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A preliminary study of haemolymph from four Venezuelan populations of *Panstrongylus geniculatus* Latreille, 1811 (Hemiptera: Reduviidae) and its epidemiological significance

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SUMMARY

SDS-PAGE profiles of both sexes of the haemolymphs of *Panstrongylus geniculatus* from different Venezuelan regions (savannas, piedmont, tropical forest and urban areas) were compared. It was determined that the haemolymphs showed a different electrophoretic profile, with proteins that ranged from 14 to 164 kDa. The most representative protein band in the profile of females was observed in two sectors: between 164 and 46 kDa and between 33 and 30 kDa. The main illustrative protein band in males was observed in one region: from 46 to 35 kDa. The Haemolymph composition of *P. geniculatus* from populations evaluated in this work expressed high homogeneity of this species with a clear difference between males and females. This similarity may be useful for control of these insects, taking into account that the genetic stability may be very important, since the use of an insecticide in a population with these characteristics is always more successful. According to the bibliographic review, this is the first study of haemolymph from *Panstrongylus geniculatus*.

Key words*:* Epidemiology, haemolymph, *Panstrongylus geniculatus*, Reduviidae, Venezuela.

Panstrongylus geniculatus Latreille 1811[1] is probably the species of triatomine, which has the high geographical distribution in the Americas. Its presence in Argentina, Uruguay, Paraguay, Perú, Bolivia, Brazil, Ecuador, Colombia, Guyana, French Guiana, Surinam, Venezuela, Trinidad, Panamá, Costa Rica and Nicaragua[2-3] has been established. In Venezuela, in nearly all federal dependencies has been described.[4] It is one of the most important vectors in the wild cycle of *Schizotrypanum cruzi* and occasional visitor of the housings arriving in directional flight attracted by the light.[4,5]

The blood of the insects is called haemolymph, which may be a clear colourless fluid. The electrophoretic (SDS-PAGE) profiles of triatomine haemolymph demonstrate a complicated composition of proteins, permitting the separation of species.[1,6] Nevertheless, it is unknown whether the composition of haemolymph varies within either species or different populations. With these considerations, the main aim of this study was to compare the haemolymph electrophoretic profiles from four Venezuelan populations of *P. geniculatus* and try to search the similarity or difference genetic among specimens from different geographical locations.

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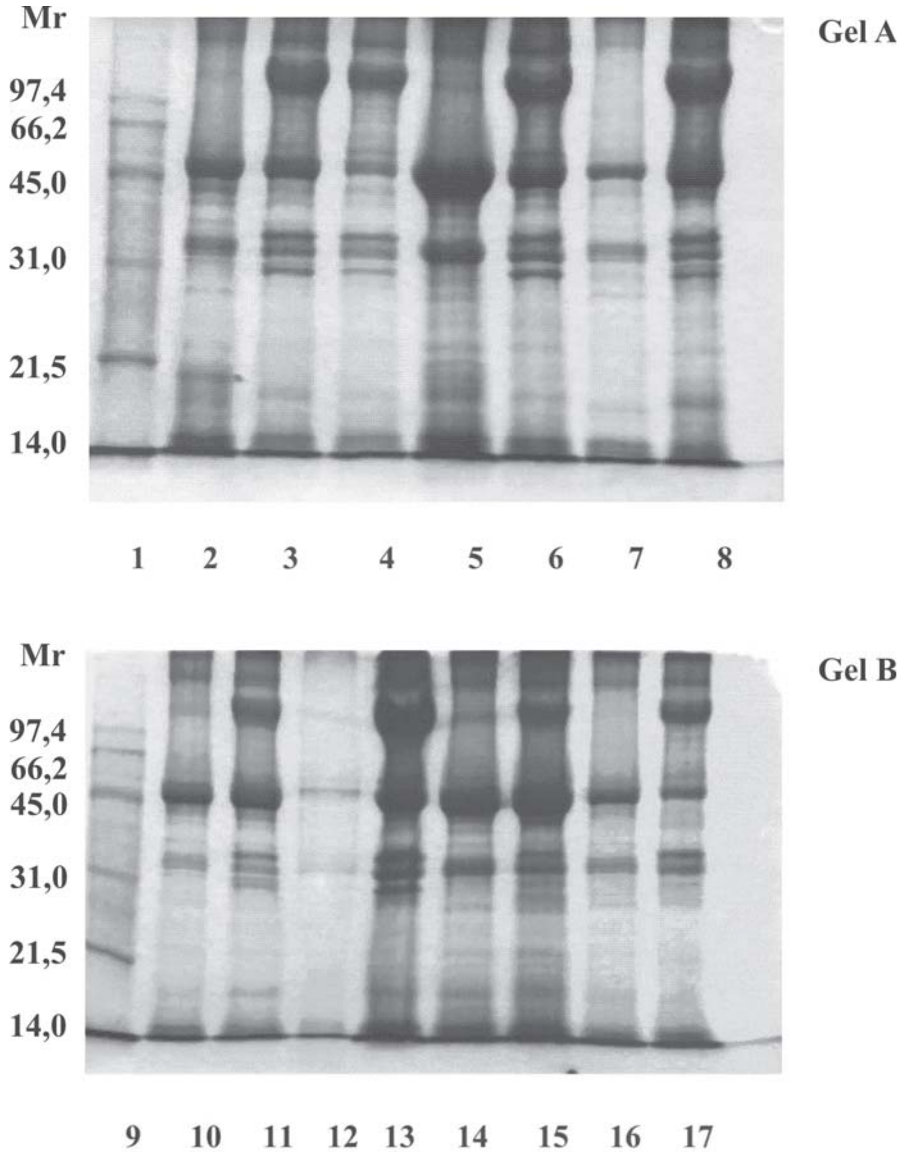
METHODS

P. geniculatus sensu stricto were captured from the next regions: Cuyagua, Aragua state, at 60 m altitude in semi-xerophytes habitats, near the seaside using a Shannon trap (light attraction); Cumboto, Aragua state at 100 m altitude, where it emerged in a humid tropical wood environment, attracted during the night by house lights. Paracotos, Miranda state at 400 m altitude at the Coastal Range piedmont, in a tropical deciduous wood; Los Anaucos, Miranda state at 600 m altitude in an urbanization located at the Coastal Range piedmont. Los Campitos and El Hatillo town, Miranda state at 1000 m altitude in a subtropical environment, where *P. geniculatus* were attracted by houses lights in urban environmental, as well as in Carmelitas, Caracas, Venezuela at 900 m altitude demonstrating a high potential for the colonization of human housings.[7]



Gel A: Lanes: 1: Low molecular weight (Mr) markers. 2: Paracotos-Sardi (M); 3: Paracotos-Sardi (F); 4: Paracotos-Cave (M); 5: Anauco (M); 6: Anauco (F); 7: Cumboto (M); 8: Cumboto (F).
Gel B: Lanes: 9: Low weight molecular weight markers; 10: Carmelitas (M); 11: Carmelitas (F); 12: Campitos (M); 13: Campitos (F); 14: Hatillo (M); 15: Hatillo (F); 16: Cuyagua (M); 17: Cuyagua (F).
(M): Male. (F): female. Only Paracotos-Cave males were described.

Fig. Female (F) and male (M) *Panstrongylus geniculatus* haemolymphs SDS-PAGE (12,5 %) electrophoresis under reduction conditions

Artificial colonies were established in the Entomological Laboratory of the Tropical Medicine Institute of the Universidad Central de Venezuela, Caracas, Venezuela, from wild-caught samples.

The insects were growing in an insectarium under relative humidity of 60-80 %, at 26 ± 2 ºC temperature. To feed the triatomines, hens were offered to the insects each 10 days until adult insects were accessible for use in the assays. Five male and five female specimens from each geographical sample were studied 1 month after their final change in the cycle.

Separated individual haemolymph cutting into the basis of the posterior femurs near the apex, using a capillary tube was collected. Eppendorf tubes filled with 0.3 mL of haemolymph were stored at -70 ºC until use. Protein concentration in the haemolymph by the micromethod of Bradford[8] was determined.

Electrophoresis using a Dual Mini Slam Kit AE-6450 (Atto Corporation, Tokio, Japan) chamber was performed. SDS-PAGE was carried out conforming to the Laemmli method,[9] using 20 % gels under reducing conditions. Molecular weight markers (Bio-Rad) were run in parallel and gels stained with Coomassie Blue R-250. *P. geniculatus* haemolymph samples to be analysed were dissolved in a proportion of 1:1 in the solubiliser solution: 0.5 M Tris.HCl, pH 6.8, with 10 % (w/v) SDS, 10 % (v/v) ß-mercaptoethanol, 10 % (v/v) glycerol and 0.05 % (w/v) brome-phenol blue, and heated at 100 °C for 10 minutes. The molecular weight was determined by Kodak Image Station 440 System (IS440CF) Analysis Software. A taxonomic matrix was achieved by the study of protein banding composition.[10]

P. geniculatus taxonomic determination was carried out at the Entomology Department of the Tropical Medicine Institute based on the analysis of external morphology and taxonomical keys.[11,12]

RESULTS

Female and male *P. geniculatus* haemolymph showed in the electrophoretic studies a multiple protein constitution with relative masses varying from 14 to 164 kDa in both sexes. Male and female electrophoretic profiles showed different protein migration patterns. The most representative female haemolymph protein bands profile was observed in two regions: between 164 to 46 kDa and between 33 to 30 kDa. The principal illustrative male protein bands profile was observed in one region: between 46 to 35 kDa (fig.: gel A and gel B).

DISCUSSION

Among Venezuelan wild triatomines, *P. geniculatus* presents the greatest capacity to adapt to the domestic environment[7] and currently is regarded as the main sylvatic vector of *T. cruzi* in the Country.[4]

After Latreille description in 1811 the Triatominae *Panstrongylus geniculatus* (Hemiptera:Reduviidae), wait almost in the forgetfulness until Pinto[1] identify it as a possible Chagas disease vector. It has been considered the responsible of the maintenance of the Chagas disease enzootic cycle.[5] While it was accomplished these projects of sanitary and medical character, the taxonomical, biological and physiological investigations on this genus have progressively grown. The triatomine is described living in a wide variety of wild ecotypes including burrows of armadillo, hollow of trees, caves, housings of opossum, palms and bromeliaceous, also peridomestic and domestic habitats in some southern areas of Venezuela and north of Brazil.[7,13]

Fundamentally there are aspects that have motivated the development of compared morphology,[14] cytogenetic, PCR, isoenzymes and saliva[15] studies in triatomines that are *Trypanosoma cruzi* vectors, causative agents of Chagas disease. Some of them related to the possibility of resistance development to insecticides, by virtue of the implications in the chemical control and the second routed to solve the controversy of the possible monophyletic or polyphyletic origin of the Triatominae subfamily.

Recently, the results obtained in these studies have reinforced the hypothesis of a monophyletic origin for some tribes and their respective groups of species within the triatomine, based on that the differences obtained in the considerate aspects have resulted little significant among the genera and within some species.[16,17] Concomitantly, these results have also been interpreted as a reflex of a reduced variability present in these groups, in other words seems to be genetically very stable.[3]

Since Wigglesworth[18] studying a Reduviidae, described the haemolymph and the process of wound healing in an insect and Jones[1] who spoke about the circulatory system of the insects, the bibliography has been increase slowly in this topic. Control and eradication of a disease transmitted by an arthropod, only is possible, when is acquired a profound knowledge of the vector biology. Nowadays, most investigations concerned to haemolymph is referred to few insects species, such as *Manduca sexta* (holometabolous), *Locusta migratoria* (hemimetabolous), scarce triatomine bug[16] but the information about *Panstrongylus* genus haemolymph as far it is known does not exist.

In the current study, morphological differences were not observed among four populations. However, it was possible to differentiate female and male populations by their haemolymph components. The examination of the total proteins did not give major differences amid the genera. However, the electrophoretic bands of the obtained proteins allowed establishing qualitative differences between male and female, of these *Panstrongylus geniculatus* strains that could be associated in the female with factors related to the storage proteins and vitellogenesis (a major component of oogenesis in insects).[20, 21] For instance, this phenomenon has been reported during eggs development in *Rhodnius prolixus,* a different species of triatomine.[22] Similarity among males and similitude among females protein composition from different locations was noticed.

There are many female hormones involved in regulation of insect reproduction. The general scheme is that the follicle cells produce oostatic hormones that acts on the neuroendocrine system, such as 21 kDa and 19 kDa proteins found in the haemolymph of the locust [23]. In most insects Juvenile Hormone is involved in the regulation of oocyte development, this occurs at the level of vitellogenin production and uptake by the ovaries. i.e. vitellogenesis.[24] The hJHBP from *M. sexta* is composed of a single subunit, has a molecular weight of 27.4 kDa.[25]

It is confirmed that lipids, proteins and carbohydrates did not show significant differences between species or/and stages.[16] Within this context and by virtue of similar studies in other triatomines, it can be concluded that the haemolymph could not be used as a diagnostic character that would allow establish the difference among different populations of a same species of triatomine. But, it could be useful to know that this genetic stability may be important for the control of the insect given that the use of insecticide, in a genetically stable population may be more thriving. The composition of the *P. geniculatus* haemolymph populations evaluated in this work express increased homogeneity of this species, in accordance with other similarities reported by *Jaramillo* et al.[27] for *P. geniculatus* Colombian populations.

New epidemic evaluations, including specimens from other regions of Venezuela, it will be necessary to ratify the actual findings about haemolymph similarities among *P. geniculatus* positioned in different geographical locations. Haemolymph as population marker would be an important indicator of genetic stability in this group of triatomines. It would be also significant to profound in the control considerations of these insect groups.

Un estudio preliminar de la hemolinfa de cuatro poblaciones venezolanas de*Panstrongylus geniculatus* Latreille, 1811 (Hemiptera: Reduviidae) y su significado epidemiológico

RESUMEN

Se enfrentaron perfiles SDS-PAGE, de los 2 sexos, de las hemolinfas de *Panstrongylus geniculatus* de diversas regiones venezolanas: sabanas, piedemontes, bosque tropical y zonas urbanas y se determinó que las hemolinfas mostraron un perfil electroforético diferente, con proteínas que variaban de 14 a 164 kDa. Las bandas más representativas en el perfil de las hembras se observaron en 2 regiones: de 164 a 46 kDa y de 33 a 30 kDa. La banda principalmente ilustrativa de las proteínas de los machos se observó en una región: de 46 a 35 kDa. La composición de la hemolinfa de *Panstrongylus geniculatus* en las poblaciones evaluadas en este trabajo, expresaron alta homogeneidad de la especie, con una clara diferencia entre machos y hembras. Esta similitud puede ser útil para el control de estos insectos, conociendo que la estabilidad genética puede ser muy importante, porque el uso de insecticidas en una población con estas características siempre es más exitoso. Este trabajo, de acuerdo con la investigación bibliográfica, representa el primer estudio de hemolinfa de *Panstrongylus geniculatus*.

Palabras clave: Epidemiología, hemolinfa, *Panstrongylus geniculatus*, Reduviidae, Venezuela.

REFERENCES

1. Pinto C. Artrópodes, parasitos e transmissores de doenças. Bibliot Scient Brasil 1930;117:1-63.
2. Luitgards-Moura JF, Borges-Pereira J, Costa J, Zauza PL, Rosa-Freitas MG. On the possibility of autochthonous Chagas disease in Roraima, Amazon region, Brazil, 2000-2001. Rev Inst Med Trop Sao Paulo 2005;47:45-54.
3. Schofield CJ. Triatominae: Biología y Control. West Sussex, London: Eurocommunica Publications; 1994. p. 1-312.
4. Pifano F. El potencial enzoótico silvestre del complejo ecológico *Schizotrypanum cruzi-Didelphys marsupialis-Panstrongylus geniculatus* y sus incursiones a la vivienda humana del valle de Caracas-Venezuela. Bol Acad Cien Fís Matem Natur 1986;46:9-35.
5. Zeledón R. Epidemiology, modes of transmission and reservoir hosts of Chagas' disease. In: Ciba Fundation Symposium 20. Trypanosomiasis and Leihsmaniasis with special reference to Chagas disease. Amsterdam:Elsevier-Excerpta Medica; 1974. p. 76-7.
6. Pereira J, Dujardin JP, Salvatella R, Tibayrenc M. Enzymatic variability and phylogenetic relatedness among *Triatoma infestans, T. platensis, T. delpontei* and *T. rubrovaria*. Heredity 1996;77:47-54.
7. Reyes-Lugo M, Rodríguez-Acosta A. Domiciliation of selvatic chagas disease vector *Panstrongylus geniculatus* Latreille, 1811 (Triatominae: Reduviidae) in Venezuela. Trans Roy Soc Trop Med Hyg 2000;94(5):508.
8. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
9. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680-5.
10. Dice LR. Measures of the amount of ecological association between species. Ecology 1945;26:297-302.
11. Carcavallo RU, Galíndez Girón I, Jurberg J, Galvao C, Lent H. Pictorial keys for tribes, genera and species of the subfamily Triatominae. Atlas of Chaga´s disease vectors in the Americas. Brazil. Editors RU; 1998. p. 107-204.
12. Ramírez-Pérez J. Chipos de Venezuela. Caracas: Publicación de la Dirección de Malariología y Saneamiento Ambiental. Caracas: Ministerio de Sanidad y Asistencia Social; 1985. p. 1-108.
13. Valente VC. Potential for domestication of *Panstrongylus geniculatus* (Latreiile, 1811 (Hemiptera, Reduviidae, Triatominae) in the municipality of Muaná, Marajó Island, State of Pará, Brazil. Mem Inst Oswaldo Cruz 1999;94(1):399-400.
14. Barth R, Muth H. Estudos anatômicos e histológicos sobre a família Triatominae (Heteroptera, Reduviidae).VII Parte: Observações sobre a superfície dos ovos das espécies mais importantes. Mem Inst Oswaldo Cruz 1958;56:197-208.
15. Barbosa SE, Diotaiuti L, Soares RPP, Pereira MH. Differences in saliva composition among three Brazilian populations of *Panstrongylus megistus*. Acta Trop 1999;72:91-8.
16. Canavoso LE, Rubiolo ER. Haemolymphatic components in vectors of *Trypanosoma cruzi*: Study in several species of the subfamily Triatominae (Hemiptera: Reduviidae). Rev Inst Med Trop Sao Paulo 1993;35(2):123-8.
17. Schofield CJ, Dujardin JP. Theories on the evolution of *Rhodnius*. Actual Biol 1999;21(71):183-97.
18. Wigglesworth VB. Wound healing in an insect (*Rodnius prolixus*, Hemiptera). J Exp Biol 1937;14:364-81.
19. Jones JC. The circulatory system of insects. In: Rockstein M ed. The physiology of insecta. New York: Academic Press; 1964. p. 1-107.
20. Chapman RF. The Insects: structure and function. Cambridge: Cambridge University Press; 1998. p. 1-131.
21. Shapiro JP, Wasserman HA, Greany PD, Nation JL. Vitellin and vitellogenin in the soldier bug, *Podisus maculiventris*: Identification with monoclonal antibodies and reproductive response to diet. Arch Insect Biochem Physiol 2000;44:130-5.
22. Wang ZW, Davey KG. The role of juvenile hormone in vitellogenin production in *Rhodnius prolixu*s. J Insect Physiol 1993;39:471-6.
23. Zhang J, McCracken A, Wyatt GR. Properties and sequence of a female-specific, juvenile hormone-induced protein from locust haemolymph. J Biol Chem 1993;268:3282-8.
24. Ishizaka S, Bhaskaran G, Dahm KH. Juvenile hormone production and ovarian maturation in adult *Manduca sexta*. In: Tonner M, Soldan T, Bennettova B eds. Regulation of Insect Reproduction IV. USA: Academia Publishing House; 1989. p. 49-58.
25. Hidayat P, Goodman WG. Juvenile hormone and haemolymph juvenile hormone binding protein titers and interaction in the haemolymph of fourth stadium *Manduca sexta*. Insect Biochem Mol Biol 1994;24:709-15.
26. Forattini OP. Biogeografia, origem e distribuição da domiciliacão de triatomineos no Brasil. Rev Saúde Publica 1980;14:265-99.
27. Jaramillo ON, Castillo D, Wolff M. Geometric morphometric differences between *Panstrongylus geniculatus* from field and laboratory. Mem Inst Oswaldo Cruz. 2002;97(5):667-73.

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