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Original Article

T. vaginalis in riverside women in Amazonia, Brazil: an experience using the EVALYN® BRUSH vaginal self-collection device

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Abstract

Introduction: The challenges related to the diagnosis of sexually transmitted infections present more complex factors in remote and hard-to-reach areas. The use of self-collection devices that facilitate the obtaining of a biological sample with high quality for sensitive molecular tests have been examined. This study aimed to evaluate the performance and acceptance of the Evalyn® Brush (Rovers® Medical Devices) for detection of *T. vaginalis* among women living in the riverside communities of Amazonas, Brazil.

Methodology: The study included 300 riverside women. They received instructions for self-collection, carried out the task, and then answered a questionnaire on the use of the device. *T. vaginalis* was detected by Polymerase Chain Reaction, using primers TVK3/TVK7.

Results: The mean age of the women was 35.8 years, and most of them presented low schooling, low income, agricultural activity and lived in a marital union. All samples were positive for human genomic DNA (100%) and the prevalence of *T. vaginalis* infection was 5.6% (n = 17). Of the 300 women, 293 (97.7%) indicated that they liked the use of the device, 287 (95.7%) reported having had no difficulty in handling it, 265 (88.3%) did not feel any type of discomfort and 228 (76%) said they preferred the self-collection to the collection made by the professional, mainly due to privacy and comfort.

Conclusions: The Evalyn® Brush proved reliable as a device for the collection of biological samples for molecular analysis and was well-accepted by women. Its use can be indicated in remote and hard to reach places.

Key words: *Trichomonas vaginalis*; Amazonas; self-collection.

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Introduction

Early detection of sexually transmitted infections (STI) has a vast and positive impact on both individual and public health: it allows the institution of appropriate treatment, reduces transmission between partners, decreases risk of death, avoids long-term complications, as well as medical and hospital expenses of the treatment in the advanced stages of the disease, among other benefits [1]. Vaginal trichomoniasis is one of the most prevalent STI [2]. Despite its high incidence, *Trichomonas vaginalis* infection has not received much attention from the Brazilian Health System, which is most likely due to its severe symptomology, but is not considered as not very serious. Only recently, *T. vaginalis* infection has been associated with a variety of adverse health effects for women (and the unborn baby), including preterm birth, low birth weight, post hysterectomy infection, inflammatory pelvic disease and infertility [2–4]. One of the most serious associations has been the role of *T. vaginalis* infection in facilitating the acquisition and transmission of Human Immunodeficiency Virus (HIV), as it has been postulated to increase the risk of contamination by 2 to 3 times [2,4–7].

Coping with the Acquired Immunodeficiency Syndrome (AIDS) epidemic and other STI (even curable ones) takes on more complex proportions in remote and hard to reach regions, as these regions exhibit adverse conditions for collection and transport of biological samples and little or no infrastructure for on-site examination [8,9]. The Brazilian Amazon region is an area of great demographic dispersion, with small isolated riverside communities, and with exclusive access by river or air (through small seaplanes). These riverside communities located in the Amazon interior have social, cultural and geographic peculiarities, with health care centralized in the urban areas and sporadic actions of health care professionals

in the riverside communities. Access to the urban area is usually limited by the financial conditions of the population, and especially the distance, which can exceed 500km and a displacement of several days [9–11].

Vaginal self-collection devices have been studied in both urban and isolated regions as methods to facilitate the collection of biological samples, mainly for Human Papillomavirus (HPV) screening. In general, they have been well-accepted by women, with reliable stability and conservation of biological material, and the results demonstrate a strong agreement with samples obtained by professionals [12–14]. In this study, the objective was to verify the prevalence of *T. vaginalis* infection in a sample of riverine women using Evalyn® Brush to collect the sample, and to evaluate its performance, acceptance and viability of use in these locations.

Methodology

Study design, population and sample

This is a cross-sectional descriptive study, carried out in the city of Coari, Amazonas, Brazil. The sample was calculated based on the population of 6,110 female inhabitants, who are over 18 years old and live in the rural area of Coari (data from the Brazilian Institute of Geography and Statistics, 2010). The expected prevalence of *T. vaginalis* infection used for the sample calculation was 12.7% [9], with a margin of error of 3% and confidence level of 95%, thus estimating a minimum number of 266 women to be included in the study.

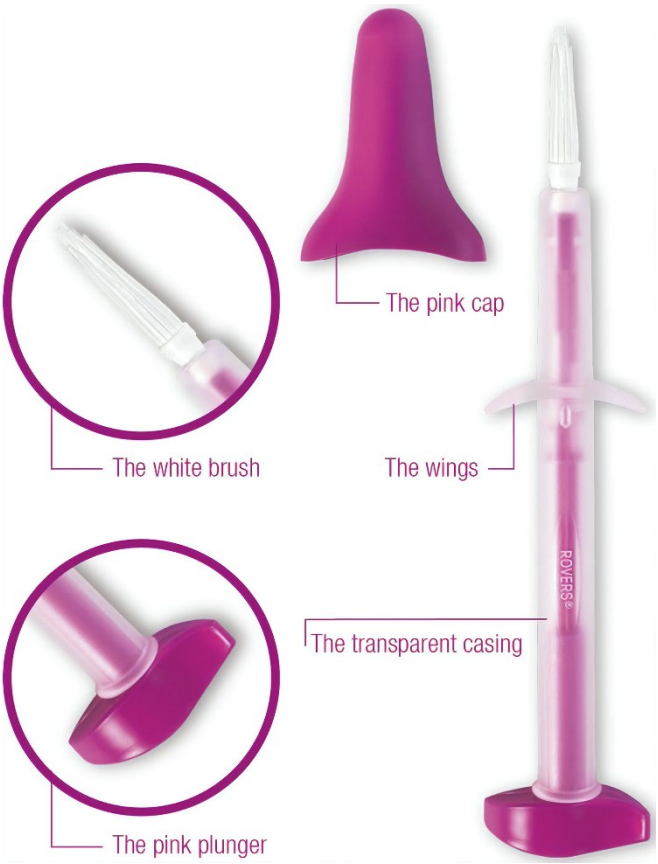
Coari city has 206 riverside communities, from which 32 were selected for convenience from August 2014 to February 2015. Women over the age of 18 that agreed to participate in the study were included. Virgins, pregnant women, those who had been using vaginal medicine in the last 7 days or menstruating at the time were excluded.

Meetings were held with the Community Health Agents (CHA) of the selected communities to explain the procedures related to the development of the research. In urban areas, a CHA is a professional that makes up a multi-professional team in basic health care services. However, because there are no permanent multi-professional teams in the riverside communities, a CHA is the single "link" between users and health services available in urban centers, and each community has a CHA. Thus, in the days before the team went to the community, the CHA was advised by radio to invite the women to an event at the Community Center or at the local school, where the research team explained the objectives, risks and benefits of the study and requested participation.

Collection of data and biological sample

In this study, the Evalyn® Brush (Rovers® Medical Devices) was applied. This device is about 20 cm long and has a plunger at one end and a brush at the other end. It is easy to use, as it has a deep indicative insertion, which is controlled by its wings (Figure 1). The number of rotations can be perceived by an audible click during collection, and the woman can perform the self-sampling lying down or standing. Initially, women who agreed to participate answered a standard questionnaire with questions about sociodemographic characteristics, clinical history, and sexual behavior. After being instructed about the technique of self-sampling with the help of an explanatory leaflet, they went to a private location for self-sampling with the Evalyn® Brush. The women then returned the brushes, which were labeled and stored. At the end of the session, the women answered a few more questions regarding the use of the self-sampling brush. The brushes were transported at room temperature to the

Figure1. Cervical-vaginal self-collecting device COARI® (Kolpast®, São Paulo, Brazil).



Molecular Biology Laboratory at the Institute of Health and Biotechnology of the Federal University of Amazonas, in the urban area of Coari City and stored in a refrigerator (4ºC) until processing (maximum 7 days).

Laboratory processing

The bristles of the brushes were cut with a sterile scalpel blade and transferred to a DNAse and RNAse free microtube. The DNA of the samples was extracted by the QIAamp DNA MiniKit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. To verify the extraction efficiency, a Polymerase Chain Reaction (PCR) was performed for human genomic DNA using PCO4 / GH20 primers (PCO4: 5′ CAA CTT CAT CCA CGT TAC ACC 3′ e GH20: 5′ GAA GAG CCA AGG ACA GGT AC 3′), for the human beta globin gene and amplified a fragment of 268 base pairs (bp) [15]. The final volume of the reaction was 25 µL, containing: 2.5 µL of buffer 10X, 0.8 µL of 50 mM $MgCl_2$, 2.0 µL of 2.5 mM dNTP, 1.0 µL of primer mix at 10 µM, 0.25 µL of Platinum Taq DNA polymerase (5U / µL) (Invitrogen, EUA), 2.5 µL of glycerol 57%, 2.5 µL cresol red, 5.0µL of DNA and water. PCR was performed on the Veriti thermal cycler (Applied Biosystems, Foster City, CA, USA), with the following thermocycling conditions: 94°C for 5 minutes, 40 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute, followed by 72°C for 10 minutes. PCR products were analyzed by 1.5% agarose gel electrophoresis, stained with ethidium bromide and visualized on ultraviolet light in a transilluminator.

To detect *T. vaginalis*, a PCR was performed using the TVK3/TVK7 pair of primers (TVK3: 5′ ATT GTC GAA CAT CAT TGG TCT TAC CCT C 3′ e TVK7: 5′ TCT GTG CCG TCT TCA AGT ATG C3′), which amplified a fragment of 300 bp [16]. The final volume of the reaction was 25 µL, containing: 0.25 µL of Platinum Taq DNA polymerase (5U/µL) (Invitrogen, Carlsbad, USA 5 mM of each primer, 2.5 µL of buffer 10X, 0.8 µl of 50 mM $MgCl_2$, 10 mM dNTP, 5.0 µL of DNA and water. Amplification was performed on the Veriti thermal cycler (Applied Biosystems, Foster City, CA, USA) under the following cycling conditions: 95°C for 5 minutes, 35 cycles of 95°C for 1 minute, 60°C for 30 seconds and 72°C for 2 minutes, and final extension at 72°C for 7 minutes. The amplified products were submitted to 2.0% agarose gel electrophoresis, stained with ethidium bromide (0.5 mg / mL) and visualized with the aid of a transilluminator. Positive and negative controls were used in all PCRs.

Statistical analysis

Data was tabulated in the Epi-Info program (7.2.1.0) and statistical analysis was performed in the BioEstat program (5.0). To verify statistical differences between the groups, the t-Student test was used. The analysis of the significance of difference in proportions as well as a subsequent evaluation of whether there was an association between variables were performed using the Pearson Chi-Square Test (χ^2) and/or Fisher's Exact Test, for cases in which the expected frequency was less than five ($p < 0.05$). The level of significance used in the tests was 5%, with a confidence interval of 95%.

Table 1. Frequency distribution of demographic and clinical variables of the riverside women sampled in Coari City, Amazonas, Brazil, 2015.

Variables (n = 300)	fi*	%
Age (years)		
< 25	75	25,0
25 |--- 35	83	27,7
35 |--- 45	69	23,0
45 |--- 60	50	16,7
60 |--- 70	19	6,3
≥ 70	4	1,3
Mean ± SD**	35,8±13,7	
Range	18 - 77	
Schooling		
≤8 years	224	74,7
>8 years	76	25,3
Occupation		
Farmer	232	77,3
Other occupations	68	22,7
Familiar income (minimum wages)* **		
≤1	286	95,3
>1	14	4,7
Marital status		
Married/stable marriage	256	85,3
Single/Divorced/Window	44	14,7
Number of pregnancies		
None	18	6,0
1 to 3	104	34,7
4 to 6	88	29,3
7 to 9	52	17,3
> 9	38	12,7
Range	0 - 21	
Abortion history		
None	209	69,7
01 or more	91	30,3
Clinical complaints	141	47,2
Pelvic pain	76	25,3
Discharge	51	17,0
Pruritus	51	17,0
Difficulty urinating	38	12,7
Others	5	1,7

*f_i = absolute simple frequency **SD = Standard Deviation; *** Minimum wage amount (at that time): $ 258.00.

Results

Demographic, clinical and behavioral characteristics

300 women participated in this study, ranging in age from 18 to 77 years old with a mean of 35.8 years old (SD = 13.7). The majority had low schooling, agricultural activities and a very low family income. The 85.3% were married or living in a stable union with a male partner. Most of the women had 1 to 3 pregnancies and 91 women (30.3%) had experienced miscarriage. At the time of the interviews, 141 women (47.2%) reported clinical complaints, the main ones were pelvic pain, discharge, pruritus and dysuria (Table 1).

The women affirmed that the age of their first sexual intercourse ranged from 10 to 25 years, with an average of 15.2 years (SD = 2.2). Most women acknowledged having had more than one lifetime sexual partner (66%), but only one sexual partner in the last 12 months (88.3%), predominating the inconsistent use of condoms (80.6%). When asked why they did not use condoms, most answered that they were "using another contraceptive method" (66.3%) (Table 2).

Device Acceptance and Performance

All women approached accepted the self-sampling of the vaginal sample, and there was no refusal of any women who met the inclusion/exclusion criteria. All samples (100%) were positive for human genomic DNA, demonstrating that all women used the Evalyn® Brush correctly and that it was efficient in collecting suitable biological material for molecular testing. When asked if they "liked" the collection technique, 293 women (97.7%) said "yes". The main reasons described by the 7 women (2.3%) who answered "no" were due to pain, "method was strange" and discomfort. When asked about the difficulty in using the brush, 287 women (95.7%) stated that they did not find it difficult.

Table 2. Characteristics related to the sexual behavior of the riverside women sampled in Coari City, Amazonas, Brazil, 2015.

Variables (n = 300)	fi*	%
Age of first sexual intercourse (years old)		
Media ± SD**	15,2 ± 2,2	
Range	10 - 25	
Number of sexual partners in the last 12 months		
None	22	7,3
01	265	88,3
02	8	2,7
≥03	5	1,7
Range	0 - 10	
Number of sexual partners in life		
01	132	44,0
02	47	15,7
≥03	121	14,3
Range	1 - 25	
Use of condom		
Never	130	43,3
Sometimes	112	37,3
Always	58	19,3
Reasons for not using condoms (n=187)		
She does not like	39	20,9
Partner does not like	21	11,2
Married or stable marriage	03	1,6
Use of other contraceptive methods	124	66,3

*f_i = absolute simple frequency **SD = Standard Deviation.

Table 3. Acceptance of the use of the self-collection device by the sampled riverside women of Coari City, Amazonas, Brazil, 2015.

Categories (n = 300)	f_i*	%
Presence of blood in the sample		
Yes	19	6,3
No	281	93,7
Women who said they had liked		
Yes	293	97,7
No	7	2,3
Reasons not to like (n=7)		
“I felt pain”	1	14,3
"I found it strange"	1	14,3
“I felt discomfort”	1	14,3
Women who could not answer why they did not like it	4	57,1
Women who found it difficult		
Yes	13	4,3
No	287	95,7
Difficulties (n=13)		
Difficulty opening the device	1	7,7
Device introduction and rotation	3	23,0
Women who did not know how to respond	9	69,2
Discomfort		
No	265	88,3
Yes	35	11,6
Causes of discomfort (n=35)		
Burning	16	45,7
Pain	15	42,9
Others	4	11,4
Preference		
Self-sampling	228	76
Collection performed by the professional	15	5
Indifferent ("whatever")	57	19
Reasons for the preference of self-collection (228)		
Privacy	146	64,1
Comfort	45	19,7
Facility	15	6,6
Safety	14	6,1
Women who did not know how to respond	8	3,5
Reasons for the preference for collection by the professional (15)		
Safety	12	80
Complete gynecological exam	3	20

*f_i = absolute simple frequency.

Of the 13 women (4.3%) who found it difficult to handle the device, 3 (23%) said they had difficulty inserting and rotating, 1 (7.7%) said they had difficulty opening the brush and 9 (69.2%) could not specify why they found it difficult. Regarding the discomfort of the collection, 265 women (88.3%) did not feel any type of discomfort. Of the 35 women (11.6%) who described feeling discomfort, the main causes of discomfort reported were burning and pain. When asked if they preferred self-sampling or collection by the professional, 228 women (76%) said they preferred self-sampling, and the main reasons cited were "privacy" and "comfort". Of the 15 women (5%) who said they preferred the collection performed by the professional, the main reasons given were that they "felt more secure" and "preferred a complete gynecological exam". 57 women (19%) were indifferent ("whatever") (Table 3).

Prevalence of T. vaginalis

The prevalence of *T. vaginalis* infection was 5.6% (n = 17). The group of infected women had a mean age lower than the uninfected group as well as the mean age of the first sexual intercourse, and both differences were statistically significant (p = 0.010 and p = 0.027, respectively). The prevalence of infection was higher in the group of single/separated/widowed women than in the group of women who were in a marital relationship; it was also higher in the group of women who had experienced miscarriage than in the group of women

Table 4. Distribution by frequency of demographic variables of the riverside women of Coari City, Amazonas, Brazil, regarding *T. vaginalis* infection. 2015.

Variables (n = 300)	*Trichomonas vaginalis*				Total	p
	Positive		Negative			
	f_i*	%	f_i*	%		
Age (years old)						0,010****
< 25	5	6,7	70	93,3	75	
≥ 25	12	5,3	213	94,7	225	
Mean ± SD**	29,2 ± 9,5		36,1 ± 13,8			
Schooling						0,574*****
≤8	14	6,3	210	93,7	224	
>8	3	3,9	73	96,1	76	
Marital status						0,717****
Married/Stable union	14	5,5	242	94,5	256	
Single/Divorced/Window	3	6,8	41	93,2	44	
Occupation						0,133****
Farmer	16	6,9	216	93,1	232	
Others occupations	1	1,5	67	98,5	68	
Familiar income (minimum wages)***						0,962******
≤1	16	5,6	270	94,4	286	
>1	1	7,1	13	92,9	14	
Abortions						0,786****
None	11	5,3	198	94,7	209	
One or more	6	6,6	85	93,4	91	
Use of condoms						0,338****
Sometimes/Never	12	5,0	230	95,0	242	
Always	5	8,6	53	91,4	58	
Clinical complaints						1,000****
Yes	8	6,0	125	94,0	133	
No	9	5,4	158	94,6	167	
Number of pregnancies						0,426******
Q_1 - Median - Q_3	2 - 3 - 5		2 - 4 - 7			
Age of first sexual intercourse						0,027****
Mean ± SD	14,3 ± 1,6		15,3 ± 2,2			
Number of partners in last 12 months						0,528******
Q_1 - Median - Q_3	1 - 1 - 1		1 - 1 - 1			
Number of partners in life						0,342******
Q_1 – Median - Q_3	1 - 3 - 5		1 - 2 - 4			

*f_i = absolute simple frequency; **SD = Standard Deviation; *** Minimum wage amount (at that time): $ 258.00; ****student t-test; ***** Fisher test; ****** Mann-Whitney test.

who did not report this experience, but these differences between the groups were not statistically significant (p = 0.717 and p = 0.786, respectively). Of the 17 infected women, eight (47.0%) reported symptoms and 53.0% were completely asymptomatic (Table 4).

Discussion

With the advent of AIDS, all STI have assumed great importance in the context of public health, since all are considered as facilitators of HIV infection [4]. Thus, new strategies that increase STI screening coverage are needed globally. Screening for syphilis and HIV through rapid tests can be performed with a simple finger puncture and are very useful for use in non-clinical settings and in places without adequate infrastructure for laboratory tests such as the riverside communities of the Amazon. But the best strategies for screening other STI - such as *T. vaginalis*, *C. trachomatis*, *N. gonorrhoeae* and Human Papillomavirus - in these isolated places and with limited financial resources are still under study.

In this study, the performance and acceptability of the Evalyn® Brush self-collection device in combination with Nucleic Acid Amplification Test (NAAT) for the screening of *T. vaginalis* in women living in Amazonian villages were evaluated. In the riverside communities, there are no medical clinics, and the presence and care of doctors, nurses, and other health professionals are rare and sporadic, especially in remote communities [11]. Another way of helping the health of the riverside residents is through occasional actions by the Brazilian Navy or Army or by non-governmental organizations [10], which navigate their ambulatory boats providing care wherever they go. However, as these actions are not systematic, not even the syndromic approach can be considered as the most viable methodology, since the riverside women do not have healthcare "when they need it" (that is, when the symptoms appear), but "when the boat passes by their village", relegating the riverside women to a situation of permanent underdiagnosis and self-care.

It is apparent that access to healthcare is multidimensional, encompassing physical and financial aspects as well as acceptability of services [17]. Physical accessibility is the greatest challenge for individuals living in rural areas such as these, and the CHA in these places is the only permanent link between the population and health services; he/she is responsible for scheduling medical appointments in the city, along with the delivery of medical exams and medication. There is no systematic transport for the communities studied in this study, so that the riverside residents can only access health services in the city if they have their own boat (small boats) or if they ask others (their neighbors) for a hide to the city. Financial accessibility is limited by the low income of these women (95.3% of them had a family income of up to 1 minimum wage - $258 at the time), making it difficult for them to access the urban area of the city frequently and/or for long periods of time. Regarding the acceptability of services and medical examinations, the appearance of non-invasive and self-collecting methods that are comparable to the samples collected by professionals in relation to the quality of the biological material collected has been very important.

Of the women approached by the team, there was no refusal to participate in the study. It was taken into consideration that the self-sampling was well-accepted by the participants, since 293 (97.7%) women answered that they liked this methodology after the collection. Regarding the device's handling, only 13 women (4.3%) said they had some difficulty, most specified that it was due to the insertion and rotation of the brush. Therefore, it is supposed that the handling of Evalyn® Brush by women was easy, since the brush closely resembles the devices commonly used for the application of vaginal creams, which most women are familiar with. It is emphasized here that the explanatory leaflet used to give the instruction for the use of the Evalyn® Brush contained many images and was explained calmly and repeatedly to the women, since most of them had a very low educational level (74.7% had up to 8 years of study). Thus, it was observed that they used the brush correctly, because there was 100% PCR positivity for human DNA, showing that there was a high-quality biological sample for molecular diagnosis for each.

When asked if they preferred the collection with a professional or the self-sampling, the majority declared a preference of self-sampling (76%). Clearly, the collection with a professional usually has different purposes, including the cytological examination for cervical cancer screening (Papanicolau). But even for the investigation of malignant and pre-malignant lesions, new screening tools based on self-sampling coupled with molecular tests for HPV DNA amplification and/or rapid tests for detection of high-risk HPV oncoproteins have been studied, exhibiting promising results [18]. The reasons most often reported by women who preferred self-collection were "privacy" and "comfort", which is in agreement with other studies, since the main barriers conveyed to IST screening include shame (especially with male professionals), fear of pain, discomfort with pelvic

examination, invasiveness of professional collection, distrust of confidentiality, and long waiting times in clinics [19–21]. On the other hand, some women indicated concern about the safety of the method (that is, it may be that they are not doing the collection correctly, which could generate a false negative result) considering the collection by a professional to be safer. Other women stated that they preferred the collection with professional because they wanted to have a "more complete examination", demonstrating knowledge of the importance of the visual inspection and the conversation with a professional to ask questions about the symptomatology, if present.

The prevalence of *T. vaginalis* infection found in this study was 5.6%. From the Brazilian studies that used NAATs, almost all of them were from samples collected by professionals, and the indices varied between 3.2% and 31.8% of prevalence, in several groups of women (pregnant, HIV-positive, women in gynecological consultation of routine, etc.) [9,22–26]. When the wet mount or culture test was administered, the prevalence found in the Brazilian studies varied between 4.1% and 16% [27–29]. To the best of the authors' knowledge, there has been only one study carried out in Brazil using a self-collection device (vaginal swab) for the detection of *T. vaginalis*, but this study did not use NAAT, but the XenoStrip *T. vaginalis* Test (Xenotopo Diagnostic, San Antonio, TX, USA), nowadays called OSOM *Trichomonas* Rapid Test (Sekisui Diagnostic, San Diego, CA, USA) [30]. This interesting study by Jones *et al*. (2013) [30] was realized both in women who had sought clinical those who received the swab for self-sampling and performed the test themselves at home (a self-fulfilling immunochromatographic rapid test). The women received 2 swabs, one for PCR (considered gold standard) and the other for the rapid test, which takes only about 10 minutes to prepare. The PCR results showed a prevalence of 3.0% of *T. vaginalis* infection in women; the specificity of the rapid test was very high (99.7-100), but the sensitivity was moderate (62.4-72.7).

In order to reduce adverse health effects, in particular the facilitation of HIV acquisition and transmission and undesirable clinical outcomes in pregnancy and perinatal morbidity [3–5], the combination of self-collection coupled with the rapid immunochromatographic test for *T. vaginalis* would be an ideal combination when utilized in remote areas such as riverside communities. The woman would apply the self-sampling in a private setting, take the test, have the result quickly, and receive the medication, going well beyond the syndromic approach [31]. It is necessary to keep in mind that most infections by *T. vaginalis* in both men and women are asymptomatic, so it is understandable that the syndromic approach is not as effective as would be desirable [2,31,32]. However, a low or moderate sensitivity of the rapid test is not ideal, and further studies of sensitivity and applicability of the test in this context are required. Other rapid tests for *T. vaginalis* are also commercially available - such as Affirm VPIII (Benton Dickinson, Sparks, Maryland, USA), AmpliVue Assay (Quidel, San Diego, CA, USA), Solana TV-Assay (Quidel, San Diego, CA, USA), GeneXpert TV Test (Cepheid, Sunnyvale, CA, USA) – but these tests also vary in sensitivity, take at least 45 minutes for the result, and need minimal laboratory structure [32], so that the sample would need to be transported along with another team trip for the delivery of the results and medication (as was the case for this study). In this scenario, the participation of CHA would be fundamental.

In the literature review published by Poole & McClelland [2], the authors cite some studies that show that, unlike other curable non-viral STI (including *C. trachomatis* and *N. gonorrhoeae*), higher rates of infection by *T. vaginalis* are found in older men and women compared to adolescents and young adults. In the study conducted by Rocha *et al*. [9], in the investigation of *T. vaginalis* infection in women living in the urban area also in Coari City, a higher mean age was observed among infected women, but without statistical significance. In contrast, in this study with rural women, it was observed that the group of infected women had a mean age lower than the non-infected group (29.2 ± 9.5 years and 36.1 ± 13.8 years, respectively), and both differences were statistically significant ($p = 0.010$ and $p = 0.027$ respectively). Other clinical and epidemiological variables (economic and educational status, marital status, number of sexual partners, consistent use of condoms, number of children, previous history of miscarriage, etc.) were not statistically related to a higher rate of *T. vaginalis* infection in this study.

It is estimated that in women, more than 80% of *T. vaginalis* infections are asymptomatic, so they can persist for several months. When symptoms are present, the most common include vaginal discharge, odor, dysuria and pruritus [2–4,33]. Of the 17 infected women, eight (47.0%) reported symptoms, which were: pelvic pain, vaginal discharge, pruritus and dysuria, with overlapping clinical complaints in some women. The remaining 53.0% were completely asymptomatic. Considering the difficulties of access of the riverside

women to the health services identified above, even the symptomatic cases do not receive the management recommended by the Ministry of Health (syndromic approach), keeping them as silent reservoirs of this pathogen.

Conclusions

It is believed that the vaginal self-sampling device Evalyn® Brush performed well as a biological sample collection instrument for molecular examination and was well-accepted by study participants. Its dry transport and stability make it a suitable device for use in remote and hard to reach places.

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