Discrepancies between *Aedes aegypti* identification in the field and in the laboratory after collection with a sticky trap

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Currently, sticky traps are regularly employed to assist in the surveillance of Aedes aegypti *infestation. We tested two alternative procedures for specimen identification performed by local health agents: directly in the field, as recommended by certain manufacturers, or after transportation to the laboratory. A total of 384 sticky traps (MosquiTRAP) were monitored monthly during one year in four geographically representative Brazilian municipalities. When the same samples were inspected in the field and in the laboratory, large differences were noted in the total number of mosquitoes recorded and in the number of specimens identified as* Ae. aegypti *by both procedures. Although field identification has the potential to speed vector surveillance, these results point to uncertainties in the evaluated protocol.*

Key words: *Aedes aegypti* - MosquiTRAP - entomological surveillance - dengue vector

One of the most important challenges faced by field entomologists is to develop a reliable and effective technique to sample the target species. Such a tool should provide significant information about several aspects of insect biology, including population density, dispersal and survival estimates. If we focus specifically on disease vectors, efficient and unbiased sampling tools are required to provide relevant insights into the effectiveness of vector control strategies and the risk of disease transmission (Service 1993).

On the American continent, the mosquito *Aedes aegypti* is the primary vector of dengue fever and is distributed from the United States of America to the Southern Cone of South America (Powell & Tabachnick 2013). This species is highly anthropophilic, living in close association with human dwellings: mosquitoes are more abundant in highly urbanised areas, feed preferentially on human blood, and lay eggs in man-made containers (Clements 1999). Although there is an extensive literature on *Ae. aegypti* sampling, the lack of a "gold standard" means that there is a need to continue the development of new surveillance techniques.

In the past decade, special attention has been focused on the design of mosquito traps. Collection of adult mosquitoes should provide better infestation indices than alternative techniques based on surveys of immature insects because the adult population is responsible for disease transmission. Sticky traps are a popular type of adult trap and many versions have been developed worldwide. One of these devices is the MosquiTRAP, which was developed in Brazil and has been subjected to exhaustive tests to determine its efficiency in collecting *Ae. aegypti* mosquitoes (Fávaro et al. 2006, Maciel-de-Freitas et al. 2008, de Resende et al. 2010, 2012, 2013). The MosquiTRAP consists of a one-litre matte black plastic cylindrical container filled with approximately 300 mL of 10% grass infusion substrate; alternatively, a synthetic oviposition attractant is employed. A sticky card is placed on the inner wall of the trap to capture gravid adult female mosquitoes attracted to the trap (Fávaro et al. 2006). One advantage of sticky traps over alternative sampling techniques is the opportunity to accelerate the surveillance procedure by counting and identifying captured mosquitoes in the field rather than in the laboratory under a microscope. In theory, this approach is possible because the specimens are fastened to an adhesive card, whereas they remain free and flying in other trap designs. This approach is included in the original MosquiTRAP protocol, which recommends that health agents perform species identification in the field to accelerate *Ae. aegypti* surveillance (Resende et al. 2010).

However, there is no consensus regarding where the identification procedure should be performed for various types of sticky traps. The site of identification has been reported to be in the field (Facchinelli et al. 2008), in the laboratory (Williams et al. 2006, Chadee & Ritchie 2008) or has even not been mentioned (Santos et al. 2012).

In this study, we tested the hypothesis that misidentification of mosquitoes may be an important source of uncertainty and measurement error, undermining the potential gain from vector control policies based on field identification alone. This problem would be especially significant if a surveillance system based on the adult sticky traps were applied in a routine large-scale program. The motivation for the present evaluation is the observation that in routine surveillance programs, working conditions are generally not favourable for the accurate identification of mosquito species in the field. We present results from a MosquiTRAP surveillance study conducted in four cities where *Ae. aegypti* identification in the field was compared with further identification of the same samples under laboratory conditions using a stereomicroscope.

The field work, performed within the scope of the Brazilian dengue control program, was conducted in Parnamirim, state of Rio Grande do Norte (December 2010-November 2011), Santarém, state of Pará (March 2011-February 2012), Nova Iguaçu, state of Rio de Janeiro (July 2011-June 2012) and Campo Grande, state of Mato Grosso do Sul (December 2011-November 2012), all municipalities representing Brazilian regions with a high incidence of dengue. All of these cities have adequate routine mosquito surveillance programs, which include laboratory teams trained in the identification of *Ae. aegypti*, *Aedes albopictus* and *Culex quinquefasciatus*. In addition to their previous experience, a specific two-day training program was conducted for all field workers before initiating the study. Each municipality had 12 health agents responsible for MosquiTRAP installation, mosquito identification in the field, trap deployment and storage of sticky cards for laboratory team identification of the same samples. The complete training lasted two days (16 working hours) and was conducted before field surveillance began. The ability of field workers to identify mosquitoes under field conditions was evaluated during every round of MosquiTRAP monitoring, in which a consultant of the Brazilian Health Ministry or one of the co-authors supervised health agents during one week. In each municipality, two or three additional field workers were trained to guarantee the quality of mosquito identification if it was necessary to replace health agents.

Monthly, during one year, three areas of 1 km² in each of the four studied municipalities received 96 MosquiTRAPs loaded with a synthetic attractant (32 traps per 1 km² area, 384 traps in the study). After informed oral consent had been received from the householder, sticky traps were installed on the premises to be sampled. After seven days, a health agent collected the trap. While still in the house, the agent used a hand magnifying glass to inspect and identify mosquitoes stuck to the card. The traps were then carefully stored and brought to the entomological laboratory where mosquitoes were identified again, this time by laboratory technicians, with the help of a stereomicroscope and identification keys (Consoli & Lourenço-de-Oliveira 1994).

The Jaccard index was used to test the degree of similarity among mosquitoes identified as *Ae. aegypti* in the field or in the laboratory (Legendre & Legendre 1998).

This index quantifies the similarity between two finite sample sets. It is defined as the size of their intersection relative to the size of the sum of the sample sets. The Jaccard index varies between 0-1; in the present work, 0 means no agreement, whereas 1 means total agreement between the field and laboratory measurements. Because the data take the form of number of mosquitoes per trap, it is not possible to assess the individual-level identification status of each specimen. To circumvent this problem, maximal agreement between field and laboratory identifications was assumed. Thus, for example, suppose that a trap contained 10 mosquito specimens, of which four and five specimens were identified as *Ae. aegypti* in the field and in the laboratory, respectively. In this case, we assumed that the four mosquitoes identified in the field belonged to the same group identified in the laboratory. This is a conservative assumption that favours field-laboratory agreement.

In general, the total numbers of mosquitoes recorded in the field were higher than those detected by the laboratory personnel (Table). This difference varied considerably among sites. A difference of < 15% between field and laboratory measurements was detected in Parnamirim and Nova Iguaçu, whereas a 70% difference was observed in Campo Grande. In Santarém, the opposite pattern was observed: laboratory measurements exceeded field records by 13%. Differences between laboratory and field measurements can be related to misidentification and to counting errors under field conditions as well as to losses or damage of insects during transportation to the laboratory that would interfere with identification. Also contributing to these differences are the difficulties involved in cleaning the sticky cards between two collection events. Because MosquiTRAPs were installed monthly and the sticky cards do not expire for 60 days, they were used twice, following advice that was intended to reduce costs. Further studies should investigate whether card reuse decreases the correctness of mosquito identification.

Overall, the amount of material not identified as *Aedes* (Table) (group "Non-*Aedes*", see also the "Non-*Aedes* total captured" column) varied between 35-70%, confirming the low to moderate specificity of MosquiTRAP for catching *Aedes* mosquitoes (Maciel-de-Freitas et al. 2008, Resende et al. 2013). In all municipalities, the proportion of these mosquitoes classified as non-*Aedes* was higher in field than in laboratory measurements. The difference between the field and the laboratory varied from 3% in Parnamirim and Nova Iguaçu to nearly 40% in Campo Grande. In Campo Grande, in addition to the difference of 70% in the number of specimens between the field and the laboratory, the contribution of the non-*Aedes* group to the total in the field assessment was almost 90%. These findings indicate specific difficulties with the Campo Grande results related to the work of the health agents in the field.

If only mosquitoes belonging to the genus *Aedes* are considered, except for Santarém, more than 80% were identified to the species level under both field and laboratory conditions (Table) [column "(aeg + alb)/*Aedes*)"].

TABLE

Total numbers and percentages of mosquitoes registered and identified in the field or in the laboratory in each locality

Municipality (state)	Identification protocol	n						%			
		Total captured	*Aedes aegypti*	*Aedes albopictus*	*Aedes* sp.	Non-*Aedes*	Non-*Aedes*/ total captured	(aeg + alb)/ *Aedes*[a]	aeg/ *Aedes*[a]	aeg/ (aeg + alb)
Santarém	Field	3,976	370	94	779	2,733	68.7	37.3	29.8	79.7
(Pará)	Laboratory	4,494	172	0	1,691	2,631	58.5	9.2	9.2	100
Parnamirim	Field	786	220	79	68	419	53.3	81.5	59.9	73.6
(Rio Grande do Norte)	Laboratory	684	256	79	1	348	50.9	99.7	76.2	76.4
Nova Iguaçu	Field	1,066	478	48	121	419	39.3	81.3	73.9	90.9
(Rio de Janeiro)	Laboratory	949	517	84	0	348	36.7	100	86	86
Campo Grande	Field	1,050	87	16	15	932	88.8	87.3	73.7	84.5
(Mato Grosso do Sul)	Laboratory	305	137	11	5	152	49.8	96.7	89.5	92.6

a: *Aedes* accounts for the sum of *Ae. aegypti*, *Ae. albopictus* and *Aedes* sp.; *Aedes* sp.: specimens identified only up to the genus level; non-*Aedes*: mosquitoes belonging to other genera or that could not be identified as *Aedes* ones. The columns "percent" exhibit ratios of non-*Aedes* mosquitoes relative to the total of caught specimens (non-*Aedes*/total captured), of *Aedes* mosquitoes identified up to the species level [*Ae. aegypti* (aeg) or *Ae. albopictus* (alb)] among those identified as *Aedes* [(aeg + alb)/*Aedes*] and of identified *Ae. aegypti* mosquitoes, both considering all specimens identified as *Aedes* (aeg/*Aedes*) and those identified up to species level, *Ae. aegypti* and *Ae. albopictus* [aeg/(aeg + alb)].

The percentage of identification to the species level was higher in the laboratory (96-100%) than in the field (81-88%). The same was true for the specimens identified as *Ae. aegypti* (Table) (column "aeg/*Aedes*"): this proportion was higher in the laboratory (75-90%) than in the field (60-75%). In all cases, a high proportion of *Ae. aegypti* mosquitoes among those identified to the species level was observed (Table) [column "aeg/(aeg + alb)"]. Lastly, the degree of similarity between *Ae. aegypti* identification in the field and in the laboratory based on the Jaccard index was low, ranging from 0.24-0.35 in the various municipalities (Figure).

The significant differences observed between *Ae. aegypti* identification in the field and in the laboratory suggest that these procedures provide discordant measurements of mosquito infestation and raise questions regarding the most appropriate protocol. Although Resende et al. (2010) reported a high level of agreement between field and laboratory identifications, the authors did not explain how the problem of potentially unidentifiable specimens was circumvented. Laboratory identification may be more accurate if health agents working in the field are subjected to multiple biotic and environmental stressors. In addition, mosquitoes adhere to the adhesive card in many different positions, a characteristic that may hamper identification that is primarily performed under field conditions. Moreover, loss or damage of material during transportation to the laboratory may be a potential problem for laboratory identification. Note that either under field or laboratory conditions, the loss or damage of identifiable specimens is inherent to sticky traps, a characteristic that introduces unforeseen uncertainties in the population estimators and might result in biased entomological indicators. Future studies should determine to what extent these differences could impact routine entomological surveillance.
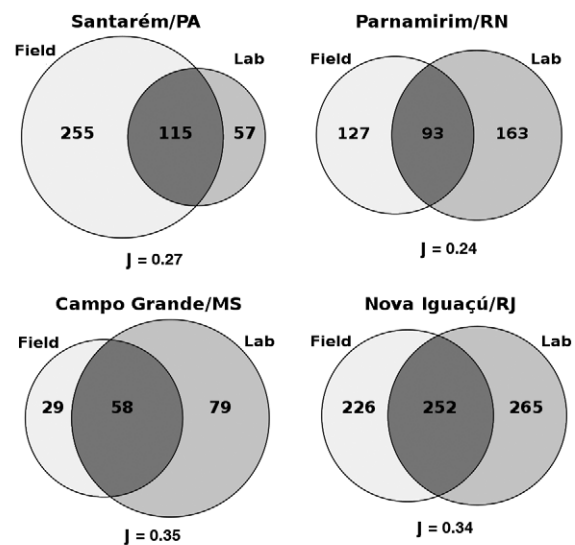
Conformity of identification in the field and in the laboratory of *Aedes aegypti* specimens caught with MosquiTRAP. For each municipality mosquitoes identified only in the field are at the left side, while those identified only at the laboratory, at the right side. The intersection represents specimens identified by both procedures, considering maximal conformity after inspection of each individual field bulletin. The Jaccard index (j), that reflects similarity between both mosquito sets, varies from 0 (completely distinct sets) to 1 (total identity). The Brazilian states: Mato Grosso do Sul (MS), Pará (PA), Rio de Janeiro (RJ) and Rio Grande do Norte (RN).

The proper use of mosquito traps requires an adequate work environment and dedicated worker teams. In this sense, it is important to invest time and resources on the training and qualification of health agents. Although traps decrease the bias in sampling resulting from variation in the motivation of health agents, these tools are still dependent on human operation and skills.

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