


## SHORT REPORT

# Human alphaherpesvirus 2 (HSV-2) among female sex workers from Brazil: cross-sectional study using respondent-driven sampling

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## ABSTRACT

**Objective** Female sex workers (FSWs) are vulnerable to human alphaherpesvirus 2 (HSV-2) infection due to their high numbers of sexual partners. The objective of this study was to evaluate the seroprevalence and risk behaviours associated with HSV-2.

**Methods** A cross-sectional study was conducted in Mato Grosso do Sul, Brazil. A total of 376 FSWS were recruited by respondent-driven sampling (RDS) methodology and answered an epidemiological questionnaire. Blood samples were collected to test for HSV-2 antibodies using commercial ELISA and for HSV-2 DNA using real-time PCR.

**Results** The seropositivity was 47.3% (178/376) for HSV-2 IgG and 10.1% (38/376) for HSV-2 IgM. HSV-2 viraemia was detected in two infected FSWS with primary infections. In bivariate and multivariate analyses, the OR for HSV-2 IgG increased with age (OR=2.53–7.90, OR=2.66–6.37) and the number of sexual partners (OR=2.30–3.25). On the other hand, daily alcohol consumption (OR=0.10) and the use of condoms during the last intercourse (OR=0.47) were protective factors against HSV-2 acquisition.

**Conclusion** Despite the impact of FSWS in public health policies with the dissemination of sexually transmitted infections, there have been few studies performed regarding the prevalence of HSV-2 in Brazil, making it difficult to implement any control or preventative measures. The results produced here using an RDS methodology demonstrated a high prevalence, risk behaviours and primary infection among the FSWS. These results reinforce the need to implement control and preventative measures for HSV-2 infection in this population.

## INTRODUCTION

Female sex workers (FSWs) stand out as an important population for the study of HIV and STI due to their social vulnerability and associated risk factors, such as high number of sexual partners, alcohol and other substance use and inconsistent condom use.

Genital herpes is an STI mainly caused by human alphaherpesvirus 2 (HSV-2). It is estimated that 267 million women were living with HSV-2 in 2012.<sup>1</sup> In Brazil, the prevalence of HSV-2 among the female population is approximately 25%<sup>2</sup> and that among FSWS varies worldwide but is generally high when compared with that of the general population.

Despite the important role of FSWS in the dissemination of sexually transmitted infections, there have been few studies performed, and the prevalence of HSV-2 is unknown in FSWS in Brazil, making it difficult to implement any control or preventative measures. Therefore, the current study aimed to evaluate the seroprevalence, presence of infection and risk behaviours associated with HSV-2 serological diagnoses.

## MATERIALS AND METHODS

The study assessed FSWS from Campo Grande who work in public places. All participants were recruited via a peer selection process known as respondent-driven sampling. Local organisations selected four participants, known as 'seeds', according to their working places and their popularity with other sex workers. Each seed recruited three new peers, and these three new participants each recruited other three participants, so the recruitment was increased in waves. Sample size was calculated as 350 participants to exhibit statistical significance. Participants were eligible for the study if they identified themselves as FSWS, aged 18 years or older, and currently living in the specified region. Participants were excluded if they presented themselves as transgender sex workers or lacked the mental and physical conditions necessary to answer the questionnaire. All eligible participants were interviewed and had their blood collected. The interviews were performed using quantitative questionnaires containing questions on demographic characteristics, sexual behaviour and risk factors. The participants received the ticket transportation.

Blood samples were analysed for HSV-2 IgG and HSV-2 IgM using commercial ELISAs (BIOELISA

HSV-2 IgG; Biokit, Barcelona, Spain) and RIDASCREEN HSV-2 IgM (R-Biopharm, Darmstadt, Germany). All anti-HSV-2-positive participants were referred to an infectious disease clinic for further clinical assessment and the provision of appropriate treatment. The serum samples reactive for HSV-2 IgM were analysed in duplicate by Taqman real-time PCR to detect HSV-2 DNA, according to Lima *et al.* This protocol exhibits detection limit of  $10^1$  copies/uL of HSV-2 DNA.<sup>3</sup>

These samples were tested to HSV-1 (IgM and DNA) to confirm a non-specific reaction<sup>3</sup> (Herpes simplex 1; Euroimmun, Lübeck, Germany).

The dataset was analysed with the Respondent-Driven Sampling Analysis Tool (RDSAT) V.5.6.0. RDSAT was used to adjust for differences in network size and for homophily to provide population-based estimates of the study population characteristics. Adjusted frequency distributions were calculated along with 95% CIs. Yates' corrected  $\chi^2$  test was used to compare the frequencies among groups. Association was expressed as prevalence OR with 95% CIs estimated by logistic regression. Statistical significance was set at a p value of 0.05.

## RESULTS

The sample size of 376 FSWs was achieved in 10 waves of recruitment. The risk factors, demographics and sexual behaviour characteristics of all FSWs are shown in table 1. Out of the 376 FSWs, 178 (47.3%) and 38 (10.1%) were reactive for HSV-2 IgG HSV-2 IgM, respectively. Of the reactive, 36.8% (14/38) were only reactive for HSV-2 IgM and 63.2% (24/38) were HSV-2 IgM and IgG positive. HSV-2 DNA was detected in two (2/38) samples (viral load:  $4.3 \times 10^2$  and  $7.4 \times 10^2$  copies/ $\mu$ L) that were only reactive for HSV-2 IgM, with high titres (394 and 543 units/ $\mu$ L). The samples were tested to HSV-1 IgM and DNA. All of them were negative to HSV-1. Unfortunately, we could not test the samples with other HSV-2 IgM assays. However, the titres of IgM positive was high, ranging from 394 to 543 units/uL.

The risk behaviours associated with seropositivity for HSV-2 IgG were older ages, the absence of condoms during the last intercourse and a high number of sexual partners in the previous week (table 1).

## DISCUSSION

In Brazil, the lack of studies evaluating specific information about HSV-2 infections in FSWs makes it difficult to identify the prevalence and behaviours associated with this infection before performing any interventions. In this study, the majority of FSWs were young (average age of 30.8 years) and had low levels of education (52.4% had elementary school educations or less). This result may be explained by difficult access to school or leaving school early due, for example, to entry into prostitution.

Approximately 52.9% of FSWs reported consuming alcoholic beverages daily, and 41.8% reported using injectable drugs. These values are higher than those found among Brazilian FSWs where the workplaces are on the indoor avenues and streets; the daily alcohol consumption was 23.4%–30.7%; and injectable drug use was 18.5%–20.3%.<sup>4</sup>

The use of drugs and alcohol is justified as a way to face the reality of prostitution. Regarding their sexual behaviours, 51.6% reported having had fewer than 7 sexual partners in the last week, but the average number of partners in the last week was 11. In a study conducted in Argentina,<sup>5</sup> FSWs had a slightly higher average number of sexual partners per week than that reported in our population (15). The majority of

FSWs (97.2%) reported having used condoms during their last intercourse, which shows their awareness of safe sex as a method of avoiding STIs. The presence of genital lesions in the last 12 months was reported by 4.5% of the FSWs, suggesting signs of an STI, but this prevalence is less than that among FSWs from Mexico (36.5%).<sup>6</sup>

The seroprevalence of HSV-2 was 47.3%, which is higher than the prevalence found in the adult population in Brazil (~30%)<sup>2</sup> and is lower than the prevalence found in previous studies conducted with FSWs from Guangxi, in China (54.9%)<sup>7</sup> and Peru (60.6%).<sup>8</sup> The diversity in the prevalence of STIs around the world could be due to differences in cultural practices, behaviours and social conditions among these countries.

This study showed statistical association between the prevalence of HSV-2 infection and increased age. In general, older FSWs have spent a longer time in a sex career, which contributes to a higher exposure to the risk of infection. Another factor associated with the increased chance of HSV-2 infection was the number of sexual partners in the last week, with an increase in the OR correlated to more partners. In agreement with our results, Chohan *et al.*<sup>9</sup> also showed that the number of sexual partners was associated with the HSV-2 infection and that the risk of acquiring HSV-2 increases with numbers of sexual partners.

Interestingly, daily alcohol consumption by FSWs was shown to be a protective factor against HSV-2 infection. The protective association between alcohol and the prevalence of HSV-2 in our population can be explained by the fact that the most of the FSWs are young. The prevalence of HSV-2 tends to increase with age, while alcohol consumption tends to be higher among younger people, creating the false impression that alcohol consumption protects against HSV-2 infection in young women.

The use of condoms during the last sexual intercourse decreased the risk of HSV-2 infection among FSWs by 90%. However, the high prevalence of HSV-2 found in this study shows that condom use is not always practised. Although among FSWs the genital transmission of HSV-2 involves contact with vaginal secretions, studies of the detection of antibodies and DNA in serum samples are important to know the prevalence of HSV-2 and to detect viraemia during primary infection by herpesviruses. In this study, FSWs with primary infections (2/376) had detectable levels of HSV-2 DNA. HSV viraemia in primary infections is considered rare because the virus normally follows the neuronal route for its transport, but HSV DNA has been detected in blood samples from some patients with recurrent oral herpes and primary genital infection.<sup>10</sup>

Of the 17 FSWs who reported having had genital lesions in the past year, HSV-2 infection was confirmed in 9 patients with HSV-2 IgG positivity. Although we cannot confirm that HSV-2 was the cause of these lesions, the diagnosis and monitoring of the genital lesions is important because genital herpes can facilitate the acquisition of HIV. Both DNA HSV-2-positive and anti-HSV-2-positive patients did not report genital lesion in the moment that the blood was collected.

This study had the following limitations: a high degree of similarity (homophily) in our population because all participants were from only two cities (Ladário and Campo Grande) due to a high preference among FSWs for recruiting participants of their own city and the potential bias involving recall and barriers concerning confidentiality and/or intimate revelations caused by face-to-face interviews. Despite these limitations, our findings have important implications for future

**Table 1** Distribution of characteristics, seropositivity prevalence (HSV-2 IgG) and  $\chi^2$  test (p value) of the female sex workers (N=376)

	HSV-2 IgG seropositivity		$\chi^2$	OR <sub>CR</sub> (95% CI)	OR <sub>AD</sub> (95% CI)
	n (%)	Seropositivity (%)			
Skin colour			0.32		
White	119 (31.6)	62 (52.1)		–	–
Black	39 (10.4)	16 (41)		–	–
Brown	212 (56.4)	99 (46.7)		–	–
Other	3 (0.8)	1 (33.8)		–	–
Age (years)			0.000		
<20	59 (15.7)	17 (28.8)		1	1
21–25	132 (35.1)	46 (34.8)		1.32 (0.67 to 2.57)	1.28 (0.62 to 2.64)
26–30	81 (21.5)	41 (50.6)		<b>2.53 (1.24 to 5.16)</b>	<b>2.66 (1.23 to 5.75)</b>
31–40	83 (22.1)	58 (69.9)		<b>5.73 (2.75 to 11.92)</b>	<b>5.03 (2.29 to 11.03)</b>
>41	21 (5.6)	16 (76.2)		<b>7.90 (2.49 to 25.0)</b>	<b>6.37 (1.85 to 21.94)</b>
Support someone					
No	104 (27.7)	49 (47.1)	0.52	1	–
Yes	272 (72.3)	129 (47.4)		1.01 (0.64 to 1.59)	–
Income per day			0.72		
<\$55	138 (36.7)	64 (46.4)		1	–
\$55–\$277	221 (58.8)	105 (47.5)		1.04 (0.68 to 2.57)	–
>\$277	12 (3.2)	7 (58.3)		1.61 (0.48 to 5.34)	–
Alcohol consumption			0.04		
No	44 (11.7)	26 (59.1)		1	–
Every day	199 (52.9)	81 (40.7)		<b>0.47 (0.24 to 0.92)</b>	–
Once a week	116 (30.9)	61 (52.6)		0.76 (0.38 to 1.55)	–
Once a month	17 (4.5)	10 (58.8)		0.98 (0.31 to 3.08)	–
Drug use			0.03		
No	219 (58.2)	113 (51.6)		1	–
Yes	157 (41.8)	65 (41.4)		0.66 (0.43 to 1.00)	–
Injectable drugs			0.55		
No	371 (98.5)	176 (47.4)		1	–
Yes	5 (1.3)	2 (40.0)		0.73 (0.12 to 4.47)	–
Most frequent sexual practice			0.14		
Oral	174 (46.3)	80 (46.0)		1	–
Vaginal	137 (36.4)	60 (43.8)		–	–
Anal	2 (0.5)	2 (100.0)		0.58 (0.32 to 1.06)	–
All	63 (16.8)	36 (57.1)		12.5 (0.00 to 52.3)	–
Condoms use in the last sexual intercourse			0.04		
No	9 (2.4)	8 (88.9)		1	–
Yes	365 (97.1)	169 (46.3)		<b>0.10 (0.01 to 0.87)</b>	–
Do not remember	2 (0.5)	1 (50.0)		0.12 (0.00 to 3.99)	–
Genital lesions in the last year			0.23		
No	359 (95.5)	(46.8)		–	–
Yes	17 (4.5)	(58.8)		–	–
Education level (years)			0.66		
0–4	18 (4.8)	11 (61.1)		–	–
5–9	179 (47.6)	84 (46.9)		–	–
10–12	164 (43.8)	75 (45.7)		–	–
>13	14 (3.7)	7 (50.0)		–	–
Age at first commercial sex (years)			0.02		
<15	33 (8.8)	15 (45.5)		1	–
18–20	151 (40.2)	57 (37.7)		0.72 (0.34 to 1.55)	–
21–25	95 (25.3)	52 (54.7)		1.45 (0.65 to 3.21)	–
26–30	48 (12.8)	28 (58.3)		1.68 (0.68 to 4.10)	–
>31	17 (4.5)	10 (58.8)		1.71 (0.52 to 5.60)	–
Age at first sexual intercourse (years)			0.37		
<12	37 (9.8)	19 (51.4)		–	–
13–15	185 (49.2)	87 (47.0)		–	–

Continued

Table 1 Continued

	HSV-2 IgG seropositivity			OR <sub>CR</sub> (95% CI)	OR <sub>AD</sub> (95% CI)
	n (%)	Seropositivity (%)	$\chi^2$		
16–18	135 (35.9)	66 (48.9)		–	–
>19	18 (4.8)	5 (27.8)		–	–
Number of sexual partners in the last week			<b>0.008</b>		
<7	194 (51.6)	81 (41.8)		1	1
8–10	48 (12.8)	26 (54.2)		1.64 (0.87 to 3.11)	1.85 (0.93 to 3.67)
11–15	53 (14.1)	33 (62.3)		<b>2.30 (1.23 to 4.29)</b>	<b>2.18 (1.11 to 4.27)</b>
>15*	77 (20.4)	36 (63.1)		<b>4.05 (1.29 to 8.89)</b>	<b>3.42 (1.85 to 7.18)</b>

Bold values are of statistical significance.

\*Numbers of partners 16–20 (n=20, seropositivity 14%) and >20 (n=57, seropositivity 22%).

HSV-2, human alphaherpesvirus 2; OR, odds ratio.

research and prevention efforts targeting these populations in Brazil.

In conclusion, this study showed a high HSV-2 seroprevalence, the presence of risk factors and two cases of primary infection with viraemia among FSWs. Once HSV-2 causes a latent infection and can be transmitted orally or sexually, pre-emptive efforts should be intensified among FSW.

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#### REFERENCES

- 1 Looker KJ, Magaret AS, Turner KME, *et al.* Global estimates of prevalent and incident herpes simplex virus type 2 infections in 2012. *PLoS One* 2015;10:e114989.
- 2 Clemens SAC, Farhat CK. Seroprevalence of herpes simplex 1-2 antibodies in Brazil. *Rev Saude Publica* 2010;44:726–34.
- 3 Lima LRP, Silva APda, Schmidt-Chanait J, *et al.* Diagnosis of human herpes virus 1 and 2 (HHV-1 and HHV-2): use of a synthetic standard curve for absolute quantification by real time polymerase chain reaction. *Mem Inst Oswaldo Cruz* 2017;112:220–3.
- 4 Damacena GN, Szwarcwald CL, Souza Júnior PRBde. Hiv risk practices by female sex workers according to workplace. *Rev Saude Publica* 2014;48:428–37. 0.
- 5 Bautista CT, Pando MA, Reynaga E, *et al.* Sexual practices, drug use behaviors, and prevalence of HIV, syphilis, hepatitis B and C, and HTLV-1/2 in immigrant and non-immigrant female sex workers in Argentina. *J Immigr Minor Health* 2009;11:99–104.
- 6 Rusch MLA, Brouwer KC, Lozada R, *et al.* Distribution of sexually transmitted diseases and risk factors by work locations among female sex workers in Tijuana, Mexico. *Sex Transm Dis* 2010;37:1–14.
- 7 Chen S, Yin Y, Chen X, *et al.* Seropositivity and risk factors for herpes simplex virus type 2 infection among female sex workers in Guangxi, China. *PLoS One* 2013;8:e69697.
- 8 Cárcamo CP, Campos PE, García PJ, *et al.* Prevalences of sexually transmitted infections in young adults and female sex workers in Peru: a national population-based survey. *Lancet Infect Dis* 2012;12:765–73.
- 9 Chohan V, Baeten JM, Benki S, *et al.* A prospective study of risk factors for herpes simplex virus type 2 acquisition among high-risk HIV-1 seronegative women in Kenya. *Sex Transm Infect* 2009;85:489–92.
- 10 Johnston C, Magaret A, Selke S, *et al.* Herpes simplex virus viremia during primary genital infection. *J Infect Dis* 2008;198:31–4.