MANGABEIRA, 1938).

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Laboratory sandfly colonies are essential for studies on biology and _Leishmania_ life-cycles. _Lutzomyia lenti_ (Diptera:Psychodidae) is a sandfly well distributed in Brazil (Young and Duncan, _Mem. Am. Ent. Inst._, 54: 158, 1994) and this species is found very often in areas of cutaneous and visceral leishmaniasis. From females captured in a CDC light trap in Betim, Minas Gerais State we initiated a colony using the methodology already established for _L. longipalpis_. This colony is now in its 6th generation and females feed well and promptly on hamsters ( _Mesocricetus auratus_ ). The average egg laid per fly is 38.0 and the development cycle from eggs to adults lost 46.6 days at 25 - 26°C. _L. lenti_ seems to be an easy species to breed in the laboratory and several attempts to infect this fly with _Leishmania sp._ have failed and it could suggest a refractoriety to _Leishmania_ infection as observed in _L. carmelinoi_ a sandfly of the same group of _L. lenti_ (Ryan et al., _Mem. Inst. Oswaldo Cruz_, 81:323, 1986).

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VE-044
SUSCEPTIBILITY OF LUTZOMYIA RENEI (DIPTERA:PSYCHODIDAE) TO LEISHMANIA INFECTIONS

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Two samples of Leishmania (Leishmania) amazonensis (MAKO/BR/78/32R and MPRO/BR/79/132R) and one of Leishmania (Viannia) braziliensis (MCAN/BR/73/BH348) were used to study the susceptibility in Lutzomyia renei. Samples 32R and 132R were isolated from rodents trapped in Caratinga, Minas Gerais State and sample BH348 was isolated from a dog in Caratinga also. Females of Lu. renei, originated from Lapinha cave, Lagoa Santa, MG were fed on lesion of hamsters experimentally infected. Lu. renei was susceptible to all the strains used although the infections rate were different. With sample BH348 13 (21%) out of 62 fed females became infected. Infections were very poor and 12 (92%) of the infected flies showed parasites attached to the hindgut. With sample 32R the indices of the infectivity was 26% (19 out of 72 fed females). The infections were rich in parasites and were restricted to the anterior midgut with large number of promastigotes moving in the gut lumen. The best infection of Lu. renei was obtained with sample 132R. From 138 fed females 75% (118) became infected. Infections were very rich in the anterior region of the gut with free parasites in the gut lumen. Only 4 (3%) flies showed promastigotes in the hindgut. The behaviour of the parasites was in accordance with that described for the subgenus Leishmania and Viannia (Lainson and Shaw, The Leishmaniases in Biology and Medicine, vol. 1, 1987).

Studies done with the same samples of Leishmania with the sandfly L. longipalpis showed similar results (Gontijo et al., Mem. Inst. Oswaldo Cruz, 90:367-373, 1995) Although Lu. renei is not incriminated as vector of leishmaniasis it was able experimentally to transmit to hamster L. mexicana by bite being considered a species potentially able to transmit Leishmania (Coelho et al., Rev. Inst. Med. trop. São Paulo, 4:220-224, 1962).

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VE-045

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In a previous communication (Brazil et al., Mem. Inst. Oswaldo Cruz, Suppl. I, vol. 86, 1991) we showed the general morphology of the salivary glands (SG) of Lutzomyia longipalpis in fresh preparations at the light microscope. At the present we are studying the histological and histochemical aspects of the SG of the same species in individuals from F1 generation of insects caught at the Lapinha Cave (Lagoa Santa, Minas Gerais). The study has been done on 3-4 mm sections of historesin (hydroxy-methyl-methacrylate) embedded insects. The basic histology was observed on H/E stained sections and confirmed the aspects described anteriorly showing an epithelium formed by a monolayer of cells. The cells exhibit large nucleus and nucleoli with loose chromatin and apparently spongy, basophilic cytoplasm (characteristic of protein secreting cells). In one of the specimens intercellular spaces could be observed and the lumen contents of the gland showed droplike particles wich suggest the presence of lipydic material. In another specimen no droplets but granules of acid nature were seen. The histochemical studies are in process.

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 VE-046
ECOLOGY OF SANDFLIES IN SERRA DO MAR, ITAGUAÍ, AN AREA OF CUTANEOUS LEISHMANIASIS, STATE OF RIO DE JANEIRO, BRAZIL (DIPTERA, PSYCHODIDAE, PHLEBOTOMINAE)

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A study on sandflies ecology was performed in an area of cutaneous leishmaniasis transmission in Itaguaí, Rio de Janeiro State, from March 1984 to February 1986. The research was performed to study the sandflies habits and their role in the disease transmission. The captures were made with different types of baits and traps, monthly, during two consecutive days, and in three collecting areas: domestic, peridomestic and forest. In the first year the captures were performed about 100 m above the sea-level, and in the second year about 300 m. There were captured 13,546 sandflies of 17 species; The most frequent species found at 100 m above the sea-level was L.intermedia, with a large prevalence over L.migonei and L.fischeri. At 300 m, the most frequent was L.migonei followed by L.longipalpis and L.fischeri. In both levels the number of females was higher than the males. L.longipalpis showed equilibrium between sex, in opposition to L.fischeri. L.intermedia and L.fischeri showed high anthropophilia and, despite other baits, the man was chosen as the best. L.longipalpis showed little interest for human blood, preferring to bite hens. L.migonei sucked man, but in comparison to other baits it preferred donkey, leaving man for final option. On hematophagy time, L.intermedia showed significant activity from 6:00 to 9:00 p.m. in the peridomestic site; from 12:00 am to 2:00 am the situation was inverted, in relation to domestic site. L.fischeri, although in smaller quantity, sucked man in the three collecting sites, keeping a considerable population in the forest. L.intermedia was highly predominant in the domestic and peridomestic areas and was not found in the forest. Once more it shows its easy adaptation to the environment changed by man. L.migonei also showed this tendency, but there is still a small population of this species in the forest. L.longipalpis was predominantly found in the peridomestic site and was not found in the forest. The most important species occurred monthly, but were more frequent in the hot and humid period - December, January and February - showing less density in the colder and dryer period - July, August and September. Analyzing the intra and extradomiciliar frequency, the transmission occurs in domestic and peridomestic sites, representing 94% of the total of species caught. L.intermedia, by its anthropophily and prevalence, is the main suspect of transmitting Leishmania(Viannia)brazilensis in the studied area. However, concerning hematophagy, the high anthropophily and eclecticism of L.fischeri must be considered, as it can be a secondary vector in the domiciliar level and can transmit the disease in its natural enzootic cycle in the forest. The presence of L.longipalpis, even showing no evidence of kalaazar cases, may bring risks to the local population, specially due to the proximity of the research area to others where cases of the reported parasitose happen.

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