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First report on sexually transmitted infections among trans (male to female transvestites, transsexuals, or transgender) and male sex workers in Argentina: high HIV, HPV, HBV, and syphilis prevalence☆

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SUMMARY

Objectives: Due to the scarce data on the prevalence of sexually transmitted infections (STIs) among male-to-female trans-sex workers (TSW) and male sex workers (MSW) in Argentina, the present study aimed to estimate the incidence of human immunodeficiency virus (HIV), and the prevalence of HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), and *Treponema pallidum*. Human papillomavirus (HPV) and *Chlamydia trachomatis* infections were tested among TSW.

Methods: Two hundred and seventy-three TSW and 114 MSW were recruited by nongovernmental organizations. HIV incidence was estimated by STARHS (serologic testing algorithm for recent HIV seroconversion). HPV and *C. trachomatis* infections were tested in anal cells from TSW.

Results: TSW showed significantly higher prevalences of HIV (34.1 vs. 11.4%), HBV (40.2 vs. 22.0%), and *T. pallidum* (50.4 vs. 20.4%) than MSW. TSW tested positive for HPV in 111/114 cases and for *C. trachomatis* in 4/80 cases. Investigation of HBV, HCV, HIV, and *T. pallidum* co-infections showed that 72% of TSW and 39% of MSW had at least one STI. *T. pallidum* was the most frequent mono-infection. The estimated HIV incidence was 10.7 per 100 person-years (95% confidence interval (CI) 3.8–17.7) for TSW and 2.3 per 100 person-years (95% CI 0–6.7) for MSW.

Conclusions: The high prevalence of STIs and the high incidence of HIV demonstrate the great vulnerability of these high-risk populations and indicate the urgent need for preventive strategies on intervention and facilitation of access to healthcare programs.

1. Introduction

The human immunodeficiency virus (HIV) epidemic in Argentina is concentrated, since HIV prevalence is consistently over 5.0% in at least one defined subpopulation.[1] Populations with the highest prevalence of infection include injecting drug users (IDUs), non-injecting cocaine users (NICUs), and men who have sex with men (MSM).[2–4] Following these subpopulations with regard to HIV prevalence, pregnant women in urban areas exhibit a prevalence of less than 1.0%, while blood donors exhibit a prevalence of 0.19%.[5,6]

Data from 1998 to 2008 in our laboratory showed high HIV prevalence in several high-risk populations: 44.3% in IDUs, 6.3% in NICUs, 13.8% in MSM (1.6% of whom were engaged in sex work), 17.1% in patients with tuberculosis, 7.2% in patients attending hospitals in Buenos Aires for consultations on sexually transmitted infections (STIs), and 3.2% in female sex workers (FSW).[2–4,7–9] No

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data on seroprevalence are available for male sex workers (MSW), while only one study so far has assessed HIV and *Treponema pallidum* prevalence (27.6% and 42.0%, respectively) among trans-sex workers (TSW).[10] TSW include male-to-female transvestites, transsexuals, and transgender individuals. This population is highly vulnerable and marginalized in Argentina, where sex work, a low level of education, and strong social stigmatization are conditions associated with a high HIV prevalence.[11]

Due to the scarce data on HIV and STIs among TSW and MSW in Argentina, the present study was considered critical to achieve a better understanding of HIV and STI prevalence in this country. Therefore, the aim of the present study was to estimate HIV incidence, and the prevalence of HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), and *T. pallidum* infections, as well as the frequency of co-infections with different pathogens in TSW and MSW from several cities of Argentina. A group of TSW was also tested for human papillomavirus (HPV) and *Chlamydia trachomatis* infections.

2. Materials and methods

2.1. Recruitment of participants

In the present study, sex workers were defined as adults who receive money or goods in exchange for sexual services, either regularly or occasionally, and who identify themselves as sex workers.[12] The study was conducted from October 2006 to December 2009 in seven cities of Argentina: Buenos Aires, La Plata, Córdoba, Mendoza, Rosario, Santiago del Estero, and Viedma. The TSW and MSW included in this study were aged 18 years and older. Individuals previously diagnosed with the pathogens under study were also included in the study. Nongovernmental organizations such as AMMAR (Asociación de Mujeres Meretrices de la Argentina), ATTTA (Asociación de Travestis, Transexuales y Transgénero de Argentina), and Nexo AC (which deals mainly with MSM) were responsible for recruiting the participants.

In each city, sex workers were identified by other peers, invited to participate in the study, and offered testing and counseling on STIs. The purpose of the study was explained to all the participants during the first encounter, and those who chose to participate were invited to read the informed consent form. After signing the form, participants were interviewed by a trained peer or social worker using a standardized questionnaire specific for each group. Results were linked to the questionnaire by a numeric code that preserved confidentiality and anonymity.

During the last 18 months of the study, HPV and *C. trachomatis* testing and HBV vaccination were offered to TSW attending Nexo AC. If the participant was found to be HBV-positive, the scheduled vaccination was reevaluated.

International and national ethics guidelines for biomedical research involving human subjects were followed (Council for International Organizations of Medical Sciences, International Ethical Guidelines for Biomedical Research Involving Human Subjects; CIOMS 2002, CIOMS 1991). This research was reviewed by the Nexo AC Institutional Biomedical Review Board (IRB 00005349) and conducted in compliance with all federal regulations governing the protection of human subjects.

2.2. Blood sample collection for diagnosis of HIV, HBV, HCV, and Treponema pallidum

Samples of anticoagulated and non-anticoagulated blood were collected in sterile conditions using standard safety precautions and protocols.

HIV screening was performed by ELISA (Enzygnost Anti-HIV 1/2 Plus ELISA, Dade Behring, Germany) and particle agglutination (Bio-Rad, Fujirebio Diagnostics, Inc., Japan). Reactive samples were subsequently confirmed by Western blot (New LAV Blot I, Bio-Rad Laboratories, Inc., WA, USA).

Syphilis infection (past or present) was determined by non-treponemal and treponemal assays (Venereal Disease Research Laboratory (VDRL), Wiener Laboratorios, SAIC, Rosario, Argentina; *Treponema pallidum* hemagglutination assay (TPHA), Biokit SA, Barcelona, Spain). An indirect immunofluorescence test (FTA-abs, Inmunofluor Biocientifica SA, Argentina) was used in the case of discordant results.

Markers of HBV infection such as surface antigen (HBsAg) and anti-core antibody (anti-HBc) were determined using ELISA (HBsAg (V2) Abbott AxSYM System, Core AxSYM System Abbott, Wiesbaden, Germany). For epidemiological purposes, a sample was considered HBV-positive if at least one of the markers was found. In order to determine HCV infection, HCV antibodies were tested by ELISA (HCV version V3.0, Abbott AxSYM System, Wiesbaden, Germany).

The participants were scheduled to return in 2 weeks to receive their HIV, *T. pallidum*, HBV, and HCV serology results and post-test counseling. Participants with a positive diagnosis were referred to clinical centers in order to receive proper evaluation and treatment. Treatment was offered to those volunteers with current *T. pallidum* infection.

2.3. Incidence estimation

The incidence of HIV infection was estimated by STARHS (serologic testing algorithm for recent HIV seroconversion), as described elsewhere.[13,14] The test consists of a modified enzyme immunoassay that allows identification of recent infections (time of infection less than 4–6 months prior to sample collection).

Samples included in this analysis were those obtained up until October 2009.

2.4. Diagnosis of HPV and Chlamydia trachomatis in samples of anal mucosa obtained by cytobrush

Due to methodological issues, HPV and *C. trachomatis* testing were offered only to TSW participants attending Nexo AC. Those who accepted were instructed on how to collect the samples (auto-sampling).

Anal brushing was performed with a cytobrush (Endocervical brush, Medisul, Argentina); this was stirred in a tube containing sterile phosphate buffered saline (PBS). The brush was removed and the tube was centrifuged at 1500 rpm for 10 min. The supernatant was discarded and the pellet was kept at −20 °C until processing. Cells were disrupted by proteinase K digestion and tested by polymerase chain reaction (PCR) for the β-globin gene to confirm the presence of adequate DNA template.[15] HPV DNA detection and typing was performed as described elsewere.[16,17]

The *ompA* gene of *C. trachomatis* was amplified using a semi-nested PCR.[18] An approximately 1-kb fragment of the *ompA* gene was amplified using the primers SERO1A and SERO2A. DNA of *C. trachomatis* L2/BU/434 (kindly provided by Sezione di Microbiologia DMCSS, Università degli Studi di Bologna, Bologna, Italy) and mock-infected cells were included as positive and negative controls, respectively.

One microliter of the first-round PCR product was used for the semi-nested PCR performed with the same reagents and conditions, except for the primers, which were SERO2A, and one nested primer: PCTM3. The second-round PCR products were checked on ethidium bromide-stained 1.5% agarose gels. This semi-nested PCR was able to detect 1–10 inclusion-forming units.

C. trachomatis genotyping was carried out in all PCR-positive samples by restriction fragment length polymorphism (RFLP).[19]

Table 1
Prevalence of sexually transmitted infections among male-to-female trans-sex workers (TSW) and male sex workers (MSW) in Argentina, 2006–2009

	TSW		MSW		*p*-Value
	n/N	% (95% CI)	*n/N*	% (95% CI)	
HIV	93/273	34.1 (28.7–39.9)	13/114	11.4 (6.7–18.7)	<0.001
HBV	106/264	40.2 (34.4–46.2)	18/82	22.0 (14.3–32.1)	<0.05
HCV	12/264	4.5 (2.5–7.9)	5/82	6.1 (2.3–13.8)	NS
Treponema pallidum	130/258	50.4 (44.3–56.4)	23/113	20.4 (13.9–28.8)	<0.001
HPV	111/114	97.4 (92.2–99.4)	ND	ND	–
Chlamydia trachomatis	4/80	5.0 (1.6–12.5)	ND	ND	–

Comparisons between proportions were analyzed using the Chi-square test or Fisher's exact test according to the sample size.
HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HPV, human papillomavirus; ND, not done; NS, not significant.

Genotypes were identified by their restriction patterns on ethidium bromide-stained 12% polyacrylamide gel electrophoresis.

2.5. Statistical analysis

Confidence intervals around proportions were calculated using the adjusted Wald method. Comparisons between proportions were analyzed using parametric and non-parametric methods. Univariate analyses were performed by Chi-square test or Fisher's exact test according to the sample size. Statistical analyses were carried out using SPSS 15.0 (SPSS Inc., Chicago, IL, USA) and Epidat 3.0 ().

3. Results

3.1. Characteristics of the study sample

The study included a population of 273 TSW and 114 MSW (166 TSW and 34 MSW from Buenos Aires City, 22 TSW and 51 MSW from La Plata, 50 TSW and two MSW from Córdoba, 13 MSW from Mendoza, five TSW from Rosario, 30 TSW and 13 MSW from Santiago del Estero, and one MSW from Viedma). Since some cities had a very small number of participants, results were not stratified by city. No significant differences were detected between cities with high numbers of participants (data not shown). Due to recruitment characteristics, no accurate tracking of individuals who were invited but did not participate was registered. However, as estimated by the recruiters, from those invited by AMMAR to participate in the study, around 90% accepted. In the case of ATTTA, this value was 50–70%. TSW participants had a median age of 29 years (interquartile range (IQR) 24–35 years), while MSW had a median age of 27 years (IQR 22–33 years). Most of the participants were born in Argentina (81.2%). A preliminary analysis of the epidemiological data from the questionnaire showed that 90% of TSW preferred male clients and that if they had a steady partner, 95% of them had a male partner. Among MSW, almost 67% preferred male clients and from those who had a steady partner, 49% had a male partner.

3.2. HIV and other STIs in TSW

In the TSW studied, HIV was detected in 34.1% of the samples, while the HIV incidence rate was 10.7 per 100 person-years (95% confidence interval (CI) 3.8–17.7; 85 HIV-positive samples from a group of 259 TSW). HBV prevalence was 40.2%, and among HBV-positive participants, only five were HBsAg-positive. HCV was present in 4.5% and *T. pallidum* infection in 50.4% (Table 1).

Regarding anal brush sample collection, 119 out of the 140 TSW participants from Buenos Aires attending Nexo AC during the last 18 months of the study agreed to self-collect the anal sample. HPV infection was searched for in these 119 samples; five tested β-globin-negative, leaving 114 samples for assessment. Of these, 97.4% were positive (Table 1). For the samples in which it was possible to determine the infecting genotype(s) (103/111), high-risk genotypes were detected in 82.5% (85/103), as shown elsewhere.[17,20]

The diagnosis of *C. trachomatis* was performed in 80 TSW. Of these, 5.0% were positive (Table 1). No venereal lymphogranuloma genotypes were detected in the samples studied.

Results of co-infections among TSW were analyzed either in the 254 participants who had the four agents tested (HBV, HCV, HIV, and *T. pallidum*) or in the 80 volunteers who had the six agents tested (HBV, HCV, HIV, HPV, *T. pallidum*, and *C. trachomatis*) since the prevalence for each of the four pathogens did not significantly differ between groups. When results were considered among the 254 TSW, 72% presented at least one infection. The frequencies of one or two pathogens were each 30%. The most frequent single infection was *T. pallidum* (13.8%) and the most frequent co-infection was HBV–*T. pallidum* (13.8%), followed by HBV–HIV–*T. pallidum* (9.8%) and HIV–*T. pallidum* (9.4%) (Figure 1). The prevalence of *T. pallidum* infection was higher among HBV- and HIV-positive TSW ($p = 0.001$ and $p = 0.017$, respectively).

When considering the 80 participants who had the six agents tested (Figure 2), the most frequent single infection was HPV (23.8%) and the most frequent co-infection was HBV–HPV–*T. pallidum* (15.0%), followed by HBV–HPV (13.8%).

3.3. HIV and other STIs in MSW

HIV prevalence was 11.4% in MSW, while the incidence rate was 2.3 per 100 person-years (95% CI 0–6.7; 13 HIV-positive samples

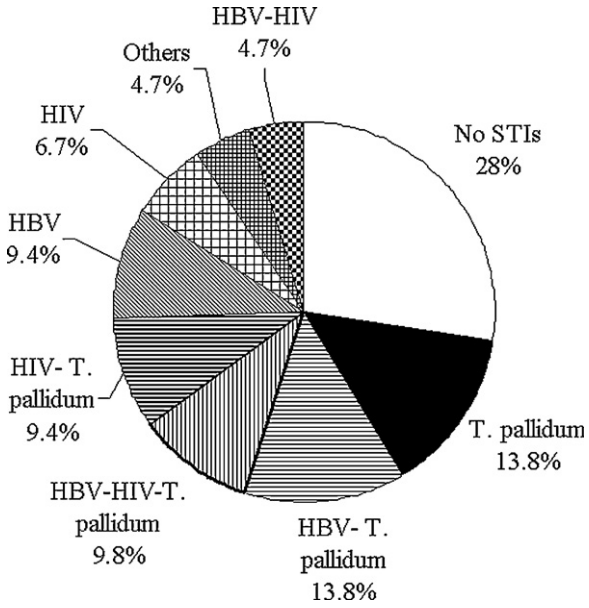

Figure 1. Frequency of co-infecting agents among 254 male-to-female trans-sex workers (TSW) with four pathogens tested (HBV, HCV, HIV, and *Treponema pallidum*).

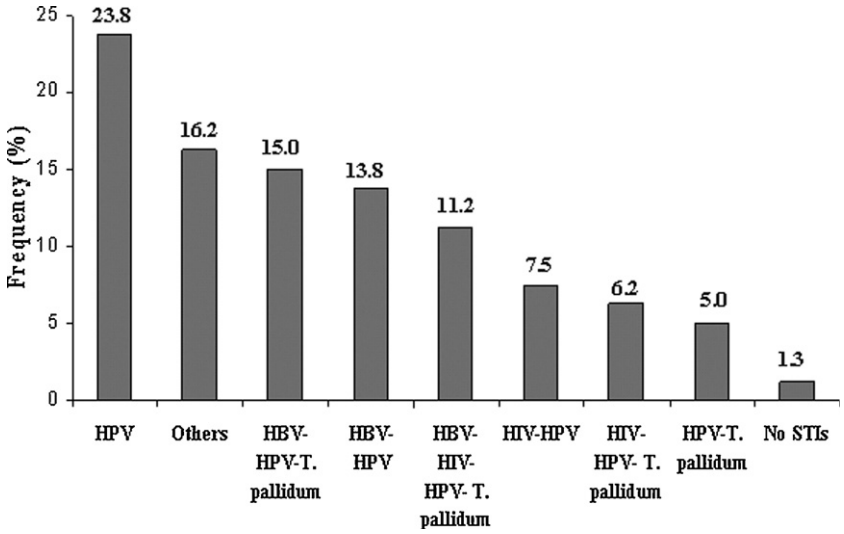

Figure 2. Number of sexually transmitted infections (STIs) and frequency of co-infections among male-to-female trans-sex workers (TSW). Results on co-infections were obtained from 80 participants with six agents tested (HBV, HCV, HIV, HPV, *Treponema pallidum*, and *Chlamydia trachomatis*).

from a group of 112 MSW). HBV prevalence was 22.0%, with only one HBsAg-positive MSW. HCV prevalence was 6.1%. In addition, *T. pallidum* infection was detected in 20.4% of MSW (Table 1).

Results on co-infections were obtained for the 82 MSW who had the four agents tested. Thirty-nine percent of them presented at least one infection, while the frequency of one pathogen was close to 20% and that of two agents was around 10%. The most frequent single infection was that with *T. pallidum* (11.0%). The most frequent co-infections were HBV–*T. pallidum* (6.1%) and HBV–HIV–*T. pallidum* (6.1%) (Figure 3). Higher HBV and *T. pallidum* prevalences were observed among HIV-positive MSW ($p < 0.001$ in both cases). HBV-positive MSW showed a higher prevalence of *T. pallidum* infection ($p = 0.002$).

3.4. Comparisons between TSW and MSW groups

HIV, HBV, and *T. pallidum* prevalence rates were significantly higher in TSW than in MSW ($p < 0.001$, $p < 0.05$, and $p < 0.001$, respectively) (Table 1).

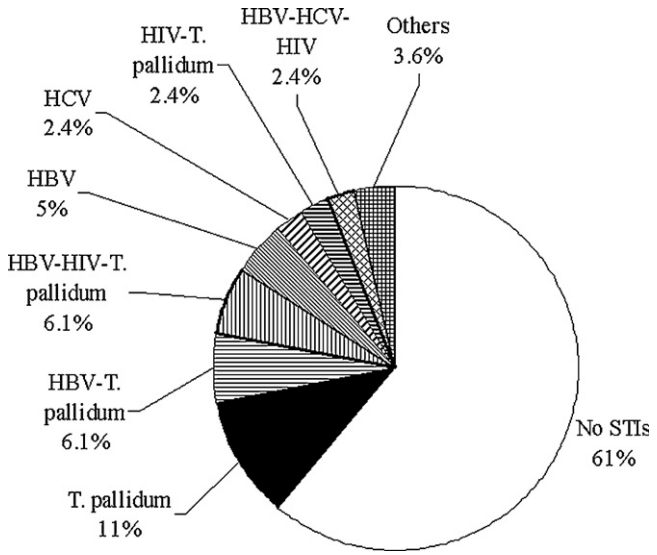

Figure 3. Frequency of co-infecting agents among 82 male sex workers (MSW) with four pathogens tested (HBV, HCV, HIV, and *Treponema pallidum*).

When the number of STIs was compared, the frequency of more than one STI was significantly higher among TSW (42.5% vs. 19.5%, $p < 0.001$).

When infecting agents were analyzed (Figures 1 and 3), *T. pallidum* was the most frequent single infection, HBV–*T. pallidum* was the most frequent double infection, and HBV–HIV–*T. pallidum* was the most frequent triple infection in both groups.

4. Discussion

This is the first report that explores HIV and other STI prevalences among TSW and MSW, since these affected populations are sparingly accounted for in the dialog concerning the HIV epidemic in Argentina.

The UNAIDS 2007 Epidemiological Update reported that HIV transmission in Latin America occurs primarily among MSM and sex workers. Unprotected sex has become the main route of HIV transmission in Argentina, with an estimated four out of five new HIV diagnoses in 2005 attributed to unprotected sex.[21] Approximately 80% of all AIDS cases reported from 2001 to 2007 were attributed to sexual transmission. Among men, around 30% of the cases resulted from homosexual intercourse and 40% from heterosexual intercourse.[1]

With few studies on the prevalence of HIV among these two subpopulations, HIV prevalence among TSW in the present study (34.1%) confirms the high prevalence (27.6%) reported by Toibaro et al.[10] On the other hand, a previous report from the capital city of Uruguay showed a significantly lower frequency of HIV infection in this population (21.5%).[22] Although the median age was similar in both samples, the difference may be explained by the years that have passed between the studies (almost a decade) and the fact that one study was carried out in only a single city. Meanwhile, the HIV prevalence among MSW in the present study does not differ significantly from that previously reported among MSM (13.8%).[4] However, a significant difference may be observed if the HIV prevalence among TSW and MSW is compared to the much lower one found among FSW (3.2%).[9]

The finding that HIV prevalence among TSW is higher than that among MSW in Argentina is consistent with the meta-analysis reported by Operario et al. Although the differences were not statistically significant, these authors found an HIV prevalence of 27.3% among TSW and a prevalence of 15.1% among MSW.[23]

The HIV incidence found among TSW (10.7 per 100 person-years) was alarmingly high, reinforcing the severity of the epidemic in this group. This was higher than the incidence observed among MSW (2.3 per 100 person-years), as well as that reported among MSM in 2006 (6.7/100 person-years) and that estimated by Viñoles et al. among TSW in Uruguay (6.03/100 person-years).[14,24] In addition to a lower HIV incidence, MSW declared safer sexual practices than TSW in the present study.

Furthermore, in spite of the existence of a free-of-charge and easy-to-deliver treatment in Argentina, the prevalence of *T. pallidum* was extremely high among TSW, confirming the results obtained by Toibaro et al. (42.0%).[10] Given that MSW and FSW (45.7%) showed a high prevalence of *T. pallidum* infection, this infection is not a public health issue exclusive to TSW.[9] These statistics are evidence of the inadequate or lack of access to healthcare services that sex workers have.

Given the high HBV prevalence in MSW and TSW, it is imperative to reach more MSW and TSW in order to provide them with early vaccination in the context of a well-designed program. Of note, only 30% of participants who received the first HBV vaccine dose returned for the second dose. In Argentina, HBV vaccination has been available since 1982 and has been recommended for all persons at high-risk of acquiring HBV, such as MSM, IDUs, heterosexuals with multiple sexual partners or with some STI, and sex partners of HBV carriers. However, only since 2000 has vaccination for HBV infection became mandatory for children and preadolescents, but still not for vulnerable populations.[25] A previous study on MSM showed that only 7.0% of a cohort population had been previously vaccinated against HBV.[26] Most MSM in the study were simply unaware of HBV risks or about the existence of a vaccine. However, when the vaccine was offered, nearly 90% of them completed the vaccination schedule.

In contrast to the other pathogens tested, HCV prevalence among MSW and TSW was relatively low. Russi et al. reported a similar HCV prevalence among TSW in Uruguay.[22] In the present study, the difference between both populations was not significant and cannot be related to the use of injecting drugs, given that only two MSW (both of them HCV-negative) and two TSW (one of them HCV-positive) reported being IDUs. Given the low chance of HCV sexual transmission, a higher number of IDUs might have been involved, although not declared, in this study sample.

This is the first study on HPV prevalence among TSW. In agreement with the findings of studies among MSM, a high HPV prevalence was found among TSW.[27]

The prevalence of *C. trachomatis* anal infection among TSW in the present study is higher than that observed (1.85%) in genital samples from STI symptomatic adult patients of both sexes in Argentina, but similar to that recently reported in an MSM population from the USA (6.8%).[28,29] A report on TSW from Indonesia showed that the prevalence of *C. trachomatis* was 3.8%, a value very similar to that found in the present study.[30] To our knowledge, this is the first report on *C. trachomatis* infection in TSW from Argentina.

T. pallidum was the most frequent single infection. Regarding the prevalence of co-infections among the four agents tested (HBV, HCV, HIV, and *T. pallidum*), detection of STIs among TSW almost doubled that found among MSW. HIV, HBV, and *T. pallidum* was a frequent association in both populations. The interaction between HIV and other pathogens should be taken into account, since disease progression may be dramatically altered.

The prevalences of HIV, HBV, HPV, and *T. pallidum* reported in the present study are alarmingly high. Regarding vulnerable populations, MSW and TSW are often excluded from research in Argentina. The high prevalence of infections observed in the present study is evidence of both the vulnerability and susceptibly of these two subpopulations. Particularly alarming are the significantly higher rates of infection in TSW. This group has suffered discrimination and stigmatization for a long time. Sex work, a job almost mandatory for this group, has led them to a situation of extreme social marginalization. This social exclusion and isolation impairs this group's ability to access public healthcare services and HIV/AIDS prevention programs, which should be designed in order to reach them. Given the high prevalence of STIs observed in this work, it is important to promote early diagnosis of STIs so that, if necessary, proper treatment can be initiated and the transmission chain stopped.

Besides, since most TSW clients are men who consider themselves heterosexuals and have female partners, they may act as an epidemiological link between TSW and the female population. Similar considerations should be taken into account as regards MSW, given that they declared sexual involvement with both men and women.

The confirmation that HIV and other STIs are concentrated in specific subpopulations, such as the ones studied in this work, calls for the urgent provision of preventive interventions and facilitation of access to healthcare programs.

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