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Rudimentary cesspits as breeding sites for *Aedes aegypti* in urban areas of Northern Brazil

Fossas negras rudimentares como locais de reprodução do *Aedes aegypti* em áreas urbanas do Norte do Brasil

Pozos negros rudimentarios como criaderos de *Aedes aegypti* en las zonas urbanas del Norte de Brasil

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ABSTRACT

We evaluated rudimentary cesspits as potential breeding sites for the mosquito *Aedes aegypti* and mosquito production compared to those officially designated common breeding sites (CBS). In November and December of 2009, immature forms of the insect were collected in water samples of cesspits as well as of CBS, the latter being any object that potentially accumulates water such as used tires, flower pots and sites of solid waste disposal. Samplings were performed in urban areas of two municipalities of Rondônia State in the Brazilian Western Amazon. Immature and adult forms of *Ae. aegypti* were observed in cesspits confirming these as novel breeding sites for the mosquito in that region. The proportion of cesspits found with *Ae. aegypti* immature forms in the two surveyed localities were as follows: Espigão do Oeste, 50.0% and Jaru, 27.3%. Moreover, the average number of insects did not differ significantly in the sampled cesspits and CBS. These results provides subsidies for change in the policies and strategies of dengue vector control in Brazil, taking into account the limited sanitation infrastructure and the local particularities of the Amazon Region. They also point to the urgency of investments and adequacy of efforts in systems of public sanitation in developing countries, to aid in the control of this important vector of viral diseases.

Keywords: Amazon Biome; Insect Vectors; Dengue; Oviposition; Urban Sewage.

The mosquito *Aedes aegypti* is the main vector of *Dengue virus* in the Americas[1] and *Ae. albopictus* was described as a potential vector[2]. In the latter species, vertical transmission of this arbovirus has been described in specimens of the mosquito collected in the field[3]. Since there is no safe and effective vaccine against the virus[4,5], dengue prevention rely on insect control. Such actions have also become more important due to the recent spread of *Chikungunya virus* and *Zika virus* in Brazil[6,7]. The main strategies of integrated control of *Ae. aegypti* aim to eliminate (i) immature forms in breeding sites through entomological surveys and (ii) adult forms through the use of insecticides[8]. A simple action of covering common breeding sites (CBS) as water tank and metal drum had the effect of diminishing the number of adult female *Ae. aegypti*, in long-term temporal scale[9]. However, failure to completely suppress vector population and disease has been observed in spite of such measures. Many factors might be contributing to this failure such as fast and disorganized urbanization, insecticide resistance of insect vector, non-synchronized top-down/bottom-up control strategies[10], or "epidemic-dependence" on planning and resource implementation[11].

As reported, the number of dengue cases is on the rise in many places, especially in tropical and subtropical regions[12]. Though in Rondônia State a recent tendency of decline was observed, with a 67% reduction in 2014

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in relation to 2013[13], the number of dengue cases has been on the rise since the first record of the virus in 1997. Virus serotype in this outbreak was not determined but in subsequent years, between 2001 and 2003, the serotype 1 was found predominant in Porto Velho[14]. Serotype 3 was detected in the period of 2004-2006 in the Municipalities of Ariquemes, Jaru, Ouro Preto do Oeste, Cacoal, Colorado do Oeste, Vilhena and Porto Velho[15]. In the year of 2009, entomological surveys were carried out due to the high number of cases reported[16]. A substantial number of immature mosquito forms was observed in cesspits, a place not officially listed as *Ae. aegypti* breeding site. Cesspits in the region are rudimentarily constructed and with loose gaskets, which freely allow insect passage. Septic tanks have been pointed as highly productive breeding places for *Ae. aegypti*[17,18,19,20]. Differently from septic tanks, however, cesspits have no outlet pipe. Therefore, waste water accumulated inside its chamber might turn cesspits even more productive. Taking into account that (i) there are no technical orientation by the National Dengue Control Program on cesspits and septic tanks during entomological surveys[21,22] and that in (ii) Northern Brazil less than 10% of households are connected to public sewage systems[23], such breeding places could be responsible for maintaining *Ae. aegypti* population density high, even with continuous and regular vector control campaigns. Hence, the present study aimed to (i) evaluate cesspits as potential and efficient breeding sites for *Ae. aegypti* by comparing measurements of infestation (presence/absence of larvae/pupae) in cesspit and CBS and (ii) measure some physicochemical parameters and evaluate suitability of conditions for insect oviposition and procreation in water samples from cesspits.

We conducted a transversal descriptive survey in urban areas of two Municipalities of the Rondônia State, Espigão do Oeste (6.4 inhabitants per km^2; 11°31'44.9"S; 61°00'49.7"W) and Jaru (17.7 inhabitants per km^2; 11°40'53.3"S; 61°10'37.6"W), Brazilian Western Amazon (Figure 1), in November and December of 2009. We randomly selected residential and commercial buildings located near the addresses of reported dengue cases in central and peripheral limits of the cities. Addresses were provided by the Municipal Health Secretary. Outdoor and indoor of households in Espigão do Oeste and Jaru (N = 8 and N = 11, respectively) were inspected by technician and researcher teams of the Research Institute of Tropical Diseases (Instituto de Pesquisas em Patologias Tropicais – IPEPATRO) and Centre for Research in Tropical Medicine (Centro de Pesquisa em Medicina Tropical – CEPEM). The area surrounding the household (peridomiciliar) up to 15 m was considered "outdoors". Water tank, plastic and metal drum, tires, pitchers, and solid waste were considered conventional CBS[24] and water samples at these sites were collected according to official guidelines[25]. Water samples in cesspits were collected three to five times at different points of the liquid surface up to 1 L final volume, with the aid of a halved polyethylene terephthalate (PET) bottle attached to an aluminum pole. Immature mosquito forms present in the liquid sample were collected with a plastic pipette, transferred to a 2 L plastic bottle and recorded appropriately (location, date, time, etc.) (Figure 2). The immature mosquito forms were then sent to the insectary of the Laboratory of Entomology at IPEPATRO/Fiocruz and reared in water containing white bowls to adult stage for subsequent identification using taxonomic keys[26]. Averaged numbers of adult forms were calculated by dividing the number of mosquitoes obtained in laboratory by the total number of containers (total number of surveyed sites, positive and negative), to minimize the effect of unequal sample size in the comparisons among localities and breeding place categories. A chi-square test with Yates's correction factor was performed to compare the proportion of samples positive and negative for *Ae. aegypti,* in cesspits and CBS. Chi-square was based on the significance level of $\alpha = 0.05$ for the comparisons and computed using the web-based software OpenEpi v. 3.01[27]. Water samples were collected from the cesspit and sent to the governmental laboratory of Water and Sewerage Company of Rondônia (Companhia de Águas e Esgotos de Rondônia – CAERD), located at Espigão do Oeste Municipality. The following parameters were measured by CAERD certified technician: pH, color (Hazen Units – HU) and turbidity (Nephelometric Turbidity Units – NTU) and compared to standard values for water supplied to households by the public water system, as recommended by the Brazilian Health Ministry[25]. The parameters measured of water from cesspits were compared to those of drinking water and served to evaluate the range of favorable conditions for insect oviposition and procreation.

A grand total of 2,288 adult mosquitoes were obtained from the 6,556 immature forms (larvae and pupae stages) collected in cesspits (Cess: N = 19) and conventional breeding sites (CBS: N = 11) at the Municipalities of Espigão do Oeste and Jaru. Ninety one percent or 6,001 larvae were collected in Cess and the remainder 9% or 555, in CBS. The collection in Espigão do Oeste resulted in 2,042 immature forms, being 81.3% (1,661) from eight Cess and 18.7% (381) from seven CBS. In Jaru, 4,514 immature forms were collected of which 96.1% (4,340) from 11 Cess and 3.9% (174) from four CBS (Table 1).

Three species of mosquitoes were identified using taxonomic keys on adults obtained from the collected immature forms: *Ochlerotatus fluviatilis*, *Culex quinquefasciatus* and *Ae. aegypti* (Table 1, Figure 3). The latter was represented by 49.0% of the total number of adult specimens obtained from the immature forms (Table 1). Almost the same percentage was observed for *Cx. quinquefasciatus* or 50.4% while *Oc. fluviatilis* had a minor frequency of 0.6%. Interestingly, *Oc. fluviatilis*, which is (i) used in studies of experimental avian malaria transmission in laboratory conditions[28]; (ii) hypothetical, but not confirmed, vector of sylvatic yellow fever; and (iii) vector of minor importance in the transmission of canine dirofilariasis[26], had no previous record of its occurrence in Rondônia State.

The relative number of adult mosquito specimens obtained from immature forms differed in respect to the mosquito species for each breeding site category. The average number of adult *Ae. aegypti* specimens obtained

from larvae collected in Cess was similar to that from CBS, while *Cx. quinquefasciatus* adult specimens originated from Cess was 16 times higher than those from CBS (Figure 3).

It was observed that the rate of site infestation by *Ae. aegypti* is similar in both Cess and CBS ($\chi^2 = 0.018$; d.f. = 1; $p > 0.05$) (Table 1). However, different localities might show different rates of infestation. For instance, while in Jaru the rate of infestation was almost the same in both Cess and CBS, i.e., three *Ae. aegypti* positive Cess from a total of 11 Cess (27%) and one positive CBS from a total of four CBS (25%) surveyed, in Espigão do Oeste the rate of infestation was 1.7 times higher for Cess (50%) than for CBS (29%) (Table 1). However, such results must be carefully interpreted since samplings were small at both localities.

1: Espigão do Oeste; 2: Jaru. The capital city of Rondônia State, Porto Velho is indicated with a star.

Figure 1 – Municipalities of Rondônia State, Brazil surveyed for *Ae. aegypti* in cesspits

Images source: Katsuragawa TH.

A: Typical cesspit in the areas of study; B: Mosquito immature forms in collection box kitchen waste water (arrows); C: Mosquito immature forms in water of cesspit (arrows); D: Larvae and pupae collected from a cesspit; E: Sampling of adult *Ae. aegypti* in a cesspit; F: Rearing of immature forms in laboratory.

Figure 2 – Water sampling of cesspits in the Municipalities of Espigão do Oeste and Jaru, Rondônia State, Brazil

Female mosquito oviposition has been correlated with turbidity[29] and pH of water[30]. While females of *Cx. quinquefasciatus* have apparently preference for water with high turbidity[29], those of *Ae. aegypti* have been long perceived to prefer clean water[31]. Such perception is reflected in government guidance for monitoring *Ae. aegypti*. Cesspits are not considered due to their obvious turbidity. Measured physicochemical properties of cesspit water samples ranged from 9.8 to 135.0 NTUs, the pH from 5.3 to 8.8, and the colour from 20.4 to 1,142.4 HUs (Table 2). For comparison purpose, the standard values of water for human consumption[25] are also listed. The measured parameters of water from cesspits compared to parameters of acceptable standards for drinking water thus indicate a wider range of favorable conditions for *Ae. aegypti* procreation and warrant changes in the official monitoring guidance for *Ae. aegypti* in Brazil.

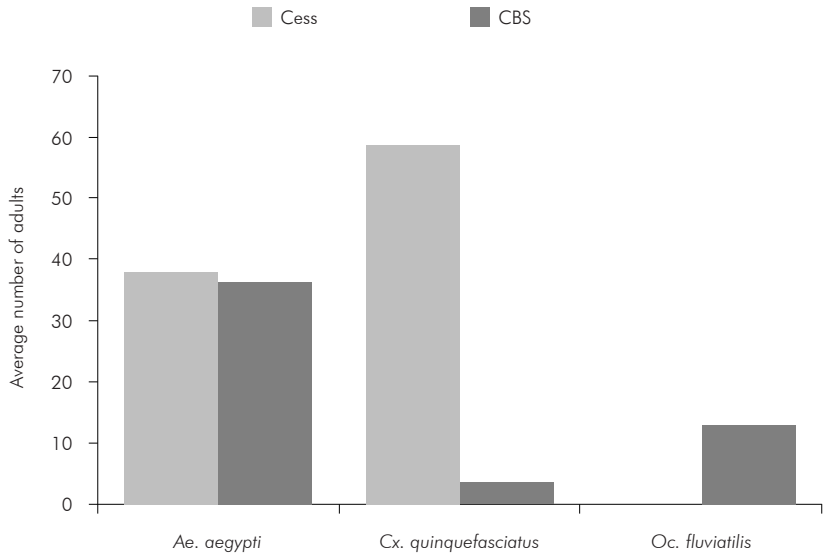

Figure 3 – Average number of reared adults of *Ae. aegypti*, *Cx. quinquefasciatus*, and *Oc. fluviatilis* per Cess (N = 19) and CBS (N = 11), in Espigão do Oeste and Jaru, Rondônia State, Brazil

Table 1 – Mosquito species identification and frequency of *Ae. aegypti* in Cess and CBS at two municipalities of Rondônia State, Brazil

	Espigão do Oeste		Jaru	
	Cess	CBS	Cess	CBS
Number of sites sampled	8	7	11	4
Sites with *Ae. aegypti*	4	2	3	1
Immatures*	1,661	381	4,340	174
Adults†	791	318	1,044	135
Ae. aegypti	211	299	511	101
Oc. fluviatilis	–	13	–	–
Cx. quinquefasciatus	580	6	533	34
Households without public sewer service (%)‡	99.6		98.9	

Cess: cesspit; CBS: conventional breeding sites. * Total number of larvae and pupae collected at each inspected site; †Total number of adult mosquitoes obtained in laboratory from collected larvae and pupae; ‡ Official survey[16]; – : numeric data equal to zero, not resultant from rounding.

Table 2 – Turbidity, pH and color of water samples collected from cesspits in two municipalities of Rondônia State, Brazil

Parameter*	Low	High	Mean	Water for human consumption†
Turbidity (NTU)	9.8	135.0	96.9	5.0 (maximum)
pH	5.3	8.8	7.7	6.0 – 9.5
Color (HU)	20.4	1,142.4	783.2	15.0 (maximum)

* Parameters of cesspit water samples were determined by CAERD, Brazil; NTU: nephelometric turbidity units; HU: hazen units; † As recommended by the Brazilian Health Ministry[18].

The difficulties experienced by some countries in integrated insect vectors control could be a combination of the opportunistic behaviour of *Ae. aegypti* and the less than optimal man-made structures of waste water disposal (mainly dictated by economic and social conditions), creating unexpectedly new ideal conditions for its proliferation. Septic tanks are referred in the World Health Organization guidelines for dengue control[32] as liquid waste storage containers that can eventually serve as procreation environment for insect vector species. In the present study, we observed indeed that cesspits are water-holding structures equally or better suited for the breeding of this mosquito species than CBS. Of the 19 cesspits inspected in urban areas of Rondônia State, seven were positive for this species. The implication of these results is that, even with a small sampling, as performed in this study, the relevance of cesspits as breeding sites for important disease vectors is evidenced. Few studies have highlighted these findings in Brazil[33,34]. Furthermore, the relative productivity of cesspits compared to other water containers requires further studies with aggregated spatiotemporal parameters.

Deficiencies in public water supply for human consumption have resulted in private construction of improvised containers for water storage that are not always adequately protected[10,35]. Human occupation of wild areas in the tropics, often with inadequate housing structures and inefficient sewage and waste disposal systems, creates favourable conditions for an unbridled raise in *Ae. aegypti* density[10,35]. In the Amazon Region, the great majority of households have no public sewage service (Table 1). Cesspits are the main alternative for domestic waste water disposal both in rural and urban areas. However, poor construction and inadequate maintenance of cesspits, causing openings and crackings in its structure (Figure 2F), might create additional favourable conditions for mosquito procreation, contributing to increased risk of dengue outbreaks, and perhaps explaining the challenges of vector control encountered in the region. In addition, our data showed that higher levels of impurities (particulate and dissolved material) affecting water turbidity and colour as well as a wide range of pH in cesspit water, did not dissuade the mosquito from laying eggs (Table 2). During the entomological surveys, we noticed adult mosquitoes coming out of cesspit chamber, being able to collect ten to 15 adult specimens per cesspit, many of them promptly identified as *Ae. aegypti* (males and females). We observed winged forms in all monitored cesspits (N = 27). To ascertain that these adult mosquitoes were directly ovipositing at those sites, ovitraps were installed nearby and inside cesspits. After 24 h, the ovitraps at the different locations presented eggs, which, after taken to the laboratory and reared to adult stage, were identified in high proportion as of *Ae. aegypti* (results not shown).

Infestation of cesspits by *Ae. aegypti* is supported by results of previous studies reporting large numbers of immature and adult forms of this species in septic tanks, which are essentially improved cesspits[18,19,30], independent of season and rainfall[20]. Septic tanks were highlighted as the main breeding site in South-Eastern Nigeria for insect's vectors of diseases, including *Ae. aegypti*[18]. In Puerto Rico, during an outbreak of dengue DEN-2, density of vector adults was restored to similar levels of pre-intervention after five weeks post-intervention, leading to the discovery of septic tanks as subterranean focus of infestation[19]. Adult mosquito presence in septic tanks was positively associated with wall cracking, unprotected openings and pH of septic water[30].

Ae. aegypti tolerance to diverse conditions and changes in its oviposition behaviour have been reported in many previous studies. For instance, it was demonstrated that larvae of some strains of *Ae. aegypti* from Africa and Central America, were very tolerant to synthetic sewage[36]. Upflow anaerobic sludge blanket (UASB) reactor effluents as well as effluents of anaerobic filter could constitute favourable insect breeding places, based on experimental evidences of *Ae. aegypti* oviposition preferences[37]. In Malaysia, *Ae. aegypti* eggs and *Ae. albopictus* immatures were found in one toilet flush tank and in a bowl of water supply to birds[38]. Natural and sylvatic breeding places have been reported as outcome of spill events of *Ae. aegypti* populations[39]. In our study, the measured parameters of water from cesspits compared to parameters of acceptable standards for drinking water indicated a wider range of favorable conditions for *Ae. aegypti* procreation and warrant changes in the official monitoring guidance for *Ae. aegypti* in Brazil.

Due to climate changes caused by increasing human activities[40] and the procreation adaptability shown by *Ae. aegypti*, new oviposition environments in conditions of natural disaster is very likely. Flood due to heavy rainfall being ever more frequent[40], potentially provides new and more numerous breeding sites for this mosquito species. Prevalence of dengue has been correlated with rainfall flood in Thailand[41], for instance. Such climate events cannot be ignored in the context of control of the mosquito. Moreover, the present studied region is characterized by periodic rainfall and occasional flooding. Considering that cesspits are the main infrastructure for waste water treatment in many parts of Northern Brazil[23], further studies are necessary to address whether the procreation of this vector species in cesspits/septic tanks is seasonally affected by the water table, taking into account the characteristic seasonal wet and dry periods in the region.

In spite of all evidences there is no recommendation to survey septic tanks/cesspits in the Brazilian Dengue Program. Public policies should effectively include cesspit/septic tank inspection/management as a regional/local priority. Our observations of *Ae. aegypti* presence in a wider range of water turbidity, add in the support of such recommendations, emphasizing as well the need for more attention by governmental agencies on building better structured public sanitation, especially in developing countries situated in tropical and subtropical regions, and where dengue and other vector transmitted diseases are recurring problems.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

Fossas negras rudimentares como locais de reprodução do *Aedes aegypti* em áreas urbanas do Norte do Brasil

RESUMO

Fossas negras rudimentares foram avaliadas como potenciais criadouros do mosquito *Aedes aegypti* em relação a locais de criadouros comuns (CBS). Nos meses de novembro e dezembro de 2009, formas imaturas do inseto foram coletadas em amostras de água em fossas rudimentares assim como em CBS, isto é, objetos que potencialmente acumulam água como pneus usados, vasos de flores e locais de disposição de lixos sólidos. As amostragens foram realizadas em áreas urbanas de dois municípios do Estado de Rondônia na Amazônia Ocidental Brasileira. Os estágios imaturos e adultos de *Ae. aegypti* foram observados em águas de fossas rudimentares, confirmando-as como novos locais de reprodução para o mosquito nesta região. A proporção de fossas rudimentares com insetos nos estágios de larvas e pupas encontradas nas duas localidades foi como segue: Espigão do Oeste, 50,0% e Jaru, 27,3%. Além disso, o número médio de insetos coletados não diferiu significativamente entre fossas negras e CBS. Estes resultados fornecem subsídios para mudanças nas políticas e estratégias de controle do vetor da dengue no Brasil, levando em conta a infraestrutura de saneamento limitado e as particularidades locais da Região Amazônica. Evidencia-se também a urgência de investimentos e adequação de esforços empreendidos em sistemas de saneamento público nos países em desenvolvimento, como uma medida adicional ao controle deste importante vetor de doenças virais.

Palavras-chave: Bioma Amazônico; Insetos Vetores; Dengue; Oviposição; Esgoto Urbano.

Pozos negros rudimentarios como criaderos de *Aedes aegypti* en las zonas urbanas del Norte de Brasil

RESUMEN

Fueron evaluados pozos negros rudimentarios como posibles criaderos del mosquito *Aedes aegypti*, en comparación con los sitios de reproducción común (CBS). En los meses de noviembre y diciembre de 2009, se recolectaron formas inmaduras del insecto mediante la inspección de agua en pozos rudimentarios, así como en CBS, específicamente objetos en donde se acumula agua, como neumáticos usados, macetas y sitios de disposición de residuos sólidos. Los pozos negros rudimentarios inspeccionados son de zonas urbanas de dos municipios ubicados en el Estado de Rondônia, al oeste de la Amazonía brasileña. Se observaron formas inmaduras y adultas de *Ae. aegypti* en el agua de pozos rudimentarios, confirmándose que estos son nuevos lugares de reproducción para el mosquito. La proporción de pozos rudimentarios en los dos locales con insectos en las etapas de larvas y pupas fue: Espigão do Oeste, 50,0% y el Jaru, 27,3%. Por otra parte, el promedio de insectos encontrados en pozos rudimentarios y CBS no difirió significantemente. Estos resultados aportan conocimiento para un cambio en las políticas y estrategias de control de vectores del dengue en Brasil, teniendo en cuenta la infraestructura de saneamiento limitado y las particularidades locales de la Región Amazónica. También se hace evidente la urgencia de mejorar los sistemas de saneamiento ambiental público en los países en desarrollo, como una medida adicional en el control de este importante vector de enfermedades virales.

Palabras clave: Bioma Amazónico; Insectos Vectores; Dengue; Oviposición; Alcantarillado Sanitario.

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