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Sexual behavior as a risk factor for *T. Gondii* infection in HIV patients

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Key words: *Toxoplasma gondii*; human immunodeficiency virus; sexual behavior; risk factor; prediction model.

Abstract

The relationship between sexual behavior and *Toxoplasma gondii* (*T. gondii*) infection has gained attention following the discovery of the parasite's presence in semen and ejaculate. Certain sexual behaviors, such as the frequency of sexual activity and inconsistent use of condoms during oral or anal sex, can increase the risk of *T. gondii* infection. This study aims to investigate the relationship between sexual behavior and *T. gondii* infection in Human Immunodeficiency Virus (HIV) patients. A cross-sectional study was conducted involving 197 HIV patients on Antiretroviral (ARV) therapy from the Jakarta, Bogor, Bekasi, and Depok regions of Indonesia, supported by four foundations. Serological status for *T. gondii* infection (IgG-positive) was determined using the Enzyme-Linked Immunosorbent Assay (ELISA) method. Demographic and sexual behavior data were collected through a structured questionnaire. The results revealed that risky sexual behavior significantly increased the probability of *T. gondii* infection with adjusted Prevalence Ratio (aPR): 6.00; 95% Confidence Interval (CI): 3.38–10.64; $p < 0.001$.

Furthermore, sexual behavior models applied to predict *T. gondii* infection showed sensitivity, specificity, and accuracy rates of 92.25%, 82.4%, and 88.83%, respectively. The study showed that risky sexual behavior, particularly unprotected oral and anal sex, is associated with an increased risk of *T. gondii* infection in HIV patients. These findings emphasize the need for screening sexual behaviors as part of routine care in HIV management, particularly in stages I and II. Early identification of high-risk behaviors can help to inform treatment decisions and guide public health interventions, including sexual health education and prevention strategies in primary healthcare settings.

Introduction

Toxoplasma gondii infection in Human Immunodeficiency Virus (HIV) patients can result in neurological disorders and death.[1,2] The majority of *T. gondii* infections are associated with the consumption of foods contaminated with the parasite, such as meat, fish, fruits, and vegetables.[3-5] There are allegations that sexual behavior is related to *T. gondii* infection. This conjecture is based on findings of *T. gondii* in semen and in both man and woman reproductive tract.[6-10]
This gave rise to the theory that oral sex may be one of the transmission routes for *T. gondii*. Oral sex, especially without the use of condoms, increases the risk of ingesting *T. gondii*. Parasites present in semen can be ingested, leading to *T. gondii* infection. Additionally, *T. gondii* infection is related to a history of wounds during sexual intercourse. The odds of *T. gondii* infection in sex workers who had been injured during sexual intercourse were 6.3 times higher than the odds of the control group with adjusted Prevalence Ratio (aOR): 6.30; 95% Confidence Interval (CI): 1.1-33.7; p=0.03.[11]
The ingestion of bradyzoites from *T. gondii* is one of the suspected causes of infection through oral sex. Bradyzoites from *T. gondii* are resistant to room temperature and digestive enzymes.[12] The presence of bradyzoites in the body marks a chronic infectious process involving *T. gondii*. Bradyzoites can reside within muscle and nerve tissues of immunocompetent individuals. They can survive in the host's body because they are protected by cysts that contain glycans and proteins, providing energy for survival. When the host's immune condition decreases, as does a

decrease in CD4 levels to below 100 cells per μL in HIV patients, bradyzoites will transform into tachyzoites. This mechanism causes *T. gondii* infection to be latent. It is one of the causes of fatal nervous system disorders in HIV patients who experience a decrease in immunity.[13]

This study aims to investigate the relationship between sexual behavior and *T. gondii* infection. In this study, the status of *T. gondii* infection is determined by measuring the titer of Immunoglobulin G (IgG) antibodies. Meanwhile, the sexual behaviors examined included oral sex, anal sex, histories of injuries sustained during sexual intercourse, and the frequency of condom use. This research hypothesizes that sexual behavior is associated with *T. gondii* infection.

Materials and Methods

This study used a cross-sectional design and involved 197 HIV patients receiving Antiretroviral Therapy (ARV) at community health centers (Puskesmas) supported by four foundations in Jakarta, Bogor, Bekasi, and Depok (Indonesia). Participants met several inclusion criteria: they had to be HIV patients undergoing treatment at these health centers, willing to take a serological test for *T. gondii* antibodies, consented to participate in the research, and attended the data collection sessions. Exclusion criteria included patients with blood or vascular disorders preventing blood sampling, pregnant or breastfeeding women, individuals with congenital immune system disorders, and those with a borderline IgG *T. gondii* Enzyme-Linked Immunosorbent Assay (ELISA) test result. The selection process for participants is shown in Figure 1.

The research was conducted from September 2022 until January 2023. To measure the IgG titer for *T. gondii*, blood samples were collected from all participants at the headquarters of the four HIV-supporting foundations. The blood was stored in tubes with anticoagulants and then sent to the Clinical Parasitology Laboratory at the Faculty of Medicine, Universitas Indonesia, Jakarta. To ensure temperature stability and prevent blood lysis, the samples were kept in an ice box with ice packs during shipping at 4°C. The IgG titer for *T. gondii* was measured using the ELISA method. Patients were considered infected with *T. gondii* if their IgG titer was ≥10 IU/mL, while those with values <8 IU/mL were considered negative. Results in the range of 8 to <10 IU/mL were classified as borderline and excluded from the study. This classification is based on the laboratory procedures.

In this research, a systematic and closed-ended questionnaire was used as a research instrument. This questionnaire is used to measure demographic and sexual behavior. Sexual behavior was measured based on 10 sexual practices, namely receiving payment for sexual intercourse, intensity of oral sex, condom use during oral sex, condom use by partners during oral sex, habit of swallowing ejaculate during oral sex, intensity of anal sex, condom use during anal sex, condom use by partners during anal sex, history of genital injuries during sexual intercourse, and history of anal injuries during sexual intercourse. Demographic status was also measured in the study, including gender, age, and the location of health facilities where ARV therapy was accessed. This research has undergone an ethical review process at the Research Ethics Committee, Faculty of Public Health, University of Indonesia, Jakarta. The number of ethical approval is Ket-549/UN2.F10.D11/PPM 00.02/2022. This code of ethics was valid from September 26th, 2022, until September 26th, 2023.

Statistical tests to determine the relationship between sexual behavior and demographic status with *T. gondii* infection were conducted using the chi-square test and Poisson regression analysis. The relationship was considered significant if the p-value $< \alpha$ (0.05). Additionally, statistical analysis was performed to assess the strength of the relationship by calculating the Prevalence Ratio (PR). PR value greater than 1 indicated that sexual behavior and demographic status increase the risk of *T. gondii* infection in HIV patients. This study also aims to develop a predictive model for *T. gondii* infection, based on sexual behavior. The modeling process was conducted using Poisson regression analysis. In addition to generating the model, Poisson regression was also used to analyze the correlation between predictors and *T. gondii* infection based on multivariate analysis.

Results

The ELISA test showed that the prevalence of *T. gondii* infection among HIV patients in Jakarta, Bogor, Bekasi, and Depok was 65.2%. This finding showed that *T. gondii* infection is a non-rare disease in Jakarta, Bogor, Bekasi, and Depok areas. Among female respondents, 70.7% (53 out of 75) were infected, while 61.9% (73 out of 118) of male respondents were infected. Among transgender respondents, the infection rate was 60% (3 out of 5). There was a significant relationship between age and *T. gondii* infection. HIV patients aged 37 to 42 years had a 1.34 times higher risk of infection compared to those under 30. Those over 42 had a 1.20 times higher risk than individuals under 30. Additionally, HIV patients under 30 had a 1.32 times higher likelihood of testing positive for *T. gondii* IgG compared to those aged 34 to 37. The incidence of *T. gondii* infection was also higher among HIV patients receiving ARV treatment in South Jakarta, with a rate 1.45 times higher than in West Jakarta. Detailed demographic data are presented in Table 1.

The general profile of sexual behavior of HIV patients having a positive IgG test for *T. gondii* were as follows: HIV patients who have never been paid to have sex have a history of oral sex with a frequency of less than 2 times a month. When having oral sex, HIV patients and their partners rarely use condoms. In addition, HIV patients infected with *T. gondii* showed sexual behavior in the form of swallowing ejaculatory fluid, and had a history of genital and anal injuries during sexual intercourse. Detailed information related to the description of sexual behavior is presented in Table 2.

The bivariate analysis identified several sexual behaviors related to *T. gondii* infection, including the frequency of oral sex, condom use by HIV patients and their partners during oral sex, swallowing ejaculatory fluid during oral sex, frequency of anal sex, condom use during anal sex by both HIV patients and their partners, and a history of genital or anal injuries during sex. *T. gondii* is also related to demographic variables, such as age, healthcare facilities, and gender. These variables were analyzed using the Poisson regression test to assess their impact and to build a *T. gondii* infection prediction model. Poisson regression test used to estimate the prevalence ratios in the cross-sectional studies of the non-rare disease.[14-14] The results of the Poisson test for the full model are presented in Table 3.

The final model included frequency of oral sex, condom use by HIV patient partners during oral sex, and condom use during anal sex, all of which were found to be predictive of *T. gondii* infection risk.[14] After four iterations, the model demonstrated an excellent fit to the data, with a Pearson Chi-Square p-value of 1 ($p > \alpha$). The β-coefficients revealed that all three variables increased the risk of *T. gondii* infection. Of these, condom use habits exhibited the strongest predictive effect. The analysis of the Prevalence Ratio (PR) showed that *T. gondii* infection was 1.23 times more prevalent among HIV patients whose partners used condoms during oral sex compared to those without this habit. Detailed results are shown in Table 4.

The model for measuring sexual behavior is:

T. gondii Infection Prediction Score = -1.54+0.14×X1+0.23×X2+0.20×X3

The modeling notation above is as follows: X1, frequency of HIV patients having oral sex; X2, habit of using condoms by couples of HIV patients during oral sex; X3, habits of condom use by HIV patients during anal sex.

In this study, sexual behavior was conceptualized as a latent variable with a numerical scale. *T. gondii* infection prediction score was calculated based on the coded values of three variables: frequency of oral sex, condom use by HIV patient partners during oral sex, and condom use during anal sex. The frequency of oral sex was categorized into five levels: never, 1 time per month, 2-3 times per month, 2-3 times per week, and 4 or more times per week, with corresponding scores of 0, 1, 2, 3, and 4. For condom use by HIV patient partners during oral sex, four categories (always use, frequently use, rarely use, and never use) were assigned scores of 1, 2, 3, and 4, respectively. The "never have oral sex" category was assigned a score of 0. Likewise, for condom use during anal sex, scores of 1, 2, 3, and 4 were assigned to the "always use," "frequently use," "rarely use," and "never use" categories. A score of 0 was applied to the "never have anal sex" category.

However, for analysis, the sexual behavior was transformed into a categorical variable. This transformation was done by calculating a cut-off value. The cut-off value was determined using Receiver Operating Characteristic (ROC) analysis, which yielded an Area Under The Curve (AUC) of 0.8977. This result indicated that the sexual behavior score can predict *T. gondii* infection with an accuracy of 89.8%.

After carrying out the above stages, the model validity test process was carried out. The basis for assessing the validity of the model was the value of sensitivity, specificity, and accuracy. In this validity test, a tabulation process was carried out with the principle of a 2 × 2 table. In this test, the prediction of *T. gondii* infections occupied the position as a predictor, while the *T. gondii* infection was the outcome variable. The results of the validity of the sexual behavior model are presented in Table 5.

Based on the *T. gondii* infection prediction model, patients with a positive prediction had a 6-fold higher risk of IgG-positive *T. gondii* serology compared to HIV patients with a negative prediction. The analysis showed that when these behaviors occur more frequently or condoms are not consistently used, the risk of *T. gondii* infection increases among people living with HIV.

In other words, the combination of these sexual behaviors contributes to a higher likelihood of acquiring *T. gondii* infection in this population.

Discussion

Several studies, both in humans and animals, reveal the presence of *T. gondii* in semen and the male reproductive tract.[6,10,17-19] When having oral sex, HIV patients can intentionally or unintentionally swallow ejaculatory fluids. So that, an HIV patient who was initially not infected with *T. gondii* got infected due to ingestion of the parasitic cyst. This route of transmission of infection is the same as the ingestion of *T. gondii* cysts, due to the consumption of livestock meat contaminated with the parasitic cysts. Especially cysts that contain bradyzoites.[20]

Low-intensity condom use, particularly by partners during oral sex, was associated with a greater potential for *T. gondii* bradyzoite ingestion and cyst transmission. Within the *T. gondii* infection prediction model, three sexual behavior variables were considered: the frequency of oral sex, condom use by the partner during oral sex, and condom use by HIV patients during anal sex. Among these, the coefficient for condom use by the partner during oral sex (0.23) was the highest, indicating that this variable contributed more substantially to the likelihood of *T. gondii* IgG seropositivity compared to the other two. This finding suggests that inconsistent or low-frequency condom use by partners during oral sex poses a greater risk for *T. gondii* transmission than either frequent oral sex or inconsistent condom use during anal sex. While higher frequencies of oral sex and reduced condom use during anal intercourse may also facilitate exposure, their relative contributions to infection risk appear smaller. Consistent condom use during oral sex, therefore, represents an important preventive factor against the ingestion of *T. gondii* bradyzoites or cysts.[20]

A habit of swallowing ejaculatory fluid because the partner does not use a condom will increase the chance of ingestion of *T. gondii* parasites from semen.[20] In modeling, ejaculatory fluid swallowing habits were not included in the variables that measure sexual behavior. One of them is because this variable is related to the use of condoms by couples of HIV patients when having oral sex. In the correlation analysis, the value of the correlation coefficient (r) between the habit of swallowing ejaculatory fluid and the use of condoms by the spouse of HIV patients during sexual intercourse reached 0.6786. In good modeling, each prediction variable must be independent. The independence between the prediction or predictor variables will prevent the model from experiencing multicollinearity problems. This is why the variable of swallowing ejaculatory fluid is not included in the variables of observation and prediction of sexual behavior, because it is correlated with the variable of condom use.

Oral sex is believed to be a sexual behavior that is not related to infectious diseases and causes low condom use when having oral sex. A study revealed that oral sex is a foreplay part of sex in the form of penetrating the penis into the vagina (penetrative intercourse).[21] A study revealed that 47% of adolescent boys aged 15 to 19 years get oral sex from their partner before having penetrative intercourse.[22] Meanwhile, 24% of men aged 15 to 24 years had oral sex before having penetrative intercourse.[21]

The understanding that oral sex does not cause pregnancy is one of the causes of low awareness of the need to use condoms when having oral sex. A study conducted in the UK revealed that the proportion of respondents who had only once had oral sex and used condoms was only 17%. Meanwhile, the proportion of respondents who have had oral sex more than once and use condoms is only 2%. Apart from the reason that the risk of infection is low and does not cause pregnancy, oral sex without a condom is also associated with pleasure. The use of condoms is thought to decrease arousal, sensuality, and pleasure when having sex.[20]

The relationship between oral sex and *T. gondii* infection has been observed by examining the sexual behavior of pregnant women. Several studies found that *T. gondii* infection in this population was not linked to consuming raw or undercooked meat or to exposure to cats. Specifically, these studies revealed no association between infection and cat ownership, handling litter, gardening, or exposure to sandboxes.[23,24] This finding raised the suspicion that sexual contact with a partner could be a source of infection for a pregnant woman. Given that penetrative intercourse is often considered a health risk to the mother and fetus, pregnant women may engage in oral sex to meet their partners' biological needs. Oral sex performed without a condom could serve as a route for *T. gondii* transmission from a partner to a pregnant woman.[20] This is based on the assumption that the infection was acquired during pregnancy. To test this hypothesis, genotyping of the parasite is required, as the three main genotypes can provide clues about the infection's origin.[25]

The suspicion of oral sex and the low use of condoms in oral sex with *T. gondii* infection is reinforced by the results of a study on the sexual behavior of 1,000 men and 1,500 women who had oral sex in the Czech Republic. Initially, this study analyzed the relationship between oral sex behavior and *T. gondii* infection. The results of the analysis revealed that there was no meaningful relationship between oral sex behavior and *T. gondii* infection, both in men and women. However, when the reanalysis was carried out, by controlling for age and passive oral sex, there was a change in the results. There was a significant association between active oral sex and *T. gondii* infection in women.[22]

The use of condoms by both HIV patients and their partners during anal sex is included in sexual behaviors related to positive serology of *T. gondii.* One of the suspected links between condom use during anal sex and *T. gondii* infection is related to the intensity of sex and the number of partners. Anal sex correlates with sexual behavior with multiple partners (promiscuity).[26] This finding is based on research involving 893 students. The study involved a population of college students, as this group comprised individuals who were sexually active and reported having multiple partners within a year. The results indicated that 23% of students who engaged in penetrative intercourse also reported engaging in anal sex. Students who had anal sex had penetrative intercourse at a younger age, compared to students who never had anal sex. In addition, students who have anal sex have a higher frequency of penetrative intercourse relationships compared to students who do not have anal sex. These findings indicate that active sex and promiscuity are related to a higher frequency of anal sex.[27]

A correlation of promiscuity with *T. gondii* infection was found in a study with a population of female sex workers. In the study, the incidence of *T. gondii* infection in female sex workers who served more than 20 customers in a week was 2.76 times higher than the incidence of *T. gondii* infection in female sex workers who served fewer than 20 customers in a week. In addition, the prevalence of *T. gondii* infection in women who served more than 500 customers in their lifetime was higher compared to the prevalence of female sex workers who served 100 to 500 customers during their lifetime, and less than 100 customers (24.2% *vs* 17.6% and *vs* 10.3%, respectively).[11]

The correlation between promiscuity and anal sex was found in a study involving 12,571 male and female respondents aged 15 to 44 years in the United States.[27] In the study, U.S. residents who had more than one partner in the past year had a higher frequency of anal sex compared to those who had only one partner in the past year. Residents of the United States who cohabited with a partner had a higher frequency of anal sex compared to the never-married, non-cohabiting population. The adjusted odds ratio revealed that the frequency of anal sex among cohabiting men and women was 1.6 times greater than among those who were never married and not cohabiting.

Men who reported having more than one sexual partner practiced anal sex with a frequency 1.8 times higher than those in a monogamous relationship. The study also found that men with multiple partners were less inclined to use condoms during anal sex. A similar trend was observed in the female population, where women with multiple sexual partners engaged in anal sex with a frequency 1.9 times higher than their monogamous counterparts. Interestingly, unlike the male group, non-monogamous women demonstrated a higher rate of condom use during anal sex.[27] These findings suggest a hypothesis that men with multiple sexual partners are more likely to engage in anal sex without a condom. This sexual behavior could potentially transmit *T. gondii* bradyzoites or cysts, as both of these *T. gondii* life stages have been found in human semen.[6,28] It is hypothesized that men who engage in promiscuous behavior have a low level of awareness regarding condom use during sexual intercourse. This promiscuous behavior in men is therefore associated with a higher risk of Sexually Transmitted Infections (STIs), such as HIV and *T. gondii*.

Conclusions

This study developed a model that predicts the risk of *T. gondii* infection based on sexual behavior screening. The model can be used at first-level health facilities offering ARV therapy to HIV patients. Positive screening results can trigger a serological test for *T. gondii*, which would guide appropriate treatment. Most HIV patients accessing ARV therapy at these facilities were at stages 1 and 2 of HIV, with CD4 levels above 200 cells per µL, meaning they still had functional immunity. Early treatment for *T. gondii* infection at this stage can assist in preventing neurological complications. To reinforce the hypothesis that semen is a source of T. *gondii* infection, future studies should focus on isolating and identifying *T. gondii* from the semen of HIV patients in the Jakarta, Bogor, and Depok areas. Unprotected oral and anal sex are considered potential routes of infection. To allow for the generalization of results to the broader population of HIV patients in these regions, subsequent research should involve a larger sample. Furthermore, a follow-up study is needed to measure *T. gondii* IgM serology, which would provide a more comprehensive understanding of infection status, particularly in determining the active phase of the infection.

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Contributions: The authors contributed equally to the conception and design of the study, data acquisition, analysis and interpretation, manuscript drafting, and critical revision of the article. The authors have read and approved the final manuscript.

Conflict of interest: the authors declare no potential conflict of interest, and all authors confirm accuracy.

Ethics approval: this research has undergone an ethical review process at the Research Ethics Committee, Faculty of Public Health, University of Indonesia, Jakarta. The number of ethical approval is Ket-549/UN2.F10.D11/PPM 00.02/2022. This code of ethics was valid from September 26th, 2022, until September 26th, 2023. The study is conformed with the Helsinki Declaration of 1964, as revised in 2013, concerning human and animal rights.

Informed consent: all patients participating in this study signed a written informed consent form for participating in this study.

Patient consent for publication: written informed consent was obtained from a legally authorized representatives for anonymized patient information to be published in this article.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

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Figure 1. Process of selecting subjects

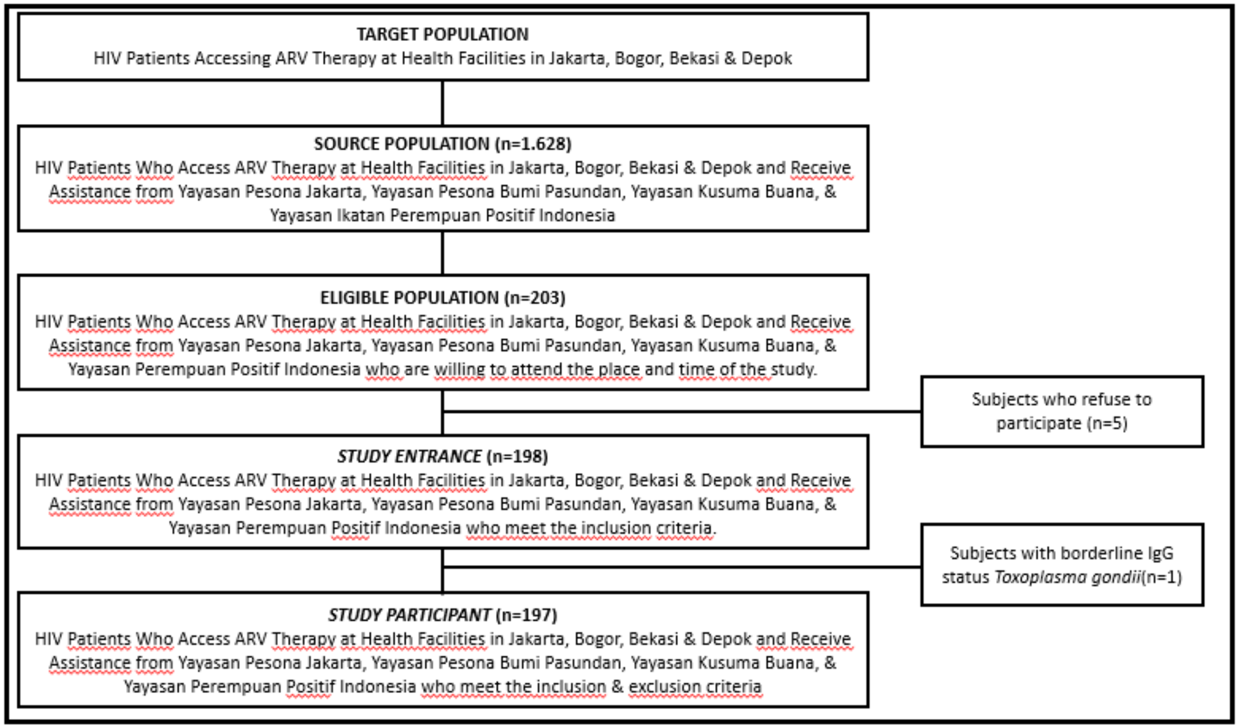

Table 1. Human Immunodeficiency Virus (HIV) patients demographic data.

Variable	***Toxoplasma gondii* infection**	**Prevalence ratio (PR)**	**95% Confidence**	**p value**

		Positive	Negative		**interval (CI)**	
Gender	Woman	53	22	1		
	Man	73	44	0.88*	0.72 – 1.08	0.228
	Transgender	3	2	0.85	0.41 – 1.76	0.661
Age	Less than 30 Years	32	19	1		
	30 – 33 Years	23	13	1.02	0.74 – 1.41	0.913
	34 – 37 years old	20	22	0.76*	0.52 – 1.11	0.156
	37 – 42 Years	27	5	1.34**	1.04 – 1.74	0.025
	Over 42 Years	27	9	1.20*	0.90 – 1.59	0.217
Healthcare facilities in accessing antiretroviral therapy	South Jakarta	33	10	1		
	Central Jakarta	11	7	0.80	0.53 – 1.19	0.269
	East Jakarta	22	8	0.96	0.73 – 1,25	0.743
	West Jakarta	28	25	0.69**	0.51 – 0.93	0.016
	North Jakarta	10	5	0.87	0.59 – 1.29	0.484
	Bekasi & Bogor	18	11	0.81*	0.58 – 1.12	0.206
	Depok	7	2	1.01	0.69 – 1.49	0.946

* : $p < 0.05$ (significant)

** : $p < 0.01$ (highly significant)

Table 2. Sexual behavior of Human Immunodeficiency Virus (HIV) patients.

Variable		Serology status		Prevalence ratio (PR)	95% Confidence interval (CI)	p value
		Positive	Negative			
Getting paid to have sex	Already	109	59	1.06	0.81 – 1.39	0.669
	No	20	9	1		
Frequency of oral sex	Never have oral sex	6	54	1		
	1 time per month	50	10	8.33	3.57 – 19.43	<0.001
	2 – 3 times per month	41	2	9.53	4.05 – 22.46	<0.001
	2 – 3 times per week	24	1	9.60	3.92 – 23.48	<0.001
	4 or more times per week	8	1	8.89	3.08 – 25.62	<0.001
Condom use when having oral sex	Never have oral sex	6	54	1		
	Always use condoms	31	5	8.61	3.59 – 20.64	<0.001
	Frequently use condoms	20	0	10.00	4.01 – 24.90	<0.001
	Rarely use condoms	39	2	9.51	4.03 – 22.47	<0.001
	Never use a condom	33	7	8.25	3.45 – 19.69	<0.001
Condom use by spouses during oral sex	Never have oral sex	6	54	1		
	Always use condoms	31	6	8.38	3.50 – 20.08	<0.001
	Frequently use condoms	12	0	10.00	3.75 – 26.64	<0.001
	Rarely use condoms	47	3	9.40	4.02 – 21.98	<0.001
	Never use a condom	33	5	8.68	3.63 – 20.72	<0.001
	Never have oral sex	6	54	1		
	Never swallow ejaculatory fluid	74	10	8.81	3.83 – 20.24	<0.001

Habit of swallowing ejaculation fluid during oral sex	Rarely swallowing ejaculatory fluid	32	4	8.89	3.72 – 21.26	<0.001
	Frequent swallowing ejaculation fluid	10	0	10.00	3.63 – 27.51	<0.001
	Always swallow ejaculation fluid	7	0	10.00	3.36 – 29.76	<0.001
Frequency of anal sex	Never have anal sex	32	58	1		
	1 time per month	44	7	2.43	1.54 – 3.83	<0.001
	2 – 3 times per month	21	2	2.57	1.48 – 4.45	0.001
	2 – 3 times per week	26	1	2.71	1.61 – 4.54	<0.001
	4 or more times per week	6	0	2.81	1.18 – 6.73	0.020
Condom use when having anal sex	Never have anal sex	32	58	1		
	Always use condoms	11	1	2.58	1.30 – 5.11	0.007
	Frequently use condoms	35	3	2.59	1.60 – 4.18	<0.001
	Rarely use condoms	13	2	2.43	1.28 – 4.64	0.007
	Never use a condom	38	4	2.54	1.59 – 4.07	<0.001
Condom use by couples during anal sex	Never have anal sex	32	58	1		
	Always use condoms	8	1	2.50	1.15 – 5.43	0.020
	Frequently use condoms	34	3	2.58	1.59 – 4.18	<0.001
	Rarely use condoms	18	2	2.53	1.42 – 4.51	0.002
	Never use a condom	37	4	2.54	1.58 – 4.07	<0.001
History of genital injuries during sexual intercourse	No	102	66	1		
	Yes	27	2	1.53	1.31 – 1.79	<0.001
	No	80	63	1		

History of anal injury during sexual intercourse	Yes	49	5	1.62	1.37 – 1.91	<0.001

Table 3. Poisson test results for sexual behavior and demographic variables.

Sexual behavior variables	**Coefficient (B)**	**Prevalence ratio (PR)**	**95% Confidence interval (CI)**	**p value**
Frequency of oral sex	0.16	1.17	0.92 – 1.48	0.177
Condom use when having oral sex	-0.11	0.90	0.61 – 1.32	0.584
Couples use condoms when having oral sex	0.31	1.36	0.91 – 2.03	0.136
Habit of swallowing ejaculation fluid	0.11	1.12	0.90 – 1.40	0.315
Frequency of anal sex	-0.09	0.91	0.74 – 1.13	0.406
Condom use when having anal sex	0.14	1.16	0.77 – 1.74	0.479
Couples use condoms during anal sex	0.08	1.08	0.72 – 1.63	0.704
History of genital wounds during sex	0.08	1.08	0.67 – 1.76	0.749
History of wounds to the anus during anal sex	0.05	1.05	0.68 – 1.63	0.817
Age	0.10	1.02	0.92 – 1.11	0.770
Healthcare facilities	0.09	0.99	0.91 – 1.08	0.867

Gender	-0.13	0.88	0.61 – 1.27	0.484
Constant	-1.61			<0.001

Table 4. Final model of sexual behavior based on Poisson regression test.

Independent variable	Coefficient (B)	Prevalence ratio (PR)	95% Confidence interval (CI)	p value
Frequency of oral sex	0.14	1.15	0.94 – 1.41	0.161
Couples use condoms when having oral sex	0.23	1.26	1.07 – 1.49	0.005
Condom use when having anal sex	0.20	1.22	1.09 – 1.37	0.001
Constant	-1.54			<0.001

Table 5. Results of the sexual behavior model validity test.

Variable		***Toxoplasma gondii* Infection**		**Instrument Reliability**			
		Positive	Negative	Sensitivity	Specificity	Accuracy	Area under the curve (AUC)
Sexual behavior	High Risk	119	12	92.25%	82.35%	88.83%	0.8977
	Low Risk	10	56				

Table 6. Results of analysis of the relationship between sexual behavior and *Toxoplasma gondii* infection.

Variable		***Toxoplasma gondii* Infection**		**Prevalence ratio (PR)**	**95% Confidence interval (CI)**	**p value**
		Positive	Negative			
Sexual behavior	High risk	119	12	6.00	3.38 – 10.64	<0.001
	Low risk	10	56			