DOI: 10.1590/1413-81232015213.23562015

ARTICLE

Evaluation of simple diffusion chlorinators for decontamination of wells in a rural settlement in Amazonia, Brazil

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Abstract *While the Amazon region has the world's largest reserve of fresh water, the lack of water services and water treatment, especially in non-urban regions, causes environmental and health problems. In isolated rural settlements supply is usually by shallow wells, and the quality of water is a concern for residents. These are situations where there are restricted options for water treatment. This study aimed to assess the use of simplified diffusion chlorinators as an alternative water treatment method. Bacteriological analyses were made of 100 samples of water from the wells, before and after application of the chlorinators, in the Rural Settlement of Rio Pardo, Presidente Figueiredo in the Brazilian State of Amazonas. The sources that were analyzed were considered inappropriate for consumption without prior treatment, and the use of the chlorinators eliminated all contamination by thermotolerant coliforms in the great majority of cases. Also, the method was well received by residents, because it does not leave a taste in the water, is relatively low-cost and handling is easy. We discuss the advantages and limitations of the use of this method of treatment for this social-environmental context and present suggestions for improvement and adaptation, for application of this methodology in other settlements.*

Key words *Water quality, Water treatment, Chlorinators, Amazônia, Rural settlements*

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Introduction

Lack of infrastructure for water treatment, capture and distribution services is a problem not only in the crowded outer regions of the large Brazilian cities but also in the rural areas[1]. In the rural areas, access to potable and safe water can be even more difficult, because of their distance from the treatment systems and distribution that works in the cities – and the degree of inequality of opportunity of access to water, and the absence of public policies for water treatment and health, are well-known[2]. In recent decades, global initiatives (such as the *Millennium Goals*) have aimed to expand access, especially for the more vulnerable populations. In spite of the advances achieved in water supply, some 36 million people in Latin America still have no access to potable water, and 80% of these are in rural areas[3,4]. In the rural areas of Brazil the deficit in coverage of water and sewerage treatment systems is high: almost 67% of the population find water in alternative sources, usually inappropriate for human consumption, and 66.5% dispose of organic waste in rudimentary septic ditches or tanks, or directly on the soil or in water courses[5].

Brazil's Amazon Region (*Amazônia*) has the world's largest reserve of freshwater, but its population suffers from problems of access to potable water and to the technologies conventionally applied in water treatment and distribution[6]. Brazil's policy of land reform in the 1970s attracted migrants to Amazônia interested in working on Amazon projects financed or encouraged by the federal government[7]. Today, more than half of the lots in rural settlements distributed by the National Land Colonization and Reform Institute (*Instituto Nacional de Colonização e Reforma Agrária*, or Incra) are in the region of Brazil legally described as Amazônia[8]. In these settlements the principal sources of water are shallow wells and springs, which are very susceptible to contamination. The risk of water-borne diseases is high, since often the wells are inadequately sealed and close to sources of contamination such as septic tanks or grazing areas occupied by animals[9].

Where there are no water capture, treatment and distribution systems, one of the alternatives proposed is the use of *home-based disinfection techniques*, in which chlorine and sodium or calcium hypochlorite are the most common materials used[10]. Some works have suggested the use of simplified diffusion chlorinators as a proposal for *social technology* to meet the demands of the population that do not have access to water treatment and distribution, such as rural communities[11-15]. Studies on social technologies have been gaining visibility due to their capacity for helping to improve living conditions of the most vulnerable groups of the population. These technologies can be defined as "a group of technologies, transformative methodologies, developed and/or applied in the interaction with the population and appropriate for that population, providing solutions for social empowerment and improvement of living conditions", directed, as priority, to emancipation of the actors involved, having at their center the producers and users of these technologies themselves[11,16]. In Brazil the National Health Foundation (*Fundação Nacional de Saúde* – Funasa) is the institution responsible for water services and environmental health in the Brazilian municipalities with population under 50 thousand, including rural areas, *quilombolas*, and riverside communities. Among the projects proposed by Funasa is the use of simplified systems for treatment of water with low-cost technology to meet the immediate demand of rural communities, such as the simplified diffusion chlorinator.

The objectives of this study are (i) to evaluate the use of this method for decontamination of shallow wells in a rural settlement far from major population centers in central Amazônia, and (ii) to discuss the advantages and limitations of its use in this social-environmental context. The plan for the survey was previously submitted to and approved by the Research Ethics Committee of the Amazonas Hematology and Hemotherapy Foundation.

Methods

Area of Study

The study was carried out in the Rio Pardo Rural Settlement, created by Incra in 1996 in the municipality of Presidente Figueiredo, in the Brazilian state of Amazonas. The municipality is 110 km to the north of Manaus, and the settlement – at coordinates 01°47′52″ S, 60°15′82″ W – is 35 kilometers from the center of the municipality (Figure 1). Access is by land, by the BR-174 highway, then by 17 kilometers of unpaved dirt road, or by water, through the Rio Pardo Creek, a small affluent of the Rio Negro River.

The settlement comprises unpaved dirt roads, referred to as 'branches' ('*ramais*'). It has a pop-

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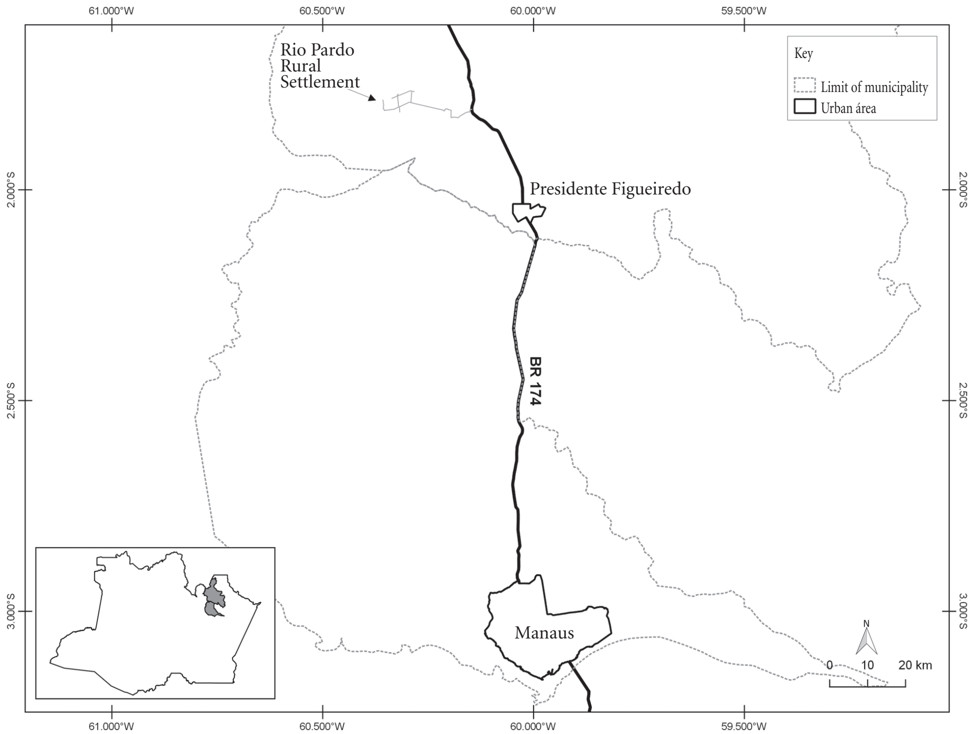

Figure 1. Rio Pardo Rural Settlement location – Presidente Figueiredo (Amazonas).

ulation of 584, with a main street, four branch streets and an area where the residents live on the banks of the creek, with access only by water. The majority of homes are made of wood, but there are constructions in masonry or wattle-and-daub (mud/straw – *pau-a-pique*).

Properties are distant from each other, the center comprising only a small group of houses, churches, small food stores, a kindergarten and a primary school, and a health post which is visited weekly by a dentist, a nurse and a doctor. Access to the residents of the creek is by canoe, and the distance between properties is two kilometers.

The principal economic activity of Rio Pardo is family farming. The main crops are banana, manioc, *cupuaçu*, *pupunha* palm, peppers and *pimenta-de-cheiro* (aromatic chili peppers). Part of the production is delivered to traders and transported to be sold in the city of Presidente Figueiredo and Manaus. A few of the settlers have cattle for commercial purposes, but many families keep domestic animals for consumption (pigs, chickens, sheep and goats).

In general, the conditions of water services in the Rio Pardo Rural Settlement are precarious, and there is no infrastructure for adequate disposal of wastes. Also, it has no network or structure for treatment of water for consumption. The water sources used in the settlement are: wide-diameter shallow wells (*cacimbões*), with diameter between 80 and 150 cm, and maximum depth of 15 meters, dug manually until the surface water table is reached, without the need for licensing or governmental authorization; a spring (a point where water flows from the soil surface); a creek (an amazon watercourse comprising a long branch of a river or canal, characterized by relatively low depth); and a deep well (capture of water from water tables situated between two impermeable layers – requiring special labor and equipment for construction).

Installation of diffusion chlorinators

After negotiation with the residents' association and the proprietors, 20 shallow wells (*cacimbões*) were selected – those in which there was sharing between households – supplying 39 families of a total of 219 properties[17]. In each well, technical staff of Funasa have installed a simplified diffusion chlorinator, recommended by the Brazilian Health Ministry as a domestic method for disinfection of water[11]. The chlorinator consists of a plastic vessel (PVC tube or PET bottle) containing 340 grams of calcium hypochlorite [$Ca(ClO)_2$] with 65% active chlorine, as disinfectant, mixed with 850 grams of washed sand, with two opposite 6-millimeter diameter holes, approximately 10 centimeters below the top (Figure 2). The sand must be washed, and contain no organic material or clay. It is not appropriate for the sand to be very coarse, nor very fine, nor should it come from brooks or rivers that receive high levels of pollutants or contaminants[18].

The calcium hypochlorite is released in supposedly homogeneous concentrations, maintaining a residual level until the end of its useful life, and the sand has the function of controlling the quantity of disinfectant released into the water. According to members of the Funasa technical staff, this mixture has capacity for disinfection of 2,000 liters of water, and can continue releasing chlorine for approximately 30 days inside the well. The equipment should be tied to a nylon line and submerged, keeping the top close to the level of the water[11].

Bacteriological analysis of the water

In each *cacimbão*, water samples were collected for analysis of bacteria, measured as: total coliforms, and *Escherichia coli*. Coliforms are divided into fecal coliforms and total coliforms, which include bacteria that are not exclusively of fecal origin, and may occur naturally in the soil, in the water and in plants[19]. Detection of *E. coli* in the water establishes an indicative parameter of better correlation with the risks of heath associated with contamination of a given environment: whether there is a possibility of existence of pathogenic microorganisms responsible for transmission of illnesses through use or ingestion of water, such as typhoid fever, paratyphoid fever, bacillary dysentery and cholera[11,20]. Samplings for quantification of total coliforms and *Escherichia coli* are specified in Ministerial Order Nº 2914/2011[21] of the Health Ministry, which regulates procedures for control and oversight of quality of water for human consumption and its standard of potability.

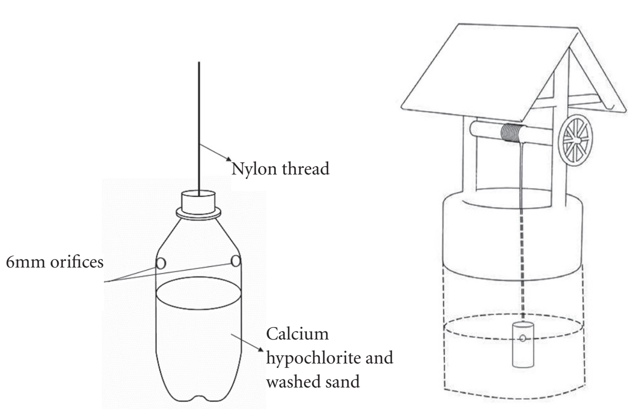

Figure 2. A) simplified diffusion chlorinator and B) chlorinator position in the well.

The samples were collected using the plastic vessels specific to the kit, sterilized, with level markers to not exceed the standard volume of 100 ml, and kept refrigerated in an expanded polystyrene box until being transported to the laboratory for processing, which took place in up to 6 hours after collection. The water sample was taken using the system installed in the property (mechanical pumps linked to hoses which draw the water from the *cacimbão*), before arrival of the water at the storage location. The analyses were made in the Mobile Laboratory of Funasa and the Support Laboratory of Fiocruz in the Settlement itself, at different times: before the intervention, and 2, 15, 30 and 90 days after the intervention, totaling 100 samples. The Colilert® and Quanti-Tray/2000 systems of IDEXX Corporation were used for analysis, based on the defined substrate method, with incubation time of 24 hours. This method, which is simplified and fast, complies with the requirements of the international bodies that regulate standards for analyses and potability of water and is cited in the *Standard Methods for the Examination of Water and Waste-water*[22]. Brazilian Health Ministry Ministerial Order 2914/2011[21] indicates that the dosage of residual chlorine should be realized during the process of decontamination. In all occasions where the water was sampled, the concentration of residual chlorine was analyzed based on the colorimetric method with tablets of DPD (N-diethyl-p-phenylenediamine) and equipment of the HACH® brand. The DPD tablets were dissolved in a sample of water containing chlorine, producing the pink coloration the intensity of which is proportional to the concentration of chlorine. By this method, the color produced by the reagent enables measurement of the concentration of residual chlorine, free or total, by the device referred to as 'chlorine comparator' through the colorimetric method. During

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the whole period of the study the properties were visited periodically and observations were made and interviews held with residents to assess the receptivity to the method and other water handling practices.

Results

At the moment of the initial diagnosis of the sources, 100% of the samples of water consumed by the population were contaminated by total coliforms and by *E. coli*. Shortly after the installation of the simplified diffusion chlorinators, on the second day, it was observed that contamination by E. coli had been eliminated in 19 of the 20 samples, and 16 of them (80%) remained decontaminated after 15 days. After 30 days, 13 of the *cacimbão* wells (65%) still showed decontaminated samples as a result of the action of the chlorinator, and, even after 90 days, five *cacimbões* were still decontaminated (Table 1), and had residual chlorine (Table 2). Only one *cacimbão*, sample 14, did not present decontamination of the water on any of the days analyzed (Tables 1 and 2).

Even with residual chlorine levels lower than the recommended one (0.2 mg/L) or at non-detectable levels, in some samples we observed reduction or even elimination of the bacteria of the total coliform group and *E. coli* (Table 2).

The chlorinators were installed in the *cacimbões* in the presence of the owners of the sources, and they were oriented on the method used. During the collection of the samples, interviews were carried out to assess users' degree of satisfaction and acceptance, or otherwise. All the residents said that the flavor of the chlorine was almost imperceptible, that the water appeared to have become more "limpid" (although this probably has no relationship with the installation of the diffusion chlorinator), and that they had greater security due to consuming water of good quality.

Discussion

When evaluating the efficiency of water treatment methods at a location it is necessary to consider the alternatives that are able to be employed

Table 1. Comparison of the Most Probable Number (MP/ml) of total coliforms and *Escherichia coli* (*E. coli*) in the 20 shallow wells (*cacimbões*) analyzed in the Rio Pardo Rural Settlement (municipality of Presidente Figueiredo, Amazonas State, Brazil) before installation of diffusion chlorinators (T0) and after 2, 15, 30 and 90 days (T2, T15, T30 and T90).

	T0		T2		T15		T30		T90	
Sample	Total coliforms	*E.coli*	Total coliforms	*E.coli*	Total coliforms	*E.coli*	Total coliforms	*E.coli*	Total coliforms	*E.coli*
1	1011.2	5.2	0	0	0	0	7.4	0	7.4	0
2	1011.2	4.1	0	0	85.2	0	0	0	0	0
3	1011.2	1.0	0	0	0	0	0	0	0	0
4	1011.2	3.1	0	0	0	0	3.0	0	3.0	0
5	1011.2	59.5	0	0	0	0	107.6	7.5	107.6	7.5
6	1011.2	396.8	0	0	0	0	326.2	126.1	326.2	126.1
7	1011.2	456.9	0	0	0	0	0	0	0	0
8	1011.2	4.1	0	0	0	0	0	0	0	0
9	1011.2	9.7	0	0	0	0	21.8	0	21.8	0
10	1011.2	4.1	0	0	1.0	0	83.6	0	83.6	0
11	1011.2	1011.2	0	0	0	0	0	0	0	0
12	1011.2	113.7	0	0	478.6	456.9	34.1	1.0	34.1	1.0
13	1011.2	6.3	0	0	52.1	1.0	1011.2	1.0	1011.2	1.0
14	1011.2	6.1	285.1	101.2	146.4	76.2	1011.2	791.5	1011.2	791.5
15	1011.2	1.0	0	0	1.0	0	85.7	0	85.7	0
16	1011.2	19.1	0	0	2.0	0	76.5	0	76.5	0
17	1011.2	1011.2	0	0	21.3	4.1	1011.2	721.5	1011.2	721.5
18	1011.2	7.2	0	0	0	0	88.4	0	88.4	0
19	1011.2	31.8	0	0	16.1	0	378.4	3.1	378.4	3.1
20	1011.2	5.2	0	0	1.0	0	123.6	0	123.6	0

Table 2. Residual concentration of chlorine found in the 20 shallow wells (cacimbões) analyzed in the Rio Pardo Rural Settlement (municipality of Presidente Figueiredo, Amazonas State, Brazil) after implantation of the simplified diffusion chlorinator.

Sample	Residual concentration of chlorine after implantation of the chlorinator (mg/L)		
	2 days	15 days	30 days
1	0.2	0	0.1
2	0.5	0	0
3	0.2	0.2	0
4	0.1	0	0
5	0.5	0.7	0
6	0.4	0	0
7	0.2	0	0.1
8	0.1	0.3	0.3
9	0.1	0	0
10	0.2	0	0
11	0.2	0.3	0.1
12	0.3	0	0
13	0	0	0
14	0.1	0	0
15	0.2	0	0
16	0.1	0	0
17	0.1	0	0.1
18	0	0.3	0
19	0	0.1	0
20	0.1	0	0

in that specific social-environmental context, and also the costs of installation, maintenance and the acceptance of the technology by the community in residents. In the case of the isolated settlements of Central Amazônia, the options are even more limited. For example, the costs of installation of a water treatment and supply network are prohibitive – due to the very large distance between the rural properties – and maintenance of a network could be made impossible by the difficulty of access, which might be totally interrupted in the rainy months (December to May) or even when there are heavy rains during the rest of the year.

Assuming this social-environmental context, the greater part of the supply in this and in other rural settlements of the region takes place on a point-by-point basis, through shallow wells excavated on each property. Most frequently, as was reported to us by the residents of Rio Pardo, the residents themselves pay the cost of opening the wells. As well as this characterizing a transfer of responsibility – since that is a duty of Incra, under Normative Instruction 15 of March 30, 2004, Article 7 – in the majority of cases, according to the residents, there is none of the due technical monitoring for construction and/or maintenance of the *cacimbões*. As a result, 100% of the wells analyzed were contaminated and not suitable for consumption (Table 1). With rare exceptions the wells presented inadequate conditions of construction[23]. Some of the characteristics frequently observed in the settlement have already been attributed as potential factors causing contamination, such as: lack of protection of the edge of the well by surfacing of the walls in masonry or concrete; use of a lid that does not provide complete closure; and the use of buckets and cords for collecting water[14,24]; also proximity of the source to the sewage system of households, especially when those systems are of the rudimentary septic tank type or simply a gully in the ground for sewage, or when there are no latrines[25]. These factors combined can increase the risk of contamination of the source by materials which are conducted into its interior, such as earth, animal feces and other contaminants[25,26].

In Rio Pardo it is very common to see animals close to the sources of water. Machado[27], working in this settlement, found strains of *Salmonella* sp. in samples of water from the creek and wells that were genetically similar to those found in feces of adults and children and feces of chickens and dogs that circulate freely in the household[27]. This evidences the cycle of contamination by *Salmonella* sp. at the location, showing the role of these animals in the contamination of the environment and of human populations[27]. A parasitological investigation of feces in the schools of the settlement found a prevalence of 80%, one of the most frequent parasites being *Giardia* sp.[28], transmission of which is principally attributed to contaminated water and food, as well as the polyparasitism found in 30% of the samples analyzed[28]. Some studies have already shown that bovine organic wastes increase the contamination of the sources of water, since these animals are reservoirs of various microorganisms such as *Cryptosporidium partum* and *Giardia* sp., potential causes of human illnesses[29]. Another parasite identified in the samples from Rio Pardo was *Ancylostoma* spp., which could be an indication of contamination of the soil by feces of animals, especially cats and dogs[28].

A systematic review of articles with different interventions concluded that the most effective measures in the prevention of diarrhea are those that reduce bacteriological contamination of the water[30], and chlorination is one of the treatment methods most used, principally due to its residual capacity[10]. The presence of free residual chlorine in the water can be used as an indicator of decontamination of sources[10]. However, its efficiency depends on various factors, such as temperature, turbidity and presence of organic material[27-29] in the water, as well as the quantity and time of the exposure to the chlorine[10]. The quantity of residual chlorine found in the samples was lower than the recommended level (Table 2), probably due to the quantity of interference factors since the chlorine reacts primarily with eth organic and inorganic material and, only afterward, acts on the microorganisms[10]. Thus it is necessary that an adjustment should be made of the quantity of calcium hypochlorite added in each chlorinator to meet the demand for the chemical reactions and maintain the viable level of residual chlorine for elimination of bacterias[10]. An excess of residual chlorine should also be avoided, because it can cause worsening of the organoleptic parameters and lead to the formation of chlorinated organic substances, some of which are toxic, such as the trihalomethanes[31-33].

The only method of water treatment adopted in the settlement is the use of sodium hypochlorite (2.5% solution) in water for consumption. Solutions of hypochlorite in 50 ml flasks are distributed monthly and free of charge by the Health Ministry, through the Municipal Health Department of the municipality of Presidente Figueiredo, Amazonas. According to the instructions on the label, two drops of the solution should be added to each liter of water, for at least 30 minutes before consumption, and the residents are the parties responsible for administration of the product. As well as this characterizing a transfer of responsibility, only 16% used the treatment correctly, because of rejection of the residual taste of chlorine in the water after the treatment[23]. To reduce the taste left by the sodium hypochlorite, most users do not administer the quantity of hypochlorite recommended by the Health Ministry[11], and add, according to information obtained in interviews or through observations at the location, less than the amount recommended to decontaminate the water. However, it is important to point out that even if the whole of the population were to use the hypochlorite correctly, this would only be a palliative measure, because other domestic uses (washing and preparation of food; personal hygiene, such as showering, washing hands and brushing teeth; washing dishes, etc.) would still be carried out with contaminated waters coming from the shallow wells.

In situations such as those described in this study, to have greater efficiency it is important to adopt an approach that includes not only the treatment of the sources, but also of the water in storage and at the moment of consumption. The simplified diffusion chlorinator showed itself to have some advantages as an alternative for treatment of the water of shallow wells. For example, in Rio Pardo, the residents said that the system minimized or eliminated the problems found in the use of sodium hypochlorite (unpleasant taste, and need for frequent application in the vessels used for consumption), which increased the community's acceptance of the method. Also, if the vessels for storage and consumption are maintained and cleaned, there is no need for further chlorination – although, based on the field observations, there is a need for a sanitary education program at the location. Thus, considering the advantages already mentioned, some aspects and limitations need to be observed before taking the decision to implement this method.

In the present study, the accompaniment of the use of the simplified diffusion chlorinator was for 90 days. It would be important to assess whether the population keeps the domestic interventions functioning correctly and consistently and whether the water remains decontaminated during a longer period. According to various authors, the diffusion chlorinator has an estimated decontamination capacity of 30 days, it not being necessary to replace the mixture of chlorine and sand in this interval[11-15]. In our study, we found that after the installation of the chlorinators, one among the 20 shallow wells analyzed did not present decontamination during the whole of the evaluation period (Table 1). This could be related to characteristics of the well itself (the internal wall is entirely of earth, not made impermeable), as well as the environmental aspects observed around the source. This household is located less than 50 meters from the creek and various fauna were observed around the well (cattle, dogs and chickens, as well as wild boars and monkeys that live with the family and can be considered 'pets'), and also animal feces. As well as the problems observed with this source, 20% and 35% of the wells were contaminated by *E. coli* after 15 and 30 days, respectively, in spite of the expectation being that all the sources would be decontaminated during this period. These results indicate that the method has potential for use in the region, but that even if the chlorinator is correctly installed this is not a guarantee of health quality and that other factors may be acting in contamination of the wells.

In the Amazon region, it is important to assess the action of the chlorinator as a function of the frequency of daily use of the water from the well and in the periods of intense rain or intermittent drought, when there is a wide variation in the volume of water in the well. As well as the periodic substitution of the mixture of sand and chlorine, the user needs to frequently verify whether the chlorinator is being maintained at the correct position for the orifices where the chlorine is released, below the water level. A potential solution to this problem would be coupling of the chlorinator to a buoy, enabling it to be in the right position independently of the volume of water in the well. However, even so, it has to be considered that the use of calcium hypochlorite solutions can result in the formation of deposits on the chlorine-release orifices, principally in contact with very hard waters[10], and this is another aspect that needs to be evaluated periodically by the user or by the provider of the service.

Another consideration to be made is that the use of the chlorinator (or of another form of chlorination), on its own, does not guarantee complete decontamination of the water. Cysts of *Giardia* and, especially, oocysts of *Cryptosporidium partum* may be resistant to chlorination[34]. Since these and other parasites tend to aggregate to solid particles in the water, the solution for reducing contamination is filtration (in the cases of residential systems, or, in more complete systems, sedimentation by coagulation or flocculation)[34]. An investigation combining techniques of decontamination of the water in the Amazon context is still necessary. In the present study, only the reduction of *E. coli* was evaluated, using the Colilert method. In spite of some studies reporting that this method is not different from the traditional techniques of bacteriological analysis of water, such as multiple tubes and filtering membrane[35], others show that the Colilert system can show rates of false-positive and false-negative results greater than other techniques[36,37].

Considering that interventions on water for consumption aim to have positive effects on health, it would be important if there were a reduction of the prevalence of intestinal parasitosis[28] and frequency of diarrheas[38] caused by contamination of water. These responses could aggregate important information for the taking of a decision on the use of this method in this and other similar contexts.

The fact of the method needing lesser maintenance than in the system currently used (addition of sodium hypochlorite to vessels for consumption) does not mean that there is not a need for involvement of the heads of families to treat water correctly and avoid recontamination. This is an alternative proposal for serving a population that historically is excluded from public policies of water treatment and distribution. A good part of the population that today occupies the Rio Pardo Rural Settlement is of migrants from the Northeast, arising from the time of construction of the Balbina Hydroelectric Plant at the end of the 1970s and of people who were settled, often in a precarious manner, in the countryside. Thus, the costs of maintenance of this method should not rest on the user, they should be incorporated by the Municipal Health System or by Incra. Considering that the annual consumption of chlorine is approximately 4kg per well, the estimated average cost of maintenance of the simplified chlorinator would be approximately R$ 70.00 per year. The results of this survey were presented to the Municipal Prefecture of Presidente Figueiredo with a view to continuity of implementation of this method and the evaluations of other important factors, as proposed in this study: (1) acceptance and maintenance of the chlorinators by the residents in the long term; (2) effectiveness of the decontamination produced by the chlorinators in periods of large daily fluctuations of water level in the wells and over the periods of the year; (3) effectiveness of the installation of buoys in the chlorinators; (4) verification of the concentration of residual chlorine in the water, and also possible formation of toxic compounds such as trihalomethanes; (5) accompaniment not only of any decontamination of the sources, but also of the water in storage and at the moment of consumption, seeking to understand the factors that contribute to contamination and/or good practices for decontamination of waters; (6) accompaniment of the indicators of health, connected to the diseases transmitted by contaminated water; (7) prevalence of parasites resident to chlorination. If it is considered cost-effective, this method could be incorporated into the public health policy of this and other municipalities, serving as a model for implementation in other rural settlements of Amazônia.

Collaborations

DC Ferreira took part in the conception and development of the study, collection, tabulation and interpretation of the data, writing of the article and adaptation to the rules of the journal. DF Buss took part in the conception of the study, interpretation of the data, discussion of the results, writing, critical revision and final approval of the version of the article to be published. SLB Luz took part in the conception of the study, definition and orientation of the methodology of work, review and approval of the final text to be published.

Acknowledgment

We would like to record our thanks to the association of residents of Rio Pardo and to all the residents involved in this survey. This work would not have been possible without the participation of the team of the Amazon State Management Team (SUEST/AM) of Funasa. We are grateful for the support of the prefecture of Presidente Figueiredo, Amazonas State, for the partnership in the work carried out in the settlement, to the laboratory technicians of the Leônidas and Maria Deane Institute/Fiocruz Amazônia (especially Michele Silva de Jesus, Ricardo Mota and Diego Lázaro Leite) for the help in the fieldwork for collection and laboratory analyses, and to Dr. Fernando Abad-Franch and Dr. Maria Luiza Garnelo Pereira for the comments that they contributed to this publication. This article is contribution number 25 of the Program of Research in Ecology of Infectious Diseases in Amazônia of the Leônidas and Maria Deane Institute of Fiocruz (PP-EDTA) and was part of the dissertation of Danielle Costa Ferreira, which enjoyed the logistical and financial support of ILMD/Fiocruz Amazônia and a study grant from CAPES.

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Article submitted 05/08/2015
Approved 04/12/2015
Final version submitted 07/12/2015