Home Self-collection to test for Human Papillomavirus and *Chlamydia trachomatis* infection in riverside women in Amazonas

Auto coleta domiciliar para testagem de Papilomavírus Humano e *Chlamydia trachomatis* em mulheres ribeirinhas no Amazonas

Auto Recolección domiciliaria para pruebas de Virus del Papiloma Humano y *Chlamydia trachomatis* en mujeres ribereñas de la Amazonía

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Abstract
Women living in rural areas of Amazonas - the riverine - have difficulty accessing adequate health care, aggravating their vulnerability in the face of various pathological processes, such as cervical cancer and sexually transmitted infections. Strategies that expand coverage of screening and early diagnosis are welcome. To evaluate the prevalence

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of Human Papillomavirus (HPV) and Chlamydia trachomatis (CT) in riverside women from Coari City, Amazonas, in samples obtained by self-collection and to verify the acceptance of this method among the participants. Fifty eight riverside communities were visited, with a "house-to-house" approach. The samples were obtained with a COARI® brush (Kolplast®) and the diagnosis of pathogens was made by Polymerase Chain Reaction. Four hundred and nineteen sexually active women aged between 18 and 70 years old (34.4 ± 12.1) participated in the study. The prevalence of CT in this population sample was 1.1% and that of HPV was 15.7%. We observed a low prevalence of CT and a high prevalence of HPV among participating women, in addition to a good acceptance of self-collection among them.
Keywords: Human papillomavirus; *Chlamydia trachomatis*; Epidemiology; Amazon region.

Resumo
As mulheres moradoras da zona rural do Amazonas – as ribeirinhas - têm dificuldade de acesso à adequada assistência à saúde, agravando sua vulnerabilidade diante de vários processos patológico, tais como o câncer cervical e infecções sexualmente transmissíveis. Estratégias que ampliem a cobertura de rastreamento e diagnóstico precoce são bem-vindas. Avaliar a prevalência de Papilomavírus Humano (HPV) e *Chlamydia trachomatis* (CT) em mulheres ribeirinhas da cidade de Coari, Amazonas, em amostras obtidas por auto coleta e verificar aceitação deste método entre as participantes. Foram visitadas 58 comunidades ribeirinhas, com abordagem "de casa em casa". As amostras foram obtidas com a escova COARI® (Kolplast®) e o diagnóstico dos patógenos foi feito por Reação em Cadeia da Polimerase. Participaram da pesquisa 419 mulheres sexualmente ativas com idade entre 18 e 70 anos (34,4 ± 12,1). A prevalência de CT nessa amostra populacional foi de 1,1% e de HPV foi de 15,7%. A maioria delas relatou não ter sido difícil usar o dispositivo de auto coleta (95%), não ter sentido nenhum tipo de incômodo (87,6%) e preferir a auto coleta em relação à coleta pelo profissional (80%). Observamos baixa prevalência de CT e alta prevalência de HPV mas mulheres participantes, além de boa aceitação da auto coleta entre elas.
Palavras-chave: Papilomavírus humano; *Chlamydia trachomatis*; Epidemiologia; Região Amazônica.

Resumen
Las mujeres que viven en la zona rural de Amazonas -las mujeres ribereñas- tienen dificultades para acceder a una atención adecuada en salud, lo que agrava su vulnerabilidad a diversos procesos patológicos, como el cáncer de cuello uterino y las infecciones de transmisión sexual. Serán bienvenidas las estrategias que amplíen la cobertura del cribado y el diagnóstico precoz. Evaluar la prevalencia del Virus del Papiloma Humano (VPH) y Chlamydia trachomatis (CT) en mujeres ribereñas de la ciudad de Coari, Amazonas, en muestras obtenidas por autorecolección y verificar la aceptación de este método entre las participantes. Se visitaron 58 comunidades ribereñas, con un enfoque "casa por casa". Las muestras se obtuvieron con el cepillo COARI® (Kolplast®) y el diagnóstico de los patógenos se realizó por Reacción en Cadena de la Polimerasa. Participaron de la investigación 419 mujeres sexualmente activas con edades entre 18 y 70 años (34,4 ± 12,1). La prevalencia de TC en esta muestra poblacional fue del 1,1% y de VPH del 15,7%. La mayoría informó que no le resultó difícil utilizar el dispositivo de autorecogida (95 %), no haber sentido ninguna molestia (87,6 %) y preferir la autorecogida a la recogida profesional (80 %). Se observó baja prevalencia de TC y alta prevalencia de VPH en las mujeres participantes, además de buena aceptación de la auto-recolección entre ellas.
Palabras clave: Virus del papiloma humano; *Chlamydia trachomatis*; Epidemiología; Región Amazónica.

1. Introduction

The Brazilian Amazon region comprises an immense territory in the northern region of the country, with a high demographic dispersion and precarious living conditions for the majority of its population. Basic health services are offered in urban centers in the interior of the state, but the limited installed capacity and low resolution in these little cities, combined with the concentration of services in the state capital - Manaus - lead to the need to refer a large number of cases to the medium and high complexity (Garnelo et al., 2017; Sousa *et al.,*, 2022; Garnelo *et al.,*, 2018). Women living in rural areas – the riverside women – even those who live geographically closer to larger urban centers, have difficulty accessing health care, even for relatively simple tests, such as the Pap smear. The gynecological collection of biological samples demands time, displacement, waiting and often causes shame in women, contributing to the low coverage of the exam (Torres *et al.,* 2018).

One of the consequences is the high prevalence of cervical cancer (CC) among women in Amazonas. According to data from the National Cancer Institute (INCA), except for non-melanoma skin cancer, CC was the first most incident among women in the North region of Brazil (26.24/100 thousand inhabitants) in 2019. Specifically for Amazonas, estimates for the triennium 2020-2022 were also worrying: for each year, around 700 new cases were expected, with a gross rate of 33.08 and

an adjusted rate of 40.18/100 thousand inhabitants (INCA, 2019). These high numbers of new cases and crude and adjusted rates are important to estimate the magnitude of the disease in this population and to program local actions.

As new screening strategies, the use of cervical-vaginal self-collection devices and the subsequent detection of high-risk Human Papillomavirus (HPV) samples have been widely studied both in advanced urban centers and in remote and difficult-to-access communities (Torres *et al.,* 2018; Marais *et al.,* 2019). In more urbanized places, instead of waiting for the woman to come to the doctor's office for the Pap smear, the health system sends the self-collection device to the woman by mail and she sends it back after the collection has been carried out. The sample is processed and the woman receives the result via email or another system (Marais *et al.,* 2019). In rural and/or remote communities, strategies have been devised to reach these women within the geographical, socioeconomic and cultural limitations imposed (Torres *et al.,* 2018; Brandt *et al.,* 2019).

In addition to screening for HPV, the use of these self-collection devices to detect other sexually transmitted pathogens, such as *T. vaginalis*, *C. trachomatis* and *N. gonorrhoeae*, has also been investigated (Rocha *et al.,* 2019; Rocha *et al.,* 2019; Camus *et al.,* 2021). In the Amazonian context, tracking the bacterium *C. trachomatis* also has enormous regional value: infection by this bacterium is often asymptomatic, allowing its ascending infection in the female genital tract, generating Pelvic Inflammatory Disease (PID), salpingitis, ectopic pregnancy, childbirth premature, low birth weight, miscarriages, neonatal death, among other gynecological and obstetric complications (Azevedo *et al.,* 2019). Medical care for riverine women and newborns in these conditions is very precarious.

This study aimed to investigate the prevalence of HPV and *C. trachomatis* in women living in riverside communities in Coari City, Amazonas, using a self-collection device to obtain the biological sample. In this city, as in all others in the state, health care is centralized in the urban area of the city, with sporadic actions by health professionals in riverside communities. Access to the urban center is carried out exclusively by waterway, and may take several days of travel, and the transfer is difficult for financial reasons. In this context, new strategies for sample collection and processing, return of results and referral for treatment should be investigated.

2. Methodology

2.1 Population and sample

Population composed of riverside women from the rural area of the City of Coari - Amazonas, a city with about 85,000 inhabitants. Of the 206 riverside communities belonging to this city, 58 were visited, selected for convenience, feasibility and access logistics. These communities are located in the regions of the Alto Solimões (Upper Solimões River), Médio Solimões (Middle Solimões River), Baixo Solimões (Lower Rio Solimões), Lago do Mamiá (Mamiá Lake), Rio Copeá (Copeá River), Lago de Coari (Coari Lake) and Ilha do Ariá (Ariá Island).

Considering the prevalence of STIs of 42% in general (Brasil. *et al.,* 2008) with a margin of error of 5% and confidence of 95%, an "n" sample of 385 women was estimated. Inclusion criteria were: sexually active women over 18 years of age. Pregnant women, menstruating women or those using vaginal ointments and creams in the 7 days prior to collection were not included. The exclusion criteria were: women who reported not having been able to collect and those women whose biological sample was negative for the presence of human genomic DNA.

2.2 Data collection and biological sample

Eleven trips were carried out to collect biological samples, 7 of them in our own vessel and 4 in the Fluvial Mobile Unit (FMU) in partnership with the Municipal Health Department of Coari City. In communities, a "house-to-house" approach was used to recruit women, ensuring that all women were invited to each community. Initially, they were invited to participate

in this study and, as accepted and met the inclusion criteria, they answered a standard questionnaire, where personal, clinical and behavioral information were recorded, as well as opinions regarding the acceptance of the self-collection device. The women received explanations about the research project and signed a Free and Informed Consent Form (FICT).

The device used for self-collection was the COARI® brush (Kolplast® Ltda, Brazil). Initially, the participants received an explanation of how they should use the brush (wash their hands; stand with one leg elevated and supported on a chair or bed; introduce the device into the vaginal canal up to the boundary flap; rotate for 5 complete turns; remove the device from the vaginal canal and close it without touching the bristles). The women also received an explanatory pamphlet with instructions on how to handle the toothbrush. Then, they collected the device and returned the device to the team, who identified the brush and sealed the package. After collection, the women also answered questions regarding the use of the cervicovaginal brush. The samples were transported and stored at -20ºC in the Genetics and Molecular Biology Laboratory of the Federal University of Amazonas, in Coari, where DNA extraction was performed. For molecular detection of pathogen DNA, the samples were sent to the Molecular Biology Laboratory of the Multidisciplinary Support Center of the Postgraduate Program in Biotechnology in Federal University of Amazonas – Manaus, Amazonas.

2.3 Laboratory processing

For DNA extraction, the bristles of the brushes were cut with a sterile scalpel blade and placed in 1.5 ml microtubes. 1 ml of distilled water was added to each microtube and these were vortexed for 10 seconds. The tubes were then centrifuged at 10,000xg for 5 minutes, after which the bristles and the supernatant were discarded. The pellet was then resuspended in 200 µl of distilled water. For DNA extraction, the ReliaPrep™ DNA Clean-Up and Concentration System, Promega® kit was used, following the manufacturer's instructions.

A duplex-PCR was performed to detect human genomic DNA and DNA from the bacterium *C. trachomatis*. The primers for human DNA were PCO3/PCO4 (Saiki *et al.,* 1985), and for the detection of *C. trachomatis* the primers used were CT05/CT06 (Bobo *et al.,* 1990). The CT05/CT06 primers amplify a 280bp region of the CT MOMP gene and the PCO3/PCO4 primers amplify a 110bp fragment of the human beta-globin gene The reaction was performed with a final volume of 25 µl, containing: water, 10x buffer, 50mM $MgCl_2$, 5mM dNTP, each primer at 5mM, 1U High Fidelity Platinum Taq DNA polymerase (Invitrogen®) and DNA. The reaction parameters were: 94ºC for 30 seconds, followed by 40 cycles of denaturation (94ºC for 30 seconds), annealing (58ºC for 1 minute and 30 seconds) and extension (68ºC for 2 minutes), with a final extension of 68ºC for 5 minutes. For HPV detection, the set of generic primers PGMY09/11 was used, which amplify a 450 bp fragment of the L1 region of several types of mucosal HPV (Gravitt *et al.,* 2000). The reaction components were: 10x buffer, 50mM MgCl2, 5mM dNTP, 5mM of each primer, Platinum Taq DNA polymerase (Invitrogen®), DNA and water. The reaction parameters were initial denaturation was at 94ºC for 5 minutes, followed by 40 cycles of denaturation at 94ºC for 1 minute, annealing at 55ºC for 1 minute and extension at 72ºC for 1 minute, with final extension at 72ºC for 10 minutes.

In all reactions, positive and negative controls were used. PCRs were performed in a Veriti® thermocycler (Aplied® Biosystem) and their products were analyzed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide (1 µg/µL), under ultraviolet light in a transilluminator (Loccus ® Biotechnology).

2.4 Statistical analysis

Data tabulation was performed using Microsoft Excel® version 2013 and data analysis was performed using SPSS® Inc. Version 22. The level of significance set in the statistical tests was 5Simple absolute frequency and relative frequency for categorical data were calculated. In the analysis of quantitative data, the mean and standard deviation (SD) were calculated. In the categorical data in relation to the PCR result for HPV and CT, the Odds Ratio (OR) was calculated, the chi-square test and

Fisher's exact test were applied to compare the risks in different regions. The prevalence of infections was calculated considering the positivity of the samples.

2.5 Ethical aspects

This research was approved by the Ethics Committee in Research on Human Beings of the Federal University of Amazonas.

3. Results

3.1 General characterization of the sample

Initially, 420 women were enrolled in our study, but one of them reported not having been able to carry out the collection, and was excluded. The research reached 58 riverside communities distributed in 6 regions (Table 1), which Médio Solimões, Lago do Mamiá and Lago de Coari are located around the urban center (up to 32 km) and Baixo Solimões, Rio Copeá and Alto Solimões are further away (between 57 and 84 km) (Gama *et al.,* 2018), with a duration of hours/days of travel depending on the vessel's power.

Table 1 - Number of communities and distance from the urban center of Coari, by regions.

Region	Average distance to urban area by region (km)	Number of communities	Participants (n)
Médio Solimões	18,5	07	79
Lago do Mamiá	23,7	12	94
Lago de Coari	32,3	09	51
Baixo Solimões (2)	57,2	02	18
Rio Copeá (16)	64,4	16	112
Alto Solimões (12)	84,0	12	65
Total		**58**	**419**

Source: Authors (2023).

The sociodemographic data showed that the age of the women participating in our study ranged from 18 to 70 years, with a mean age of 34.3 years (SD=12.1) and a predominance of women in the age group of 21 to 30 years (36.3%). Most of them reported being married or living in a stable relationship with a steady partner (84.7%). Low schooling predominated among them, as 48.9% had only incomplete elementary education, that is, less than 08 years of study. 84.5% of women reported that they worked in subsistence agriculture, with a family income below 01 minimum wage (R$954.00 in 2018, equivalent to about $190.00). Table 2 shows details about the sociodemographic aspects surveyed.

Regarding sexual behavior and clinical history (Table 03), the data showed that the mean age of first sexual intercourse was 15.3 years (SD = 2.5) and most of them (83.1%) reported having had up to 5 sexual partners in a lifetime. The mean age at first pregnancy was 16.9 (SD = 4.4), and the mean number of pregnancies was 4.7 (SD = 3.3) per woman. Regarding reproductive characteristics, 27.7% of women reported having had a miscarriage experience. Most women who had a steady partner (n=388) reported not using condoms (67.5%), while all women who had casual partners (100%) used condoms. Regarding clinical complaints at the time of collection, 313 (74%) of the women interviewed reported one or more, especially discharge (155 reports), dysuria (110 reports) and pruritus (110 reports). Table 3 provides detailed information about these variables.

Table 2 - Distribution according to sociodemographic data of riverside women in Coari City, Amazonas, Brazil.

Variables	n=419	
	f_i	%
Age		
18 |--- 20	40	9,5
21 |--- 30	152	36,3
31 |--- 40	104	24,8
41 |--- 50	62	14,8
51 |--- 60	51	12,2
≥ 61	10	2,4
Mean ± SD	34,4 ± 12,1	
Amplitude	18-70	
Marital status		
Married/Stable Union	355	84,7
Single/Widow/Divorced	64	15,3
Scholarity		
Unschooled	30	7,2
Incomplete Elementary	205	48,9
Complete Elementary	93	22,2
Complete High School	83	19,8
Complete Higher Education	8	1,9
Occupation		
Housewife	31	7,4
Community Health Agent	11	2,6
Fisherwoman	10	2,4
Farmer	354	84,5
Other	13	3,1
Family income		
Up to 1 MW*	369	88,1
1 to 2 MW*	43	10,3
2 to 3 MW*	7	1,7

fi = simple absolute frequency; SD = standard deviation; *MW = Minimum wage (R$ 954,00 in 2018, corresponding to $190.00). Source: authors (2023).

When asked about performing the preventive exam, most of them said they had performed it less than 1 year ago (46.8%), but 61 women (14.6%) reported never having had the exam. Among the women who had never had the exam, the most cited reasons were: fear/shame (42.6%) and difficult access to the exam (23%).

3.2 *C. trachomatis* infection

C. trachomatis DNA was detected in 5 women (1.1%). Sociodemographic, clinical and behavioral data were crossed between infected and uninfected groups; were statistically significant: the highest mean age at first pregnancy among those infected (p=0.0009), the highest number of lifetime sexual partners among positive women (p=0.0002), and "single" marital status among infected women (p=0.0052) (Table 4). Among the infected women, 3 (60%) had 1 or more clinical complaints, which were: pelvic pain, pain after sexual intercourse, discharge, dysuria and bleeding after sexual intercourse.

Table 3 - Distribution according to data on sexual behavior and clinical history of riverside women in Coari City, Amazonas, Brazil.

Variables	n = 419	
	f_i	%
Age of 1st sexual intercourse		
Mean ± SD	15,3 ± 2,5	
Amplitude	9 - 29	
Age of 1st pregnancy (n = 404)		
Mean ± SD	16,9 ± 4,4	
Amplitude	12 - 32	
Number of pregnancies (n=419)		
Never got pregnant	15	3,6
1 to 5	259	61,8
6 to 10	117	27,9
≥ 11	28	6,7
Mean ± SD	4,7 ± 3,3	
Amplitude	0 - 20	
Number of lifetime partners		
1 to 5	348	83,1
6 to 10	59	14,1
11 or more	12	2,9
Condom use with a steady partner (n = 388)		
Yes	126	32,4
No	262	67,5
Condom use with casual partner (n= 4)		
Yes	4	100,0
Number of spontaneous abortions		
0	303	72,3
1	93	22,2
2	15	3,6
3 or more	8	1,9
Last Pap smear		
Never performed	61	14,6
Less than 1 year	196	46,8
Between 1 and 2 years	115	27,4
Between 2 and 3 years	27	6,4
Between 3 and 4 years	6	1,4
5 years or more	10	2,4
Clinical complains		
Yes	313	74,7
No	106	25,3
Signals and symptons (n=313)*		
Pelvic Pain	93	
Discharge	155	
Dysuria	110	
Pruritus	110	
Pain after sexual intercourse	93	
Bleeding After Sexual Intercourse	10	
Genital warts	6	
Genital ulcers	6	

SD = standard deviation; fi = simple absolute frequency. * The results do not add up, as most women had more than one clinical complaint. Source: Authors (2023).

Table 4 - Distribution according to data related to socioeconomic variables and sexual behavior of riverside women with detectable and non-detectable samples for CT. Coari, Amazonas, Brazil.

Variables	*Chlamydia trachomatis*					
	Positive (n= 5)		Negative (n= 414)			
	f_i	%	f_i	%	Total	p
Age						0,579*
Media ± SD	31 ± 12		34 ± 12			
Amplitude	21 - 51		18 - 70			
Age of 1st sexual intercourse						1,000*
Media ± SD	15 ± 3		15 ± 3			
Amplitude	11 - 18		12 - 29			
Age of 1st Pregnancy (n=403)	Positivo (n=5)		Negativo(n=398)			0,0009*
Media ± SD	17 ± 1		14 ± 4			
Amplitude	16 - 18		14 - 32			
Marital status						0,0052**
Single/Widow/Divorced	3	60	61	14,7	64	
Married/Stable union	2	40	353	85,3	355	
Scholarity						0,212**
Unschooled	-	-	30	7,3	30	
Incomplete Elementary	1	20	204	49,3	205	
Complete Elementary	3	60	90	21,7	93	
Complete High School	1	20	90	21,7	91	
Family income						0,407**
Up to 1 MW	5	100	364	87,9	369	
2 or more MW	-		50	12,1	50	
Number of Lifetime Sexual Partners						0,0002**
1 to 5	1	20,0	347	83,8	348	
6 or more	4	60,0	67	16,2	71	
Condom use with a steady partner (388)						0,040***
Yes	4	80	122	31,9	126	
No	1	20	261	68,1	262	
Number of spontaneous abortions						0,379**
0	5	100	298	72,0	303	
1	-	-	93	22.5	93	
2 or more	-	-	23	5,5	23	

SD = standard deviation; fi = simple absolute frequency; *MW = Minimum wage (R$ 954,00 in 2018, corresponding to $190.00);
* Student's t test; **Pearson's chi-square test; *** Fisher's exact test. Source: Authors (2023)

3.3 Human Papillomavirus Infection

The prevalence of HPV infection was 15.7% (66/419). In regions closer to the urban center - up to 30 km away - (Médio Solimões, Lago de Coari and Lago do Mamiá) the prevalence of HPV infection was 14.7%, and in regions further than 30 km (Rio Copeá, Alto and Baixo Solimões), the prevalence was 16.8% (p=0.66). The mean age of infected women was lower than that of non-infected women (32±12 and 35±12, respectively) but the difference was not statistically significant (p=0.064). As shown in table 5, other sociodemographic, clinical and behavioral variables were crossed in relation to the infected and non-infected groups, but there was almost no difference between the groups. Regarding clinical complaints, 80% of women infected with HPV reported signs and symptoms, which were: discharge, pelvic pain, dysuria, pruritus, pain after sexual intercourse, genital warts and ulcers, and bleeding after sexual intercourse.

Table 5 - Distribution according to data related to socioeconomic variables and sexual behavior of riverside women with detectable and non-detectable samples for HPV. Coari, Amazonas, Brazil.

Variables (n= 419)	Human Papillomavirus					
	Positive (n=66)		Negative (n=353)			
	f_i	%	f_i	%	Total	*p*
Age						0,064*
Media ± SD	32 ± 12		35 ± 12			
Amplitude	18 - 63		18 - 70			
Age of 1st sexual intercourse						1,000*
Media ± SD	15 ± 2		15 ± 3			
Amplitude	10 - 19		9 - 29			
Age of 1st Pregnancy (n=403)	**Positivo (n=64)**		**Negativo (n=339)**			1,000*
Media ± SD	17 ± 4		17 ± 5			
Amplitude	14 - 25		13 - 32			
Marital status						0,087**
Married/Stable union	50	75,7	305	86,4	355	
Married/Stable union	16	24,3	48	13,5	64	
Scholarity						0,903**
Unschooled	6	9,1	24	6,8	30	
Incomplete Elementary	32	48,5	173	49,0	205	
Complete Elementary	15	22,7	78	22,1	93	
Complete High School	13	19,7	78	22,1	91	
Family income						0,717**
Up to 1 MW	59	89,4	310	87,8	369	
2 or more MW	7	10,6	43	12,2	50	
Number of Lifetime Sexual Partners						0,314**
1 to 5	52	78,8	296	83,9	348	
6 or more	14	21,2	57	16,1	71	
Condom use with a steady partner (388)						0,082**
Yes	26	41,9	100	30,7	126	
No	36	58,1	226	69,3	262	
No steady partner	4	6,1	27	7,6	31	
Number of spontaneous abortions						0,602**
0	48	72,7	255	72,2	303	
1	16	24,2	77	21,8	93	
2 or more	2	3,1	21	6,0	15	

SD = standard deviation; fi = simple absolute frequency; *MW = Minimum wage (R$ 954,00 in 2018, corresponding to $190.00); * Student's t test; **Pearson's chi-square test; ***Fisher's exact test. Source: Authors (2023).

3.4 Acceptance of self collection

Of the 419 women included in the study, 398 (94.8%) reported that they had no difficulty handling the self-collection device, and 367 (87.4%) reported not having felt any discomfort during use. Among the women who had already had the Pap smear (n = 358), 93.6% reported that they preferred self-collection over collection performed by a professional, and the most cited reasons for this were greater privacy (40%), greater ease (36%) and less discomfort (18%).

4. Discussion

Although Brazil has a public and universal health system (Sistema Único de Saúde – SUS), the principle of Universality has not been implemented for a significant part of Brazilians, in particular, populations living in rural areas, which represents about 16% of its population (Garnelo *et al.,* 2018). The State of Amazonas has an estimated population of about 4,341,175 inhabitants (IBGE 2022), distributed in 62 municipalities comprising urban and rural areas. In rural areas of the state, we find small riverside communities living in situations of geographic isolation, leading to difficult access not only to health services, but also to educational, vocational technical courses, banking, commercial and communication services, among

others. Residents live relegated to a situation of self-care in health for most of their lives, and it is common for many of them to spend a long period without going to the nearest urban centers. There is a great concentration of medical services in the state capital (Manaus City), and a huge discrepancy between the number of doctors in the capital and in the interior, requiring the increase of more public policies to attract and retain doctors in these regions (Garnelo, Sousa & Silva 2017; Garnelo *et al.,* 2018; Garnelo *et al.,* 2020; Sousa *et al.,* 2022).

The Fluvial Mobile Unit (FMU) has been a strategy used in the Brazilian Legal Amazon and in the Pantanal, financed by the federal government, to bring systematic health actions to the riverside people, as being a "land between rivers", the "rivers are the roads". It works as an "ambulatory boat", with a multidisciplinary team composed of a nurse, doctor, dentist, nursing technician, biochemist, vaccinator, among others (Garnelo *et al.,* 2020). However, the monthly trips of the FMU have been organized to serve different micro-regions each month, and even so, they are insufficient to cover the immense territory intended, so that the riverside people do not receive periodic or specialized medical care, only sporadic and generalist. Official guidelines from the Ministry of Health recommend that the FMU should visit each community at least every 60 days (Brasil 2014; Garnelo *et al.,* 2020), which has not been possible in this region.

For the collection in more distant regions, our team of researchers made some trips together with the health team at FMU. However, despite the relative good structure of the UMF laboratory, it was not possible to detect *C. trachomatis* and HPV during these trips. But we found that it is possible to be carried out on site, but with adjustments in relation to the delivery of results and referral of patients, as the laboratory processing of the samples takes about 48 hours to be done, and the FMU usually stays only 1 day or only 1 period. of the day (morning or afternoon) in a community, moving to the next. Following this logistical plan, the result of the examination would have to be delivered to the Community Health Agent (CHA) a few days later and this same professional would be responsible for scheduling the return for women who thus need follow-up and additional investigations in the urban area of the city.

For collection in the communities closest to the urban center of Coari, we travel in small boats rented by the team itself. In these communities, we count on the collaboration of the local CHA. The CHA works as an arm of Primary Health Care in remote places, a link between the gateway to the local health system and the most isolated users of the territory. He is a person who lives in the community, knows its problems and enjoys the trust of other community members (Garnelo *et al.,* 2020). Studies suggest that when ACS are well trained, they become important allies for improving the quality of services in the Amazon region, emphasizing the importance of these professionals in remote and rural areas (Sousa *et al.,* 2022). The rate of refusal to participate in this research was very low, practically non-existent, as the CHA was part of the process of invitation to participate. He was also part of the return of the result to the patients: they received the result, delivered it to the women and already referred those infected by CT or HPV to gynecological care in the urban region of the city. In this type of screening for STIs, the ACS can be the bridge for the delivery of the self-collection device to women, forwarding the device to the laboratory, delivery of results and referral of infected patients.

The profile of the women participating in our study revealed a predominance of women aged between 21 and 30 years; most women reported being married/stable union, had low education, low income, working in subsistence agriculture. Our results also showed that women had low adherence to condom use and an average number of children above the national average. Other studies carried out with riverine women showed similar results (Gama *et al.,* 2018; Rocha *et al.,* 2019a; Rocha *et al.,* 2019b; SEADE 2021). When we analyze this profile, the condition of vulnerability in which these women live become clear, as they are deprived of health, education and various forms of public policies that can help them overcome many of the geographic, economic and cultural barriers to improving their quality of life (Garnelo *et al.,* 2020). We take as an example the lack of public policies to increase the flow and commercialization of their rural production, perpetuating subsistence

agriculture, low income and the lack of access to the consumption of industrialized products. Many communities also lack electricity and sanitation (Gama *et al.,* 2018).

With regard to reproductive issues, the lack of quality education, access to condoms and family planning contribute to increasing female vulnerability, making them more susceptible to acquiring STIs and the occurrence of many pregnancies. Our results showed that the mean age of first sexual intercourse was 15.3 ± 2.5 years, with a record of girls who reported having had their first sexual intercourse at 9 years of age. Similarly, the mean age at first pregnancy was also low (16.9 ± 4.4 years), showing a high prevalence of teenage pregnancy, with reports of girls being pregnant for the first time at 12 years of age. This makes clear the state of neglect in which many riverside children and adolescents live, many of them being victims of sexual abuse for years in communities, where the insufficient action of the guardianship councils and the absence of other control groups by the public power perpetuate the certainty of impunity (Fernandez, Tavares, & Pinheiro 2016; Vieira, Oliveira & Sókora 2017).

The Pap smear has been used as the main screening tool for cervical cancer by the Brazilian government. Despite the high coverage and easy access to this test in larger urban centers, in remote rural areas this access suffers from many restrictions, especially those imposed by the physical distance between users and health services (Lopes & Ribeiro 2019). In addition, the number of unsatisfactory slides, low sensitivity and false negatives limit the process, leading to dissatisfaction on the part of users and sometimes loss of patient follow-up. Unscreened women are certainly the main sources of new CC cases, and in our survey we found that 61 of them (14.6%) had never had the test. The most cited reasons for this were fear/shame (42.6%) and difficult access to the exam (23%). Most of the women (46.8%) reported having had the preventive exam less than 1 year ago, but we must consider the fact that the exam was being carried out on collection trips in partnership with the Municipal Health Department at the FMU, from so that we do not have information about the last time they had performed the exam before that, to evaluate if it was being done with the correct frequency.

Some characteristics related to the reproductive life and sexual behavior of women found in our study are risk factors for the development of CC, such as multiparity, early sexual initiation, non-use of condoms and multiple partners. Thus, considering the aforementioned high rate of CC among women in Amazonas, the prevalence of HPV infection found in our population sample (15.7%) reinforces the need for greater coverage of STI screening services in these women. Other studies also carried out in this same Amazon region bring worrying results in relation to HPV infection. Rocha et al (2013), analyzing 364 women undergoing routine gynecological examination in the urban area of Coari, found HPV infection in 29.1% of them, through collection carried out by a professional and molecular detection by PCR, with a predominance of HPV-16 (58.1%) and HPV-58 (20%), both types of high oncogenic risk. In our study, genotyping of HPV-positive samples was not performed to verify the prevalence of high-risk oncogenic HPV, which is one of its limitations. The importance of genotyping is not to cause unnecessary alarm to women infected with low-risk HPV, since these (as the name implies) are not related to the development of CC and many infections will have a clearance in a few years.

In the interesting study carried out by Torres et al (2018) with 412 riverside women from Coari and also using a self-collection device (Evallyn® Brush, from Rovers®) to obtain the biological sample, the authors found (by PCR) the prevalence of 18.7% of HPV among the participants. In genotyping, performed by hybridization with probes in the Papillocheck TM assay, they found a variety of viral types including 51, 16, 53, 18, 31, 70, 52, 56, 66, 82, 58, 11, 40, 45 and 69, almost all of them at high risk, with some cases of co-infections. As an innovation, these authors also used a rapid test to detect oncoprotein E6 from high-risk HPVs 16 and 18, finding 1.4% of detection, especially in women who had altered results in cytopathological and histopathological exams, showing high specificity. The authors consider this strategy (self-collected sample plus high-risk HPV detection by rapid test) to be a promising strategy to increase screening coverage in remote areas. International studies also suggest this protocol in poorly screened women (Marais *et al.,* 2019).

In regions closer to the urban center - up to 30 km away - (Médio Solimões, Lago de Coari and Lago do Mamiá) the prevalence of HPV infection was 14.7%, and in regions further than 30 km (Rio Copeá, Alto and Baixo Solimões), the prevalence was 16.8% (p=0.66). Although this difference was not statistically significant, the higher prevalence of HPV in more remote communities is very worrying, since the greater the distance, the more impediments these women have to medical care in the city.

In addition to the detection of HPV, the biological sample from the self-collection brush has also been shown to be suitable for the detection of other sexually transmitted pathogens (Marais *et al.,* 2019; Rocha *et al.,* 2019a; Rocha *et al.,* 2019b; Camus *et al.,* 2021). Camus *et al*., (2021) carried out a large study with more than 1,000 women with the objective of showing the non-inferiority of the sample obtained by self-collection in relation to that taken by the professional for the detection of genital infections in general, such as vaginosis bacterial infection, sexually transmitted infections, and group B streptococci. For pathogen detection, the authors used culture and real-time PCR. They found concordant positivity rates in all groups of microorganisms studied and argue that their study provides evidence that self-collected samples can be used as universal biological specimens for detecting infections in the lower female genital tract. Similarly, Marais *et al*., (2018) evaluated 3 types of samples obtained from 675 women: a self-collection performed at home, a self-collection performed at the medical clinic and a collection performed by a professional, and found concordant indices for HPV, CT, *Trichomonas vaginalis* (TV), *Neisseria gonorrhoeae* (NG) and *Mycoplasma genitalium* (MG) among the 3 samples.

In our research we found a prevalence of 1.1% of infection by CT using PCR for the diagnosis of the bacteria. This prevalence is lower than that found by Rocha *et al.,* (2019a) in riverside women living in communities also in the Middle Solimões region, whose prevalence was 3.7%. These authors used the EVALYN® self-collection brush (Rovers®) and also the CT05/CT06 primers in PCR. Rocha *et al*., (2014) found a higher prevalence (6.4%) also in women in the same region in samples collected by a professional. Azevedo *et al*., (2018), studying pregnant women living in the urban center of the city of Coari, in the second trimester of pregnancy, found 18% positivity for CT using both a cervical sample collected by a professional and urine.

In our study, only the analysis of the biological material collected through the COARI® self-collection device was performed, and the professional did not perform a cervical-vaginal collection. It could be considered that this low prevalence found is due to the fact that the self-collection brush is not efficient to collect endocervical cells (which have a high tropism for the CT bacteria), and the sample collected by a professional would be better indicated for this. However, as previously mentioned, studies show a high agreement in the CT detection rate when comparing the two types of samples: Camus *et al*., (2021) found 3.2% of CT infection in a sample collected by the professional and 3.3% in a self-collected sample, studying more than 1,000 women. detected 1.1% of TC in self-collected samples and 1.6% in clinician-collected samples. Other authors also found concordant results (Fang *et al.,* 2008). Thus, we believe that, in fact, there is a low prevalence of this bacterium in this population sample studied. Although 27.7% of women reported having experienced a miscarriage – which is related to CT infection – other factors may be related to this high number of miscarriages, as other pathogenic microorganisms and other causes can also cause miscarriages.

Our results regarding the acceptance of self-collection showed that it was well accepted by them, and most women reported not having felt discomfort or having any difficulty in handling the brush, which is in line with several studies carried out on the subject with different female audiences (Marais *et al.,* 2019; Brandt *et al.,* 2019; Rocha *et al.,* 2019a; Camus *et al.,* 2021). These cited studies also bring reasons similar to those cited by the participants of our study for even preferring self-collection to collection carried out by professionals: in addition to greater comfort and ease of handling, greater privacy and less pain were also cited. The use of the Coari® self-collection brush was, therefore, well accepted by the participants and showed stability in the conservation of the collected biological material and suitability for non-refrigerated dry transport.

5. Conclusions

In our population sample studied, we found a high prevalence of HPV infection and a low prevalence of CT infection. The self-collection device used was well accepted by the participants and proved to be suitable for collecting biological samples in regions of difficult access. The optimization of the use of this device should be further studied in riverside communities covering a greater number of sexually transmitted pathogens for a better understanding of its applicability in the region.

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Conflict of interest

The authors declare that they have no conflict of interest.

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