Original article

External quality assessment of entomological identification of triatomines carried out in the Pernambuco Public Health Laboratory network, Brazil, 2017

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Abstract

Objective: To assess the quality of triatomine identification in the laboratory network of the state of Pernambuco, Brazil. **Methods:** The participating laboratories received support material with dichotomous keys and a panel made up of seven triatomine insects known in the Pernambuco, in order to identify specimen status on receipt (intact or damaged structure), as well as species and sex. **Results:** Nine out of 12 laboratories took part of the study. The proportion of correct answers was 56/63 for sex identification, and 45/63, for species. The answers did not present a direct relationship with occurrence of damage to morphological structures during insect transportation. *Panstrongylus megistus* identification was correct for all specimens (9/9 laboratories), while correct identification of species of the Rhodnius genus was the lowest (3/9 laboratories). **Conclusion:** Despite the good performance in entomological identification, the weaknesses observed may guide improvements in the laboratory network and will be essential for Chagas disease vector control programs.

Keywords: Health Human Resource Training; Chagas Disease; Insect Vectors; Public Health Surveillance.

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Introduction

Triatomines, commonly known as *barbeiros* in Brazil, are blood-sucking insects that are vectors of the *Trypanosoma cruzi* protozoan parasite, the etiological agent that causes Chagas disease, an illness that can become chronic and be potentially fatal. The Triatominae subfamily is currently distributed into five tribes, 18 genera, 151 recent species and three fossil species; mostly in the Neotropical region, although they can be found in a non-endemic form in the United States, Southeast Asia, Central and Southern Africa and Northern Australia.[1-3] These vectors are widely distributed over the Brazilian territory: there are records of 64 species in Brazil and 14 in the state of Pernambuco.[3-5]

The species representing greatest risk of vector transmission in Brazil are *Triatoma sordida, Panstrongylus megistus, Triatoma brasiliensis* and *Triatoma pseudomaculata*, all of which are found in Pernambuco. Another triatomine that has been frequently found in field research is *Panstrongylus lutzi*, a species considered to be sylvatic which is likely to occupy empty nests left by species eliminated through vector control measures. It is noteworthy that *P. lutzi* has a natural infection rate higher than that of the other species found in Pernambuco.[1,2]

> ***External quality assessment makes it possible to track improvement opportunities in a laboratory. It is of great importance for diseases of public health concern, such as Chagas disease.***

Several factors contribute to reliable laboratory test results, ranging from technical competence through to infrastructure and service management. A laboratory needs to have tools that are adequate for analyzing non-conformities and for implementing corrective and preventive actions.[6] One of these tools is external quality assessment, which enables the tracking of opportunities for improvement in a laboratory or a laboratory diagnoses network. This is of great importance when one considers diseases of interest to public health.[7] Participation in external quality assessment programs is one of the requirements of the operating standards for health laboratories set by the Brazilian Association of Technical Standards (*Associação Brasileira de Normas Técnicas* - ABNT). Such standards contribute to the development of laboratory quality management systems, for confirmation or recognition of their competence.[8]

Epidemiological monitoring of Chagas disease is based on entomological surveillance and vector control, some of which are recognized for their greater risk of vector transmission. Moreover, there is no effective vaccine or treatment for this disease.[9]

Considering the importance of correct taxonomic identification of triatomines, and the urgency of putting into practice an analytical quality control program for this form of screening, the Endemic Diseases Laboratory of the Pernambuco State Health Department Central Public Health Laboratory organized the first external assessment of triatomine quality, with the objective of analyzing the quality of entomological identification of Chagas disease vectors in the Pernambuco laboratory network.

Methods

This is a cross-sectional study the objective of which was to assess the laboratories that identify triatomines in the state of Pernambuco, in 2017. The insects received at the laboratories for species identification are caught by health workers who operate exclusively in households (indoors and around households and their outhouses), or are handed in by the population at triatomine information points. This study was carried out by the Pernambuco Central Public Health Laboratory (LACEN/PE), based on guidelines established in specific ABNT standards for laboratory quality management.[8]

The study consisted of an external quality assessment, based on the voluntary participation of laboratories that answered the invitation letter sent by the provider, namely LACEN/PE, guaranteeing impartiality, independence and confidentiality with regard to the identification of participating laboratories. Following their acceptance, the insects were sent to the participating laboratories, along with the instructions required to meet the evaluation needs.

The assessment panel was comprised of triatomine specimens bred in an insectarium and provided by the National and International Triatomine Taxonomy Reference Laboratory of the Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, RJ. The

following triatomine species were used to comprise the panel: *Panstrongylus megistus, Rhodnius neglectus, Rhodnius nasutus, Triatoma infestans, Triatoma pseudomaculata, Triatoma sordida,* and *Triatoma tibiamaculata.*

The seven species were selected according to the following criteria: four species of epidemiological importance and three primarily sylvatic species, with greater or lesser potential for invading households and immediate surrounding areas, all of which are present in the state of Pernambuco.[10] Due care was taken with their morphological similarities, essential for the identification of each specimen. The specimens were fastened to polystyrene sheets with entomological pins and then placed inside wooden cases. Each case, containing all the specimens, was covered with plastic bubble wrap and then wrapped with ordinary paper for sending to the participating laboratories.

For analysis, laboratories used stereoscopic microscope or manual magnifier. The participants were instructed to use dichotomous keys as the main identification tool. The final deadline for sending the results to LACEN/PE was within 30 days with effect from the receipt of the panel by the laboratory.

Laboratories located in Pernambuco were invited to take part in the study. Pernambuco is a state located in the Mideast area of Brazil's Northeast region. Its estimated population in 2020 was 9,616,621 inhabitants and, according to the 2010 Census, 1,744,238 of them lived in rural areas more exposed to contact with vectors.[11-13] The state is divided into 12 health regions for management purposes. Geographically, Pernambuco is classified in mesoregions (subdivisions) defined by geomorphological differences: the Recife (state capital) metropolitan region, which is coastal and characterized by remains of Atlantic Rainforest; Mata, which is a region comprised of remains of Atlantic Rainforest and sugarcane plantations; Agreste, an area of transition between the Atlantic Rainforest and the Caatinga (brushwood); and São Francisco and Sertão, which correspond to the semiarid regions of the Caatinga.[14,15] In view of their very classification, each one of these geographic regions has different species of triatomines.

Pernambuco has 13 laboratories that screen for triatomines. Most of them are state-level laboratories subordinated to the 12 health regions (regional laboratories); only two laboratories are subordinated to municipal health departments, namely Caruaru and Garanhuns (Figure 1). The LACEN/PE is the reference laboratory for the state of Pernambuco. It is responsible for monitoring the quality control of laboratories that perform diagnostic tests of interest for health surveillance. LACEN/PE is responsible for the technical coordination of the state's other entomology laboratories, the main objective of their activities being to identify disease vectors and etiological agents, including triatomines. LACEN/PE performs triatomine identification for the I and XII health regions of Pernambuco, so that their participation in the evaluation would not have been justified.

Variables used in the analysis were:

- Specimen status on receipt (intact or damaged structure – if they were damaged, the laboratories were asked to record this);
- Identification of equipment used (hand-held magnifying glass; stereoscopic microscope);
- Sex of the identified specimen (male; female);
- Taxonomic identification of the specimen to the level of species.

A standardized form was used for data collection and was filled in by the technician of each participating laboratory.

The species and sex identification results provided by the laboratories were compared with the LACEN/PE template and rated either as correct or incorrect. If there was no answer, this was considered to be an incorrect answer, for the purposes of calculating the proportion of correct answers.

With the aim of controlling bias, each participating laboratory received a sample of each species, without the taxonomic composition of the panel being revealed beforehand. The samples were identified randomly by drawing numbers and their identity was only known by LACEN/PE. Each laboratory received a standardized form for recording the results, with a format similar to the form used by them to record routine analyses, as well as support material for analyzing the specimens: an iconographic atlas with dichotomous keys; and a set of illustrated cards of all the species recorded in Brazil.

Microsoft Excel 2016 was used for the descriptive analysis of the data obtained.

Results

Nine of 12 invited laboratories took part in the external quality assessment, eight of which were regional laboratories (II, III, IV, V, VIII, IX, X and XI health regions) and one was a municipal laboratory (Caruaru), involving 17 technical health workers.

Eight laboratories had a stereoscopic microscope; Laboratory A performed its analysis with a hand-held magnifying glass. Of the nine participating laboratories, only one reported that the panel was open when it was received; the remaining laboratories confirmed adequate conditions for analysis, although some samples were damaged. Out of the total specimens sent, damage was found in the following structures: legs, antennae, tarsi, tibia and pronotum. Only two laboratories (A and I) reported that all specimens had intact structure (Table 1). Only two of the samples with incorrect identification of the *R. nasutus* and *R. neglectus* species were damaged; however, the damaged structures (antennae and tibia) did not make it impossible to identify the species. Species identification was correct for the rest of the samples recorded as being damaged.

The total proportion of the laboratories' correct answers for identification of sex was greater (56/63) than for identification of species (45/63).

Figure 2 shows the proportion of correct answers for identification of triatomine species and sex. Only Laboratory D had 7/7 correct answers both for species and for sex. Although Laboratories A, B, C and G had 7/7 correct answers for identification of sex, the proportion was lower for identification of species: respectively, 2/7, 4/7, 6/7 and 4/7. It should be noted that Laboratory A only provided answers for 3 of the 7 samples with regard to identification of species.

The answers of the participating laboratories identifying the triatomine species are summarized in Figure 3. The specimen belonging to the *R. neglectus* species was incorrectly identified as *T. pictipes* by Laboratory B, *R. brethesi* by Laboratories C and G, and as *R. domesticus* by Laboratory I.

With regard to the number of correct answers for identification of triatomine species (Figure 4), the only species with 9/9 correct answers was *P. megistus*. The species of the *Triatoma* genus were correctly identified by the largest number of participants: 8/9 correct answers for *T. infestans* and *T. tibiamaculata*; and 7/9 correct answers for *T. pseudomaculata* and *T. sordida*.

Discussion

Adherence to the external evaluation of the quality of entomological identification of triatomines carried out in Pernambuco's public laboratory network was good and thus enabled identification of differences between laboratories. External quality evaluation programs

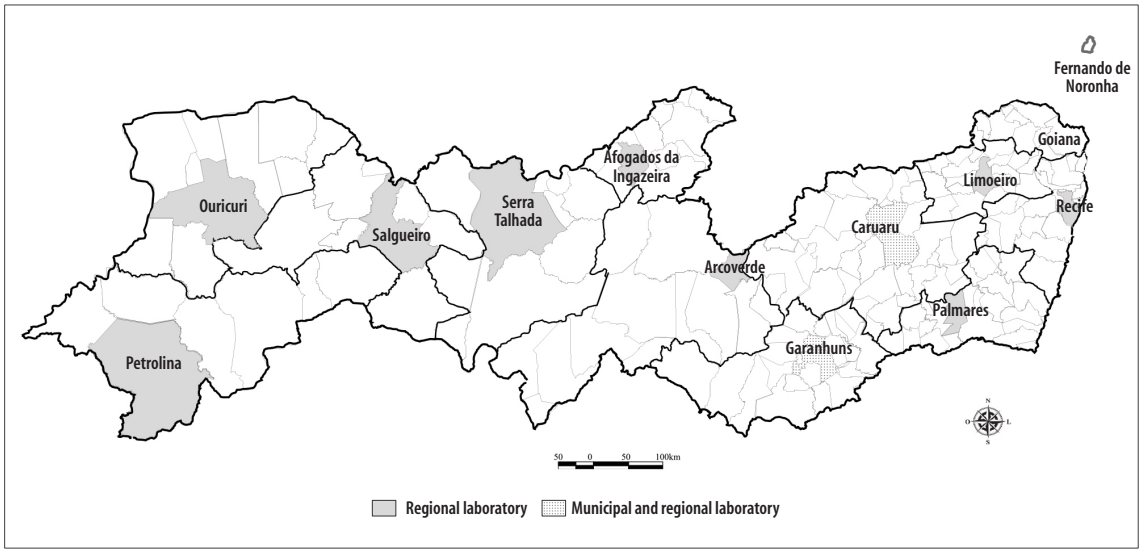

Figure 1 – Location of the triatomine entomology laboratories, according to regional or municipal health administration, Pernambuco, 2017

Table 1 – Record of specimen status on receipt, for each laboratory taking part in the external quality assessment of triatomine identification (n=63), Pernambuco, 2017

Laboratory	Intact structure	Damaged structure	Description of damaged structure	No answer
A	7	–	–	–
B	5	1	Legs	1
C	4	3	Antennae, tarsi, legs	–
D	6	–	–	1
E	3	3	Antennae	1
F	2	5	Antennae, tibia, tarsi	–
G	5	2	Antennae	–
H	5	1	Antenna and pronotum	1
I	7	–	–	–

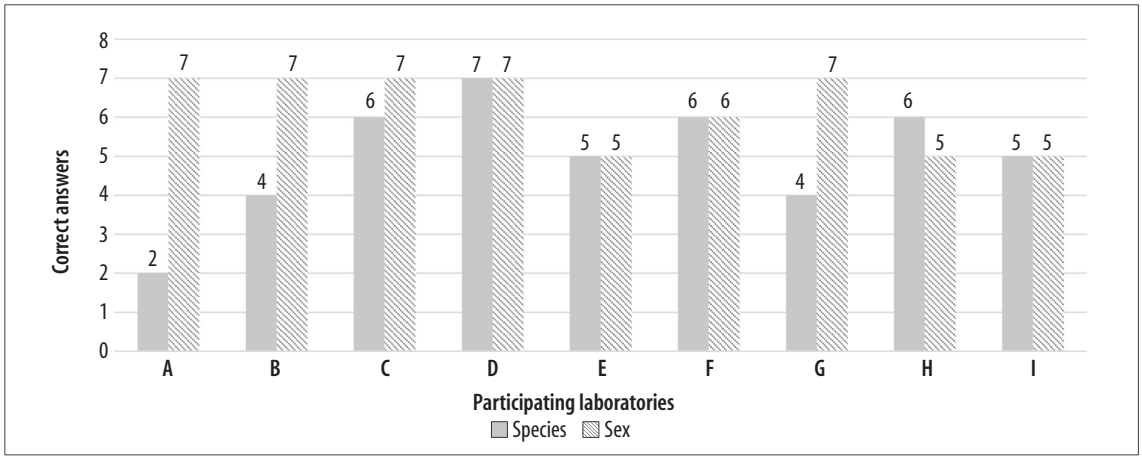

Figure 2 – Proportion of correct answers in triatomine species and sex identification, as per each laboratory taking part in the external quality assessment of triatomine identification (n=63), Pernambuco, 2017

Species (reference samples)	Answers from the laboratories								
	A	B	C	D	E	F	G	H	I
Panstrongylus megistus	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Rhodnius neglectus	–	(-) *Triatoma pictipes*	(-) *Rhodnius brethesi*	(+)	(-) *Rhodnius*	(+)	(-) *Rhodnius brethesi*	–	(-) *Rhodnius domesticus*
Rhodnius nasutus	(-) *Rhodnius domesticus*	(-) *Rhodnius robustus*	(+)	(+)	(-) *Predador*	(-) *Rhodnius prolixus*	(+)	(+)	(-) *Rhodnius robustus*
Triatoma infestans	–	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Triatoma pseudomaculata	–	(-) *Triatoma maculata*	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Triatoma sordida	–	(+)	(+)	(+)	(+)	(+)	(-) *Triatoma lenti*	(+)	(+)
Triatoma tibiamaculata	(+)	(+)	(+)	(+)	(+)	(+)	(-) *Triatoma melanocephala*	(+)	(+)

Legend: (+) Correct answer; (-) Incorrect answer, with indication of the answer given; – No answer.

Figure 3 – Answers given by the laboratories in the external quality assesment of triatomine identification, by species (n=63), Pernambuco, 2017

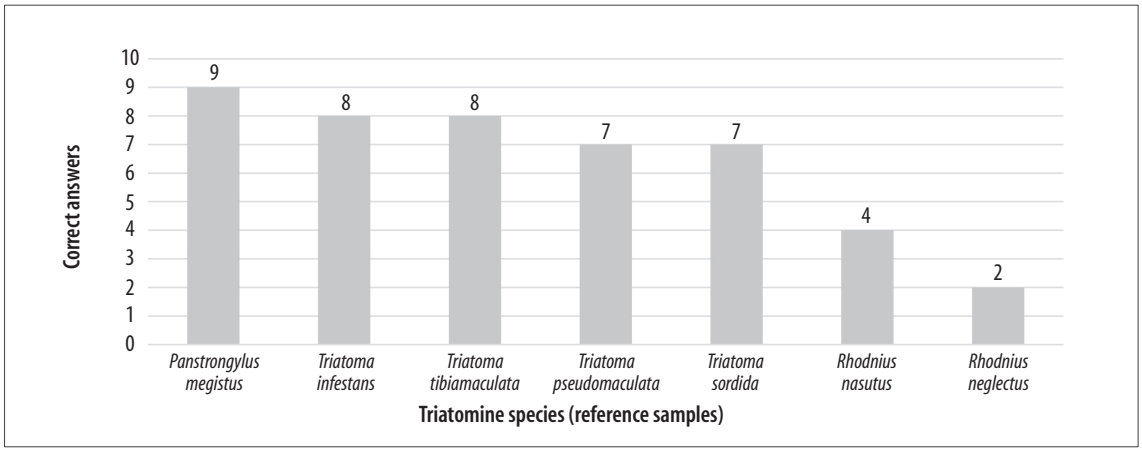

Figure 4 – Total of correct answers as to triatomine identification, according to external quality assessment of triatomine identification, as per reference samples (n=63), Pernambuco, 2017

enable results of one laboratory to be compared to those of other participating diagnosis laboratories, by a third independent laboratory.[16] The results of this study point to the existence of health workers with different levels of knowledge about correct use of dichotomous keys and the triatomine species that occur in the state of Pernambuco. Health workers with greater performance difficulties may fail to record the occurrence of new species in the state during their assessment.[17,18] Corrective measures should be implemented in order to achieve better results.

All the laboratories that perform entomological identification in Pernambuco are public. In general, one of the biggest problems they face is staff turnover, resulting from temporary employment contracts and staff retiring, without immediate replacement, in addition to lack of equipment maintenance. Taxonomic identification is an eminently technical challenge and as such requires development of professional competence. Incorrect results in species identification have a strong tendency to be associated with the health worker dimension, whereby lack of continuing training and capacity building is a determinant factor of this limitation.[19,20]

Unavailability of equipment that is adequate for laboratory activities is another barrier to diagnostic quality. This was reflected in the results obtained by Laboratory A: namely, its larger number of identification errors and being the only laboratory not to have a stereoscopic microscope with which to analyze the insects.[20]

The morphological similarities between the *Rhodnius* genus are considerable. In many cases, molecular techniques need to be used in order to differentiate species considered to be cryptic (isomorphic). This is the case of *R. prolixus* and *R. robustus*, the species of the genus used in the assessment, which are considered to be easy to differentiate. Notwithstanding the specimens of the genus had a lower number of correct identifications. The study also recorded a mistake in the nomenclature used to identify the genus in the case of *T. pictipes*. It should be noted that such an answer is impossible to obtain when using dichotomous keys.[21,22]

Apart from morphological similarities, there are other possible causes of identification errors: lack of attention when checking the results; support material insufficient or out of date; insufficient knowledge about the nomenclature of the insects' external morphology; and difficulty in regulating equipment. The correct identifications found for insects with damaged morphological structure should be interpreted as a positive and considerably relevant result, given that in their work routine the staff of these laboratories receive a large number of damaged triatomines caught by the population and handed in to triatomine information points.

The methods used in this research proved to be useful to evaluate the quality of the identification of triatomines. It can be used in the laboratory networks of other states and, moreover, in the implementation of a national program with this objective. Generally speaking,

the participating laboratories had good performance; however, the results of the study indicated weaknesses, so that corrective actions are recommended in order to enhance the service provided. The actions recommended extend to the provider, LACEN/PE, as the responsible for monitoring the public laboratory network, and also to the participating laboratories as those which perform the analyses.

Provider responsibility actions include the maintenance of the external quality assessment program and the continuing training of all Pernambuco's entomology laboratories, with the aim of keeping their technical staff up to date and encouraged to use the dichotomous key in order to obtain correct specific identification. Actions indicated as being the responsibility of the participating laboratories are aquisition and periodic maintenance of stereoscopic microscope, effective and continuing participation in the external quality assessment program, and in the trainings and capacity building courses held by the Endemic Diseases Laboratory of the Pernambuco State Health Department Central Public Health Laboratory. Finally, special attention should be dedicated to diagnosis of species of the *Rhodnius* genus and to studying the insects' external morphology.

Authors' contributions

Silva MBA, Ferreira GMOG and Borba RFB contributed to the design of the study, data analysis and interpretation and drafting the first version of the manuscript. Rocha DS and Medeiros CA contributed to data analysis and interpretation and critically reviewing the manuscript. All the authors have approved the final version and are responsible for all aspects thereof, including the guarantee of its accuracy and integrity.

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