

TOXOPLASMA AND VIRAL ANTIBODIES AMONG HIV PATIENTS AND INMATES IN CENTRAL JAVA, INDONESIA

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Abstract. In Indonesia, *Toxoplasma* and its associations with blood-borne viruses have been poorly studied. In order to study the association between anti-*Toxoplasma* antibodies and blood-borne viral antibodies, blood samples from 497 participants (375 inmates from four prisons in Central Java, Indonesia and 122 HIV patients at a Voluntary Counseling and Testing Clinic in Surakarta, Indonesia) were tested for serological markers of *Toxoplasma*, human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and human T-lymphotropic virus types I and II (HTLV-1/2). Anti-*Toxoplasma* IgG and IgM positivity rates were 41.6% and 3.6%, respectively. One point two percent of participants was positive for both anti-*Toxoplasma* IgG and IgM antibodies. Sixteen point five percent, 11.3%, 2.6% and 2.8% of participants were positive for anti-*Toxoplasma* IgG combined with anti-HCV antibodies, anti-*Toxoplasma* IgG combined with anti-HIV antibodies, anti-*Toxoplasma* IgM combined with anti-HIV antibodies and anti-*Toxoplasma* IgG combined with both anti-HIV and anti-HCV antibodies, respectively. Anti-*Toxoplasma* IgM seropositivity was associated with anti-HIV (aOR=4.3; 95% CI: 1.112-16.204, $p = 0.034$). Anti-*Toxoplasma* IgG antibodies were associated with anti-HCV (aOR=2.8; 95% CI: 1.749-4.538, $p < 0.001$) and history of injection drug use (aOR=3.1; 95% CI: 1.905-5.093, $p < 0.001$). In conclusion, we recommend patients with HIV, HCV infection and injection drug users should be screened for *Toxoplasma* infection in Indonesia.

Keywords: *Toxoplasma gondii*, HIV, HCV, Indonesia

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INTRODUCTION

Toxoplasma gondii is a parasite found worldwide with seroprevalence rates of 2% to 63% in humans (Frenkel, 2000; Montoya and Remington, 2000; Dubey, 2010). Toxoplasmosis is an important opportunistic infection in human immunodeficiency virus (HIV) (Nissapatorn, 2009). The prevalence of latent (asymptomatic) *Toxoplasma* infections among HIV/AIDS

patients varies widely around the world and is unknown in many countries (Nisapatorn and Sawangjaroen, 2011). Toxoplasmosis may hasten disease progression with HIV infection, especially among individuals with a CD4+ T-cell count less than 200 cells/ μ l (Bertschy *et al*, 2006; Miro *et al*, 2006).

Co-infections between *T. gondii* and HBV (hepatitis B virus), HCV (hepatitis C virus) and HTLV-1/2 (human T-lymphotropic virus type I/II) have not been well studied in Indonesia. One study did show a significant association between toxoplasmosis and HBV infection (Shimelis *et al*, 2009). Serum from patients with anti-HBc antibody are more likely to have anti-*Toxoplasma* IgG antibodies (Shimelis *et al*, 2009). One study from China found the seroprevalence of HIV/HCV/*Toxoplasma* triple co-infections to be 7.7% among HIV-infected former plasma donors (Zhang *et al*, 2008). Our previous study found the prevalence of anti-HCV/anti-*Toxoplasma* IgG antibodies among men who have sex with men (MSM) in Surakarta, Indonesia to be 11.2% (Prasetyo *et al*, 2014). Some of the MSM in the study mentioned above tested positive for anti-*Toxoplasma* IgG combined with anti-HIV antibodies and anti-*Toxoplasma* IgG combined with anti-HTLV-1/2 antibodies (Prasetyo *et al*, 2014).

MATERIALS AND METHODS

Study population

We have been studying the epidemiology of human blood-borne pathogens (including *T. gondii* and blood-borne viruses) by collecting epidemiological-clinical data and blood samples from high-risk communities in Central Java, Indonesia. To determine the prevalence of *Toxoplasma gondii* antibodies and its possible association with viral antibod-

ies in the samples collected from high risk communities, 497 blood samples were collected during 2009-2011 from four prisons in Central Java, Indonesia ($n=375$) (Prasetyo *et al*, 2013) and from HIV patients presenting to the Voluntary Counseling and Testing (VCT) Clinic, Dr Moewardi General Hospital, Surakarta, Indonesia, ($n=122$). These samples were examined for *Toxoplasma*, HIV, HBV, HCV, HDV and HTLV-1/2. The participants in this study with HIV (18 prisoners and 122 HIV VCT Clinics patients) received an antiretroviral therapy (ART) regimen of zidovudine (ZDV), lamivudine (3TC), and nevirapine (NVP).

Written informed consent was obtained from all participants prior to being included in the study. Ethical approval for the study was obtained from the institutional ethics committee review boards of the Faculty of Medicine, Sebelas Maret University and the Dr Moewardi General Hospital, Surakarta, Indonesia. All procedures were conducted according to the principles of the Declaration of Helsinki.

Serological studies for *Toxoplasma*, HIV, HBV, HCV, HDV and HTLV-1/2 antibodies

The serum samples obtain from each participant were checked for: anti-*Toxoplasma* IgM and IgG antibodies using the DRG *T. gondii* IgM Elisa Kit (DRG International, Springfield, NJ) and the DRG *T. gondii* IgG Elisa Kit (DRG International), respectively. Anti-HIV antibodies were detected using the Determine HIV-1/2 Kit (Abbott Diagnostics Japan, Tokyo, Japan); positive results were confirmed using the Vironostika HIV Uniform II Antigen Ag/Ab test (BioMérieux, Marcy l'Étoile, France). HBsAg was detected using the SERATEC Hepatitis B Quick Test (Gesellschaft für Biotechnologie GmbH, Göttingen, Germany). Anti-HCV, anti-HDV and anti-HTLV-1

and 2 antibodies were detected with the Ortho HCV PA II test (Ortho Diagnostics, Tokyo, Japan), HDV Ab ELISA test (Diagnostic Automation, Calabasas, CA) and MP Diagnostic HTLV-I/II ELISA 4.0 test (MP Biomedicals Asia Pacific, Pioneer Place, Singapore), respectively. All assays were performed according to manufacturer's instructions in duplicate.

Statistical analysis

Statistical analysis was conducted using SPSS, version 21 software (IBM, Armonk, NY). Chi-square and Fisher's exact tests were used to calculate the seroprevalence of anti-*Toxoplasma* IgG and IgM antibodies and other blood-borne viral antibodies in the studied population. Differences on bivariate analysis were considered significant if the *p*-value was < 0.05. The odds ratio (OR) was calculated to evaluate the association between the antibodies found and study subject risk behavior (Rosner, 2010). Logistic regression analysis was used to assess associations between antibodies found and the following factors: gender, place of residence, education level, risk behavior and previous viral infections. Variables with a *p*-value < 0.25 on bivariate analyses were included in the multivariate logistic regression analysis. Adjusted odds ratio (aOR) and 95% confidence interval (CI) were calculated as the result of the multivariate logistic regression analysis. Factors with a *p*-value < 0.05 on multivariate analysis were considered significant.

RESULTS

Demographics and seropositivity of blood borne viruses

Four hundred ninety-seven respondents were included in the study, 351 (70.6%) were males. Three hundred eighty-seven (77.9%) were drug abusers:

85.5% of males and 59.6% of females. One hundred six (21.3%) were injection drug users (IDUs); 23.1% of males and 17.1% of females. Two hundred fifty-one (50.5%) had a history of a body piercing; 29.9% of males and 100% of females (note: ear piercing is a common custom for Indonesian women). One hundred ten respondents (22.1%) had tattoos; 27.6% of males and 8.9% of females. Anti-HIV, HBsAg, anti-HCV and anti-HTLV-1/2 antibodies were found in 28.2%, 3.6%, 28% and 2.8% of participants, respectively (Table 1). No participants were found to have anti-HDV antibodies.

Toxoplasma antibodies

Anti-*Toxoplasma* antibodies were detected in 219 participants (44%). Anti-*Toxoplasma* IgM antibodies were seen in 18 participants (3.6%) and both anti-*Toxoplasma* IgM and IgG antibodies were seen in 6 participants (1.2%). The seroprevalence of anti-*Toxoplasma* IgM antibodies in the four Central Javan prisons was 1.3% and at the VCT Clinic at the Dr Moewardi General Hospital was 10.7%. There was no significant difference between males and females in anti-*Toxoplasma* IgM antibodies positivity (OR=3.2, 95% CI: 1.218-8.157; aOR=2.3, 95% CI: 0.719-7.049, *p* = 0.163).

The anti-*Toxoplasma* IgG positivity rate was 41.6% (207/497) with no significant difference between males and females (OR=1.2, 95% CI: 0.832-1.814). The overall seroprevalence of anti-*Toxoplasma* IgG antibodies among participants in the four prisons was 43.7% and at the VCT clinic was 35.2%. A history of injection drug use (OR=3.8, 95% CI: 2.413-5.996; aOR=3.1, 95% CI: 1.905-5.093, *p* < 0.001) was significantly associated with anti-*Toxoplasma* IgG positivity.

Viral antibodies associated with *T. gondii*

Of the anti-HIV positive subjects

Table 1
Characteristics of study participants by presence of anti-*Toxoplasma* antibodies.

Characteristics	Number (%)	Anti- <i>Toxoplasma</i> IgM antibody positivity, n (%)	Anti- <i>Toxoplasma</i> IgG antibody positivity, n (%)
Gender			
Male	351 (70.6)	8/351 (2.3)	141/351 (40.2)
Female	146 (29.4)	10/146 (6.8)	66/146 (45.2)
History of drug abuse	387 (77.9)	6/387 (1.6)	167/387 (43.2)
History of injection drug use	106 (21.3)	2/106 (1.9)	71/106 (67.0)
History of hepatitis contact	15 (3.0)	-	5/15 (33.3)
History of blood transfusion	14 (2.8)	-	6/14 (42.9)
History of oral operation	28 (5.6)	-	13/28 (46.4)
History of surgery	13 (2.6)	-	6/13 (46.2)
Has tattoo	110 (22.1)	2/110 (1.8)	38/110 (34.5)
Has piercing	251 (50.5)	11/251 (4.4)	100/251 (39.8)
History of sex with a foreigner	13 (2.6)	-	5/13 (38.5)
Anti-HIV positive	140 (28.2)	13/140 (9.3)	56/140 (40.0)
HBsAg positive	18 (3.6)	-	5/18 (27.8)
Anti-HCV positive	139 (28.0)	1/139 (0.7)	82/139 (59.0)
Anti-HTLV-1/2 positive	14 (2.8)	-	8/14 (57.1)

($n = 140$), 9.3% had anti-*Toxoplasma* IgM antibodies (OR=7.2, 95% CI: 2.519-20.618; aOR=4.3, 95% CI: 1.112-16.204, $p = 0.034$). There was no significant difference between males and females in anti-*Toxoplasma* IgM/anti-HIV positivity (OR=2.9, 95% CI: 0.956-8.770; aOR=1.3, 95% CI: 0.350-4.610, $p = 0.716$). Of the anti-HIV positive subjects, 40.0% had anti-*Toxoplasma* IgG antibodies. The anti-*Toxoplasma* IgG/anti-HIV positivity also had no significant difference between males and females (OR=1.2, 95% CI: 0.637-2.105).

Of the anti-HCV positive subjects ($n = 139$), 59% had anti-*Toxoplasma* IgG antibodies (OR=2.7, 95% CI: 1.794-4.008; aOR=2.8, 95% CI: 1.749-4.538, $p < 0.001$). Finding both anti-*Toxoplasma* IgG and anti-HCV antibodies was significantly more likely in males than females (OR=2.3, 95% CI: 1.229-4.175; aOR=2.2, 95% CI: 1.034-4.612, $p = 0.041$). Anti-*Toxoplasma* IgG/anti-

HCV positivity was significantly associated with a history of drug abuse (OR=28.9, 95% CI: 3.967-209.842; aOR=30.0, 95% CI: 3.424-263.329, $p = 0.002$) and injection drug use (OR=4.4, 95% CI: 2.657-7.309; aOR=2.5, 95% CI: 1.260-4.751, $p = 0.008$). The anti-*Toxoplasma* IgG/anti-HIV/anti-HCV antibodies positivity rate was 2.8% (14/497), with no significant difference between males and females (OR=1.04, 95% CI: 0.321-3.374). Anti-*Toxoplasma* IgG/anti-HIV/anti-HCV positivity was associated with a history of injection drug use (OR=10.1, 95% CI: 3.094-32.827; aOR=8.6, 95% CI: 2.308-31.718, $p = 0.001$) and having a tattoo (OR=3.7, 95% CI: 1.265-10.756; aOR=6.1, 95% CI: 1.694-22.289, $p = 0.006$).

There were other *T. gondii* combined viral antibody associations that could not be statistically analyzed because of an insufficient number of respondents who tested positive. The rates for find-

ing the combinations of anti-*Toxoplasma* IgM/anti-HCV, anti-*Toxoplasma* IgG/HBsAg, anti-*Toxoplasma* IgG/anti-HTLV-1/2, anti-*Toxoplasma* IgM/anti-*Toxoplasma* IgG/anti-HIV and anti-*Toxoplasma* IgM/anti-*Toxoplasma* IgG/anti-HCV were 0.2%, 1%, 1.6%, 0.4% and 0.2%, respectively. The rates for finding the combinations of anti-*Toxoplasma* IgM/anti-HIV/anti-HCV, anti-*Toxoplasma* IgG/anti-HIV/anti-HTLV-1/2, anti-*Toxoplasma* IgG/HBsAg/anti-HCV, anti-*Toxoplasma* IgG/anti-HCV/anti-HTLV-1/2 and anti-*Toxoplasma* IgM/anti-*Toxoplasma* IgG/anti-HIV/anti-HCV were 0.2%, 0.8%, 0.6%, 1.2% and 0.2%, respectively. Finding a combination of anti-*Toxoplasma* IgG/anti-HIV/anti-HCV/anti-HTLV-1/2 antibodies occurred in 0.4% of participants.

DISCUSSION

Sociodemographic data regarding blood-borne pathogens, especially *T. gondii* and certain viral infections, are limited in Indonesia. In the present study, we evaluated the seroprevalence of anti-*Toxoplasma* and viral antibodies along with some possible risk factors. The anti-*Toxoplasma* IgG positivity rate found in this study was higher than our previous study among MSM (Prasetyo *et al*, 2014), but lower than a study from Jakarta and Surabaya (Konishi *et al*, 2000; Terazawa *et al*, 2003). A prevalence rate of 70% was reported among patients of private practitioners and hospitals in Jakarta in 2003 (Terazawa *et al*, 2003). A prevalence rate of 58% was reported among patients attending the Emergency Unit of the Dr Soetomo Hospital in Surabaya based on data collected during 1999-2000 (Konishi *et al*, 2000). Neither of these two latter studies evaluated epidemiological or risk behavior associated with *Toxoplasma* infection.

Further studies are needed to identify risk factors associated with antibodies in Jakarta and Surabaya. Risk behavior may be more common in connection with urban housing and squatter settlements (note: Jakarta and Surabaya are large cities and densely populated), which can increase the risk of transmission of *T. gondii* leading to a high seroprevalence in those cities.

The anti-*Toxoplasma* IgG positivity rate in our study was 41.6%, and the anti-*Toxoplasma* IgM positivity rate was 3.6%. This suggests most participants had a latent infection due to previous exposure to *T. gondii*. Latent infection typically remains for life (Hajsoleimani *et al*, 2012). In our study, the anti-*Toxoplasma* IgM seroprevalence in the four Central Javan prisons was 1.3% and the rate at the VCT Clinic at the Dr Moewardi General Hospital was 10.7%. The prevalence of anti-*Toxoplasma* IgM positivity in the four prisons was not as high as it was at the VCT Clinic. No study has yet investigated the underlying reasons for the high seroprevalence of anti-*Toxoplasma* IgM antibodies at the VCT Clinic (note: all the respondents derived from the VCT clinic in the present study were positive for anti-HIV antibodies). One study reported an anti-*Toxoplasma* IgM seropositivity rate of 1.8% among 628 prisoners at Kayseri Prison in Turkey in 2009 (Yaman *et al*, 2009). The anti-*Toxoplasma* IgM seroprevalence at the four Central Javan prisons in our study is similar to the prevalence reported at Kayseri Prison. In a previous study, we reported an anti-*Toxoplasma* IgM seropositive rate of 1.4% among MSM in Surakarta, Indonesia (Prasetyo *et al*, 2014), which is similar to the seroprevalence at the four Central Javan prisons. This may reflect the fact that acute *Toxoplasma* infection, as indicated by anti-*Toxoplasma* IgM antibodies, is uncommon

in both respondents from prisons and MSM but quite common among subjects at the VCT clinic. Detection of anti-*Toxoplasma* IgM antibodies, usually considered to be a marker of acute infection may be a false-positive result and anti-*Toxoplasma* IgM antibodies may be positive for months or years after primary infection; thus, IgM is not recommended as a test of an acute infection for *Toxoplasma* (Villard *et al*, 2013). We recommend further study of *T. gondii* transmission among HIV patients who attend VCT clinics, especially using histological analysis and molecular assays, in order to confirm this finding since the sensitivity and specificity of a serological assay can be influenced by the correct performance of the test procedure. Studies of the prevalence of *Toxoplasma* infection in prisons and VCT clinics are rare. An IgG avidity assay or differential agglutination (AC/HS) test should be used to distinguish between acute and latent infections with *Toxoplasma*.

Injection drug use is a risk factor for *Toxoplasma* infection (Bailey and Brown, 1990). In the present study we also found a significant association between anti-*Toxoplasma* IgG antibodies and injection drug use, suggesting *Toxoplasma* testing should be considered in persons with a history of injection drug use. Having a history of a blood transfusion has been reported to be a risk factor for toxoplasmosis (Derouin *et al*, 2008), but we found no significant association in the present study. *Toxoplasma* screening is recommended for all blood donors, since tachyzoites of *T. gondii* can survive in stored blood and infect blood recipients (Sarkari *et al*, 2014).

A previous study reported anti-*Toxoplasma* IgG but not IgM antibodies are significantly associated with HIV infection (Daryani *et al*, 2011). In the present study, 40% of HIV infected participants had anti-

Toxoplasma IgG antibodies and 42.3% of participants who were HIV negative also had anti-*Toxoplasma* IgG antibodies with no significant difference between the two groups. However, anti-*Toxoplasma* IgG screening is important in HIV patients. If the test is positive a further confirmatory test needs to be performed, such as an IgG avidity assay or a differential agglutination (AC/HS) test in order to determine if the infection is active or latent. Anti-*Toxoplasma* IgM antibodies were significantly associated with HIV infection in our study participants ($p = 0.034$) although only 13 respondents were positive for both anti-*Toxoplasma* IgM and anti-HIV antibodies. Further studies on the association between anti-*Toxoplasma* IgM antibodies and anti-HIV antibodies are needed due to the small number of subjects with both these antibodies in the present study. anti-*Toxoplasma* IgM antibodies appear sooner during infection than IgG antibodies and usually disappear more quickly (Hill and Dubey, 2002). However, anti-*Toxoplasma* IgM antibodies should not be used alone as an indicator of acute infection (Villard *et al*, 2013). Further testing is needed in order to determine this.

Reactivation of latent *Toxoplasma* infection can occur in immunocompromised patients, enabling tachyzoites to replicate more rapidly as in acute toxoplasmosis (Walle *et al*, 2013). Both acute and reactivated infections due to *T. gondii* can usually be controlled by the body's immune response, through CD4+ T cells, which are responsible for suppressing the replication of tachyzoites (Pifer and Yarovinsky, 2011; Suzuki *et al*, 2011). During HIV infection, the CD4+ T cell count drops (Rinaldo, 2013) resulting in uncontrolled replication of tachyzoites. This suggests persons with HIV infection and low CD4+T cell counts who are co-infected

with *T. gondii* are more likely to develop toxoplasmosis. Active toxoplasmosis may also speed progression of AIDS-related diseases, especially in those with a CD4+ T-cell count below 200 cells/ μ l (Bertschy *et al*, 2006; Miro *et al*, 2006).

Anti-HCV was significantly associated with anti-*Toxoplasma* IgG antibodies in our study, suggesting people with anti-*Toxoplasma* IgG antibodies should be tested for HCV antibodies and people with anti-HCV antibodies should be tested for anti-*Toxoplasma* IgG antibodies. Studies regarding toxoplasmosis/hepatitis C co-infection are limited. *T. gondii* and HCV share some risk factors, such as injection drug use (Bailey and Brown, 1990; Backmund *et al*, 2005). Further studies regarding this co-infection are needed.

Male gender, history of drug abuse and a history of injection drug were significantly associated with anti-*Toxoplasma* IgG/HCV antibodies in our study, possibly because many of the respondents in our study reported a history of drug abuse, injection drug use and were male (data not shown). No studies have studied the link between male gender and history of drug abuse with the presence of anti-*Toxoplasma* IgG/HCV antibodies although having a history of injection drug use has been reported to be associated with *Toxoplasma* and HCV antibodies (Bailey and Brown, 1990; Backmund *et al*, 2005). Having a tattoo, reported more among men than women in our study and having a history of injection drug use, were significantly associated with anti-*Toxoplasma* IgG, anti-HIV and anti-HCV antibodies. Injection drug use has also been predicted to be a risk factor for HIV infection (Des Jarlais *et al*, 2009). Further studies need to be conducted checking for the presence of antigens to confirm current infection since our study only evaluated antibodies.

In conclusion, we suggest HIV and HCV infections should be checked for patients who test positive for *Toxoplasma* infection. *T. gondii* infection should be considered in intravenous drug users. Having a history of drug abuse, injection drug use and tattoos were associated with the presence of anti-*Toxoplasma* and some anti-viral antibodies. Education regarding *Toxoplasma* infection and various associated viral infections should be provided to at risk groups.

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