

## Molecular epidemiology of HIV, HBV, HCV, and HTLV-1/2 in drug abuser inmates in central Javan prisons, Indonesia

Afiono Agung Prasetyo<sup>1,2,3</sup>, Paramasari Dirgahayu<sup>2,3,4</sup>, Yulia Sari<sup>2,3,4</sup>, Hudiyono<sup>1,3</sup>, Seiji Kageyama<sup>5</sup>

<sup>1</sup>Department of Microbiology Faculty of Medicine, Sebelas Maret University, Jl. Ir. Sutami 36A, Surakarta, Indonesia

<sup>2</sup>Biomedical Laboratory Faculty of Medicine, Sebelas Maret University, Jl. Ir. Sutami 36A, Surakarta, Indonesia

<sup>3</sup>Center of Biotechnology and Biodiversity Research and Development, Sebelas Maret University, Jl. Ir. Sutami 36A, Surakarta, Indonesia

<sup>4</sup>Department of Parasitology Faculty of Medicine, Sebelas Maret University, Jl. Ir. Sutami 36A, Surakarta, Indonesia

<sup>5</sup>Department of Microbiology and Immunology (Division of Virology) Faculty of Medicine, Tottori University, 86 Nishi cho Yonago, Japan

### Abstract

**Introduction:** This study was conducted to determine the current molecular prevalence of human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and human T lymphotropic virus-1/2 (HTLV-1/2) circulating among drug abuser inmates incarcerated in prisons located in Central Java, Indonesia.

**Methodology:** Socio-epidemiological data and blood specimens were collected from 375 drug abuser inmates in four prisons. The blood samples were analyzed with serological and molecular testing for HIV, HBV, HCV, HDV, and HTLV-1/2.

**Results:** The seroprevalence of HIV, HBsAg, HCV, HDV, and HTLV-1/2 in drug abuser inmates was 4.8% (18/375), 3.2% (12/375), 34.1% (128/375), 0% (0/375), and 3.7% (14/375), respectively. No co-infections of HIV and HBV were found. Co-infections of HIV/HCV, HIV/HTLV-1/2, HBV/HCV, HBV/HTLV-1/2, and HCV/HTLV-1/2 were prevalent at rates of 4% (15/375), 1.3% (5/375), 1.1% (4/375), 0.3% (1/375), and 2.1% (8/375), respectively. The HIV/HCV co-infection rate was significantly higher in injection drug users (IDUs) compared to non-IDUs. Triple co-infection of HIV/HCV/HTLV-1/2 was found only in three IDUs (0.8%). HIV CRF01\_AE was found to be circulating in the inmates. HBV genotype B3 predominated, followed by C1. Subtypes adw and adr were found. HCV genotype 1a predominated among HCV-infected inmates, followed by 1c, 3k, 3a, 4a, and 1b. All HTLV-1 isolates shared 100% homology with HTLV-1 isolated in Japan, while all of the HTLV-2 isolates were subtype 2a.

**Conclusion:** Drug abuser inmates in prisons may offer a unique community to bridge prevention and control of human blood-borne virus infection to the general community.

**Key words:** HIV; HBV; HCV; HDV; HTLV-1/2; drug abuser inmates

*J Infect Dev Ctries* 2013; 7(6):453-467. doi:10.3855/jidc.2965

(Received 30 August 2012 – Accepted 23 October 2012)

Copyright © 2013 Prasetyo *et al.* This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### Introduction

Adequate molecular epidemiology databases of infectious agents are important for infection prevention and treatment programs. However, to the best of our knowledge, an adequate database of that type, particularly one that includes the human blood-borne viruses, is not available in Indonesia, a large developing country with a large population. In addition, community-based active surveillance is difficult to perform in Indonesia due to the characteristics of its geography, demographics, population, and culture.

Correctional facilities are already known as high-risk places for virus transmission, including blood-borne viruses. Blood-borne virus infection in drug

abuser inmates may reflect an increased frequency of high-risk behavior in the community outside of prison, and such behavior should be of concern. Optimal preventive and treatment measures should be applied inside as well as outside of prison. Incarceration may be an opportunity to offer preventive health-care services, such as vaccination [1-2]. The seroprevalence rates in correctional facilities are reported to be 6-19% for HIV and 19-30% for HCV [3-4]. Approximately 38-90% of prisoners incarcerated in correctional facilities who are positive for anti-HIV antibodies are also infected with HCV [5-6]. It is believed that hepatitis B and C occur 100- to 200-fold more frequently among prisoners than in the normal population [7]. In addition, the prevalence of HIV

infection is approximately fivefold higher in prisoners than among the general population [8]. The prevalence of hepatitis viruses and HIV infections among prisoners of different countries varies substantially [9-11]. Reports indicate that the highest prevalence of HBV, HCV, and HIV infections in inmates was found in a Brazilian prison (68.1%, 41%, and 13.7%, respectively) [12]. The lowest HBV and HCV prevalence reported was in an Indian jail (11.1% and 5%, respectively) [13], and the lowest HIV prevalence was seen in a prison in Hungary (0.04%) [14]. HTLV-1/2 usually is a low-prevalence infection among prisoner populations [15-17]. However, in Indonesia, there is a lack of information concerning the prevalence of blood-borne virus infections as well as the viral genotypes circulating among Indonesian high-risk communities, including that of drug abusers. Blood-borne virus prevalence data among Indonesian inmates is also very limited, and prevalence data for Central Java has not previously been determined. The absence of this information is a barrier to the development of appropriate public health interventions, including immunization policy and health protection measures. Information on prevalence is also required to inform approaches to securing appropriate clinical services for infected prisoners. Based on these conditions, we performed a cross-sectional study of drug abuser inmates in correctional facilities in Central Java, Indonesia, to determine the status of a select group of human blood-borne viruses (HIV, HBV, HCV, HDV, and HTLV-1/2), describe the viral genotypes, and determine whether any sociodemographic or epidemiological characteristics of the inmates were associated with these infections.

## **Methodology**

### *Study population*

This study was performed from August 2009 until October 2009 in four prisons (District Sragen, Klaten, Kedung Pane Semarang, and Bulu Semarang Women's Prison) in Central Java, Indonesia. These facilities serve as incarceration venues for prisoners accused of many types of crimes. A list of drug abuser prisoners was obtained from the staff of the correctional facilities, and all these individuals were recruited (n = 375). All subjects were informed that the study was voluntary and that there would be no negative consequences for refusal; however, all subjects were willing to join the study. Approval was obtained from the institutional ethical committee review boards of the Faculty of Medicine of Sebelas Maret University and Dr. Moewardi General Hospital,

Surakarta, Indonesia. Written informed consent was obtained from all individuals participating in the study. The results of the tests were given to the prisoners by the prison doctor. Blood samples collected from the prisoners were fractionated, aliquoted, and kept frozen until analyzed. All the procedures were conducted according to the principles of the Declaration of Helsinki.

In total, 375 prisoners agreed to participate. The study participants completed an interviewer-administered questionnaire that assessed general sociodemographic and epidemiological characteristics associated with HIV, HBV, HCV, HDV, and HTLV-1/2 infections. The questionnaire was administered by a specially trained interviewer. Data including age, gender, birthplace, marital status, occupation before incarceration, number and duration of incarcerations, history of hepatitis, history of hepatitis in family members or occupational exposures to hepatitis patients, transfusions, surgery, acupuncture, ear piercing, tattooing, history of traumatic injury, sexually transmitted diseases, sexual promiscuity, homosexuality, lack of condom use, hemodialysis, national and international trips, health status, socioeconomic level, consumption of alcohol, and drug abuse history were obtained from all 375 subjects.

### *Serological markers for HIV, HBV, HCV, HDV, and HTLV-1/2 infections*

Subject plasma was separated from whole blood with EDTA and subjected to the following tests. HIV antibodies were detected using a Determine HIV-1/2 Kit (Abbott Diagnostics Japan, Tokyo, Japan). A SERATEC Hepatitis B Quick Test (Gesellschaft für Biotechnologie GmbH, Göttingen, Germany), Ortho HCV PA II (Ortho Diagnostics, Tokyo, Japan), HDV Ab ELISA (Diagnostic Automation, Calabasas, CA), and MP Diagnostic HTLV-I/II ELISA 4.0 (MP Biomedicals, Singapore) were used for the detection of HBsAg, anti-HCV, anti-HDV, and anti-HTLV-1/2 antibodies, respectively. All assays were performed according to the manufacturers' instructions.

### *Nucleic acid extraction and molecular detection*

Nucleic acid (DNA and RNA) was extracted from 200 µl of plasma by using a PureLink Viral RNA/DNA Kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The nucleic acids were then aliquoted, and one aliquot was reverse-transcribed according to the Superscript III First-Strand cDNA Synthesis Supermix Kit protocol using

random hexamers (Invitrogen). Molecular detection was performed by PCR using an Amplitaq Gold 360 DNA Polymerase Kit (Invitrogen). A portion of the HIV *gag* gene encoding the p24 region was amplified using the primers H1G777/H1P202 in the first round and H1gag1584/g17 in the second round [18]. A portion of the *pol* gene encoding integrase (*int*) was amplified with the primers unipol 5/unipol 6 in the first round and unipol 1/unipol 2 in the second round [18]. A portion of the HBsAg gene was amplified using the primers HBS1F/HBS1R in the first round and HBS2F/HBS2R in the second round [19]. A portion of the NS5B region of the HCV genome was amplified using the primers hep31b/hep32 in the first round and hep33b/hep34b in the second round [20]. A portion of the E1-E2 region, including hyper variable region-1, were amplified using primers Lqz188/Lqz187 in the first round and Lqz 189/Lqz187 in the second round [21]. HDV RNA was detected by amplification of a 400-nucleotide (nt)-long region of the HDV genome proposed for the classification of HDV genotypes using the primers A/EF3 [22]. For HTLV-1/2 molecular detection, nucleic acids were extracted from peripheral mononuclear blood cells (PBMCs). The LTR regions of the HTLV-1 genome were amplified using the primers LTR1/LTR3 in the first round and LTR1/LTR2 in the second round [23]. The LTR regions of the HTLV-2 genome were amplified using the primers VS1/VS2 in the first round and VS3/VS4 in the second round [23]. Appropriate internal amplification controls were included in all of molecular assays to exclude the false negative results. PCR products were subjected to electrophoresis in 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet illumination.

#### *Determination of nucleotide sequences and phylogenetic analysis*

The PCR products were purified from agarose gels, and the nucleotide sequences were determined using the primers H1gag1584/g17 for the HIV *gag* region, unipol 1/unipol 2 for the HIV *pol* (*int*) region, HBS2F/HBS2R for the HBsAg region, hep33b/hep34b for the HCV NS5B region, Lqz189/Lqz187 for the HCV E1-E2 region, LTR1/LTR2 for the HTLV-1 LTR region, and VS3/VS4 for the HTLV-2 LTR region, respectively. The sequences were then submitted to the Blast program to check their similarity to related strains deposited in Genbank/EMBL/DDBJ. The reference strains with the highest homology score to each analyzed strain were retrieved from the GenBank/EMBL/DDJB databases and aligned with

the tested sequences using ClustalW [24]. All the HIV, HBV, and HCV sequences isolated in Indonesia deposited in GenBank were also included in the alignment analysis for each tested sequence. The frequency of nucleotide substitution at each base was estimated by the Kimura two-parameter method. A phylogenetic tree was constructed by the neighbor-joining method, and its reliability was estimated by 1000 bootstrap replications. The phylogenetic tree was constructed using the MEGA version 4 software package [25].

#### *Statistical analysis*

Statistical analysis was performed using SPSS version 16 software (SPSS, Chicago, IL, USA); 95% confidence interval (CI) was used for all data analysis.

#### *Accession numbers*

The sequences described in this article have been deposited in GenBank/EMBL/DDBJ under accession numbers HQ174799-HQ174802, HQ174804-HQ174807, JN132140-JN132147, JN202355-JN202397, JN230828-JN230857, and JN247455-JN247462.

## **Results**

#### *The socio-epidemiological data of the prisoners*

Central Javan prisons are classified based on capacity. There are two prisons, nine prisons, and four prisons with class I, class IIA, and class IIB status in Central Java, respectively. One class I prison (Semarang Kedung Pane Prison), two class IIA prisons (Sragen Prison and Bulu Semarang Women's Prison), and one class IIB prison (Klaten Prison) received special permission to be involved in the study from the Directorate General of Correctional Facilities of Indonesian Ministry of Law and Human Rights Central Java Regional Office.

In total, 289 men and 86 women participated in the study. The mean age of the inmates studied was 32.3 years (range 18 to 67 years). Of the total study population, 137 were born in Central Java (36.5%), 147 in Jakarta (39.2%), 87 in West Java (23.2%), and four outside of Java Island (1.1%). Fifty-five percent of the inmates lived in Central Java before incarceration, while 35%, 3.2%, 2.7%, and 2.1%, lived in Jakarta, East Java, Yogyakarta, West Java, and somewhere other than Java Island, respectively (Figure 1, Table 1). A total of 67.2% inmates had been incarcerated only in the prisons in Central Java, and the rest (32.8%, 123/375) had been incarcerated previously in Jakarta prisons. Interestingly, most

**Figure 1.** Map of Indonesia and the region of origin of the drug abuser inmates in the Central Javan prisons involved in the study

A: Nangroe Aceh Darussalam; B: North Sumatera; C: Lampung; D: Jakarta; E: West Java; F: Central Java; G: Yogyakarta; H: East Java; I: Maluku, J: Papua

**Table 1.** Region of origin and history as an injecting drug user (IDU) for drug abuser inmates in Central Javan prisons

Region of Origin	IDU	Non-IDU	Total
Central Java	5.3% (11/207)	94.7% (196/207)	55.2% (207/375)
East Java	8.3% (1/12)	91.7% (11/12)	3.2% (12/375)
Jakarta	54.9% (73/133)	45.1% (60/133)	35.5% (133/375)
Lampung	0% (0/1)	100% (1/1)	0.3% (1/375)
Maluku	0% (0/1)	100% (1/1)	0.3% (1/375)
Nangroe Aceh Darussalam	0%	100% (1/1)	0.3% (1/375)
North Sumatra	0% (0/1)	100% (1/1)	0.3% (1/375)
Papua	0% (0/1)	100% (1/1)	0.3% (1/375)
West Java	12.5% (1/8)	87.5% (7/8)	2.1% (8/375)
Yogyakarta	80% (8/10)	20% (2/10)	2.7% (10/375)
Total	94	281	100% (375/375)



inmates (77.7%, 73/94) with a history as an injection drug user (IDU) were derived and transferred from Jakarta prisons. The median duration of incarceration was 15.5 months (range 1 to 168 months). A history of hepatitis was present in 2.9% inmates (11/375). A history of hepatitis in family members or occupational exposure to hepatitis patients was present in 15 inmates (4%). Fourteen of the 375 inmates (3.7%) had received blood transfusions, 3.5% (13/375) had undergone surgery, 0.8% (3/375) had acupuncture, 47.5% (178/375) had piercings (86 of those were women and ear piercing for women is common in Indonesia), 26.4% (99/375) had tattoos, 1.6% (6/375) had a history of traumatic wounds, and none had ever undergone hemodialysis. Most inmates who had tattoos (82.8%, 82/99) also had piercings. A history of sexually transmitted diseases was present in 5.1% (19/375) inmates. With respect to sexual behavior, 128 inmates (34.1%) had more than one sexual partner and 36 (9.6%) had used condoms. Thirteen (3.5%) inmates had sexual partners from abroad. None of the prisoners had a history of international trips. None of the prisoners were serologically screened upon arrival in the correctional facility. The health status was good in all of the inmates.

#### *HIV, HBV, HCV, HDV, HTLV-1/2 infections*

Eighteen of the 375 inmates had HIV-1/2 antibodies with 72.2% (13/18) of the anti-HIV-1/2 positive samples derived from inmates with IDU history (OR 8.9, 95% CI 3.067-25.590). The prevalence of HIV antibodies in those living in Jakarta and other places were 10.5% (OR 7.0, 95% CI 2.255-21.730) and 1.6% (OR 0.1, 95% CI 0.046-0.444), respectively. The HBsAg positive rate was 3.2% (12/375) and only one of the HBsAg positive samples was derived from IDUs. Oral operations (OR 1.2, 95% CI 0.145-9.715) and a history of sex with a foreigner (OR 2.6, 95% CI 0.303-22.331) were associated with HBV infection in non-IDU inmates. All inmates who were HBsAg positive were more than 25 years old. The anti-HCV antibody-positive rate was 34.1% (128/375) and significantly higher among the IDUs (OR 2.5, 95% CI 1.528-3.989); however, 28.8% of the anti-HCV antibody-positive samples were derived from the non-IDUs. HCV infection among non-IDUs was more common in inmates living in Central Java (72.8%, 59/81) than in inmates from other areas. Monthly income less than 100 USD (OR 1.1, 95% CI 0.610-2.044), tattoos (OR 3.2, 95% CI 1.514-6.689), and piercings (OR 3.6, 95% CI 1.469-28.308) were associated with HCV infection in non-IDUs. The

prevalence of HCV antibodies in males and females was 37% (OR 1.8, 95% CI 1.053-3.144) and 24.4% (OR 0.5, 95% CI 0.318-0.949), respectively. No sample was positive for anti-HDV antibodies. The anti-HTLV-1/2 antibody-positive rate was 3.7% (14/375), with ten of the anti-HTLV-1/2 antibody-positive samples derived from the non-IDUs (OR 0.8, 95% CI 0.254-2.712). For IDUs, the OR was 1.2 (95% CI 0.369-3.935). Oral operation history (OR 1.3, 95% CI 0.160-10.948) and piercings (OR 1.6, 95% CI 0.430-5.639) were associated with HTLV-1/2 infection in non-IDUs. No co-infections of HIV and HBV were detected. Co-infection of HIV and HCV was significantly higher among IDUs (OR 13.6, 95% CI 3.737-49.211) than non-IDUs (OR 0.1, 95% CI 0.020-0.267). Co-infection of HIV and HTLV-1/2 was found only in inmates from Jakarta, and IDUs had a higher risk for co-infection (OR 4.6, 95% CI 0.757-27.956). Triple co-infection of HIV, HCV, and HTLV-1/2 was found in only three IDUs (Table 2). Unless otherwise stated, no statistical associations could be drawn.

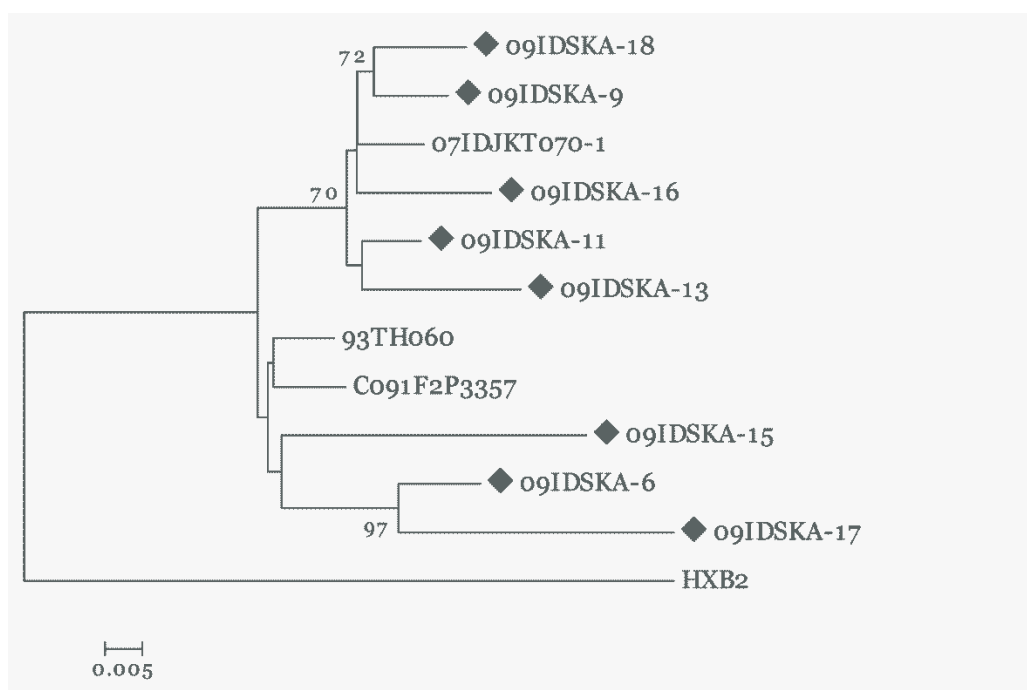
#### *HIV circulation among drug abuser inmates*

A portion of the HIV *gag* gene could be amplified by nested RT-PCR in eight samples. The amplified portions of the HIV-1 *gag* region (position 1577-2039 on HIV-1 reference HXB2) of five HIV isolates (09IDKA-9, 09IDSKA-11, 09IDSKA-13, 09IDSKA-16 and 09IDSKA-18) were clustered together in the phylogenetic tree and shared 97-98% nucleotide homology with HIV-1 isolated in Jakarta (07IDJKT070-1). The 09IDSKA-15 sample shared 95% homology with C091F2P3357 from Thailand, while 09IDSKA-6 and 09IDSKA-17 shared 96% and 94% homology with isolate 93TH060 from Thailand, respectively (Figure 2). The amino acids methionine 378 of HIV-1 *gag* (position on HXB2) was absent in the 09IDSKA-15, 09IDSKA-6 and 09IDSKA-17 (data not shown). In addition, amino acid valine 390 was absent in all of the HIV isolates (data not shown). With respect to the region of origin of the inmates, 09IDSKA-6 and 09IDSKA-13 were isolated from inmates with Central Java and Yogyakarta origin, respectively, while 09IDKA-9, 09IDSKA-11, 09IDKA-15, 09IDSKA-16, 09IDSKA-17, and 09IDKA-18 were isolated from inmates with a Jakarta origin.

The portion of the HIV *pol* gene encoding integrase (corresponding to 4493-4780 nt in HIV-1 HXB2) could be amplified in eight samples. However, only four out of eight were derived from the HIV *gag*

**Table 2.** Serological status of HIV, HBV, HCV, HDV, and HTLV-1/2 of drug abuser inmates in Central Javan correctional facilities based on history as an injecting drug user (IDU)

	IDU	Non IDU	Positive rates
Anti-HIV-1/2 (+)	13.8 % (13/94)	1.8% (5/281)	4.8% (18/375)
HBsAg (+)	1.1% (1/94)	3.9% (11/281)	3.2% (12/375)
Anti-HCV (+)	50% (47/94)	28.8% (81/281)	34.1% (128/375)
Anti-HDV (+)	0% (0/94)	0% (0/281)	0% (0/375)
Anti-HTLV-1/2 (+)	4.3% (4/94)	3.6% (10/281)	3.7% (14/375)
Anti-HIV-1/2 (+) + HBsAg (+)	0% (0/94)	0% (0/281)	0% (0/375)
Anti-HIV-1/2 (+) + Anti-HCV (+)	12.8% (12/94)	1.1% (3/281)	4% (15/375)
Anti-HIV-1/2 (+) + Anti-HTLV-1/2 (+)	3.2% (3/94)	0.7% (2/281)	1.3% (5/375)
HBsAg (+) + Anti-HCV (+)	0% (0/94)	1.4% (4/281)	1.1% (4/375)
HBsAg (+) + Anti-HTLV-1/2 (+)	0% (0/94)	0.4% (1/281)	0.3% (1/375)
Anti-HCV (+) + Anti-HTLV-1/2 (+)	3.2% (3/94)	1.8% (5/281)	2.1% (8/375)
Anti-HIV (+) + Anti-HCV (+) + Anti-HTLV-1/2 (+)	3.2% (3/94)	0% (0/281)	0.8% (3/375)

**Figure 2.** Phylogenetic analysis of HIV isolates obtained from the drug abuser inmates on the basis of HIV-*gag* nucleotide sequences (◆).

GenBank Accession Numbers of the reference isolates in the phylogenetic tree are as follows: 07IDJKT070-1: AB468573; C091F2P3357: GU458775, 93TH060: AB220946. HIV-1 reference sequence HXB2 (GenBank Accession Number: K03455) were also included in the phylogenetic analysis.

positive samples (09IDSKA-6, 09IDSKA-9, 09IDSKA-11, and 09IDSKA-13). An amplified portion of the HIV-1 *int* region (position 4471-4806 on HXB2) of all HIV isolates shared 95-97% homology with that of HIV-1 isolated previously in Jakarta (ID17 or ID12). Five HIV isolates (09IDSKA-5, 09IDSKA-9, 09IDSKA-11, 09IDSKA-12, and 09IDSKA-13) shared 96-97% homology with HIV-1 isolated in Thailand (AE-Int-280, FD0837IN, AE-Int-61, FD0047IN, FD0864IN, respectively), and shared 95-97% homology with HIV-1 isolated in Jakarta (ID17 or ID12). The 09IDSKA-6 isolate had 97% homology with HIV-1 isolated in Germany (1755) and had 96% homology with HIV-1 isolated from Indonesia (ID17). The 09IDSKA-7 strain had 95% homology with HIV-1 isolated in the United Kingdom (INT-379) and 95% homology with ID17. One HIV isolate (09IDSKA-14) had 93% homology with HIV-1 isolated in Cameroon (A1646). With respect to the inmates' hometowns, inmates with 09IDSKA-5, 09IDSKA-7 and 09IDSKA-14 strains were from Jakarta, while inmates with the 09IDSKA-12 strain were from West Java. Intriguingly, all HIV isolates belonged to CRF01\_AE; however, based on the amplified portion of the HIV *int* region, seven out of eight isolates clustered within the same taxa (Figure 3). The substitutions T736V, T749A, and I759V were found only in the seven isolates, while G747S, R751K, and G758N were found in all of the HIV isolates (data not shown).

#### *HBV circulation among drug abuser inmates*

A portion of the HBV preS2/S gene was successfully amplified in nine out of twelve HBsAg-positive samples. In particular, 417 nucleotides (corresponding to 255-671 nt in HBV ADW GenBank accession number V00866) of the strain 09IDSKAB-14 were closely related to HBV genotype C1 isolated in Malaysia (M31). Five HBV isolates were clustered together with HBV genotype B3 isolated in Malaysia (M43), and one HBV isolate (09IDSKAB-7) was closely related to M36 (isolated in Malaysia). Two HBV isolates (09IDSKAB-11 and 09IDSKAB-12) shared 99% homology with HBV genotype B3 isolated in Papua, Indonesia (NMB09010), and Java, Indonesia (1813Java, EI444AJava, and 1839Java). The following subtypes were found: adw (8/9) and adr (1/9) (Figure 4).

#### *HCV circulation among drug abuser inmates*

A total of 30 HCV RNA samples collected in the study were amplified successfully by RT-PCR and subjected to the determination of the NS5B nucleotide

sequence (328 bases, position 8279-8606 in H77 strain). HCV genotype 1a was found in 14 subjects, genotype 1c in 5 subjects, genotype 3a in 4 subjects, genotype 3k in 4 subjects, genotype 4a in 2 subjects, and genotype 1b in 1 subject (Figure 5). The NS5B-positive samples were further subjected to the determination of the E1-E2 sequence including the hyper-variable region-1 (538 bases, position 1322-1859). HCV genotype 1a was found in 13 subjects, genotype 1c in 5 subjects, genotype 3k in 5 subjects, genotype 3a in 4 subjects, genotype 4a in 2 subjects, and genotype 1b in one subject (Figure 6). A mismatch of genotype (possible chimeric) genotyping was found during the NS5B and E1-E2 analysis. In particular, 09IDSKAC-8 was subtyped as 1a according to analysis of NS5B. However, the strain was subtyped as 3k according to an analysis of the HCV E1-E2 region.

#### *HTLV-1 and HTLV-2 detected in Central Javan prisoners*

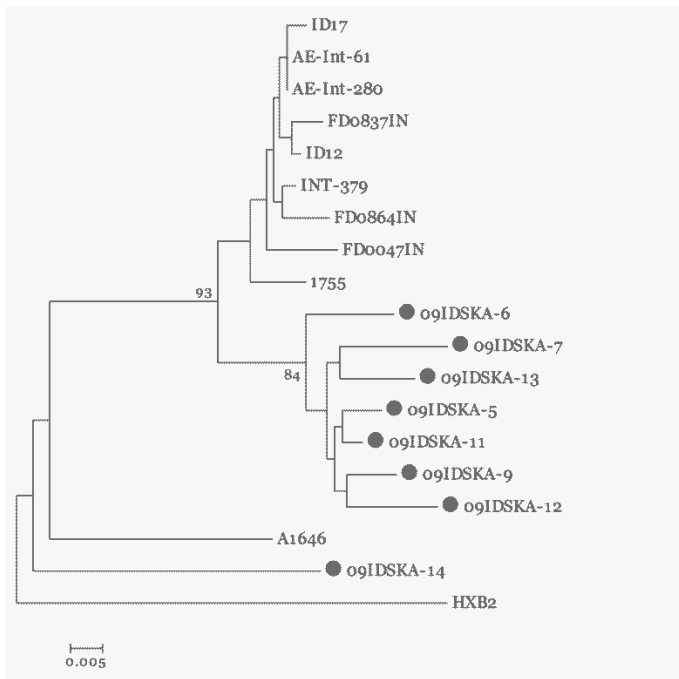
HTLV-1 RNA was detected in 3 out of 14 anti-HTLV-1/2 antibody-positive samples. Based on 317 nucleotides from the HTLV-1 LTR region, all HTLV-1 isolates shared 100% homology with B1033-2009 (HTLV-1 isolated in Japan). HTLV-2 RNA was detected in 5 out of 14 anti-HTLV-1/2 antibody-positive samples. Based on 666 nucleotides of the HTLV-2 LTR region, 09IDSKAH-2-1 shared 99% homology with HTLV-2 isolated in the USA (GenBank Accession Number: AF412314). The 09IDSKAH-2-2, 09IDSKAH-2-3, 09IDSKAH-2-4, and 09IDSKAH-2-5 strains had 99% homology with HTLV-2 isolated in Japan (GenBank Accession Number: M10060). All of the HTLV-2 isolates were subtype 2a (Figure 7).

## **Discussion**

This is the first molecular epidemiology study of human blood-borne viruses (HIV, HBV, HCV, HDV, and HTLV-1/2) in Indonesian correctional facilities, especially among drug abuser inmates imprisoned in correctional facilities in Central Java, Indonesia. Moreover, prior to this study, there were only limited data about molecular epidemiology profiles of blood-borne viruses in Indonesia and none of the molecular epidemiology data derived from the correctional facilities, to the best of our knowledge.

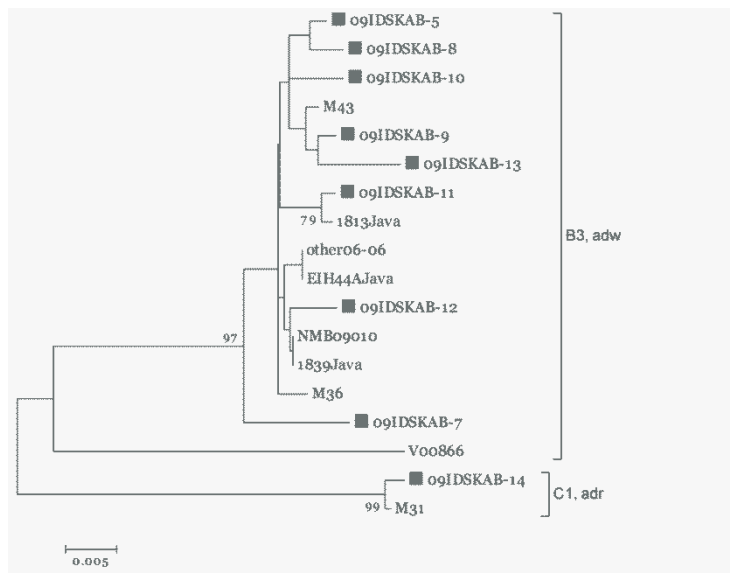
In our study, we found that history as an IDU was a very strong risk factor for HIV and HCV but not for HBV or HTLV-1/2. However, of the 375 drug abuser inmates, 32.8% were transferred from Jakarta prisons,

**Figure 3.** Phylogenetic analysis of HIV isolates obtained from the drug abuser inmates on the basis of HIV-*int* nucleotide sequences (●).



GenBank Accession Numbers of the reference isolates in the phylogenetic tree are as follows: AE-Int-280: HM150858, ID17: AY214050, 1755: FJ183574, INT-379; GU217226, FD0837IN: GU345083, ID12: AY214049, AE-Int-61: HM150817, FD0047IN: GU345047, FD0864IN: GU345079, A1646: EU618405; and HIV-1 reference sequence HXB2 (GenBank Accession Number: K03455) were also included in the phylogenetic analysis.

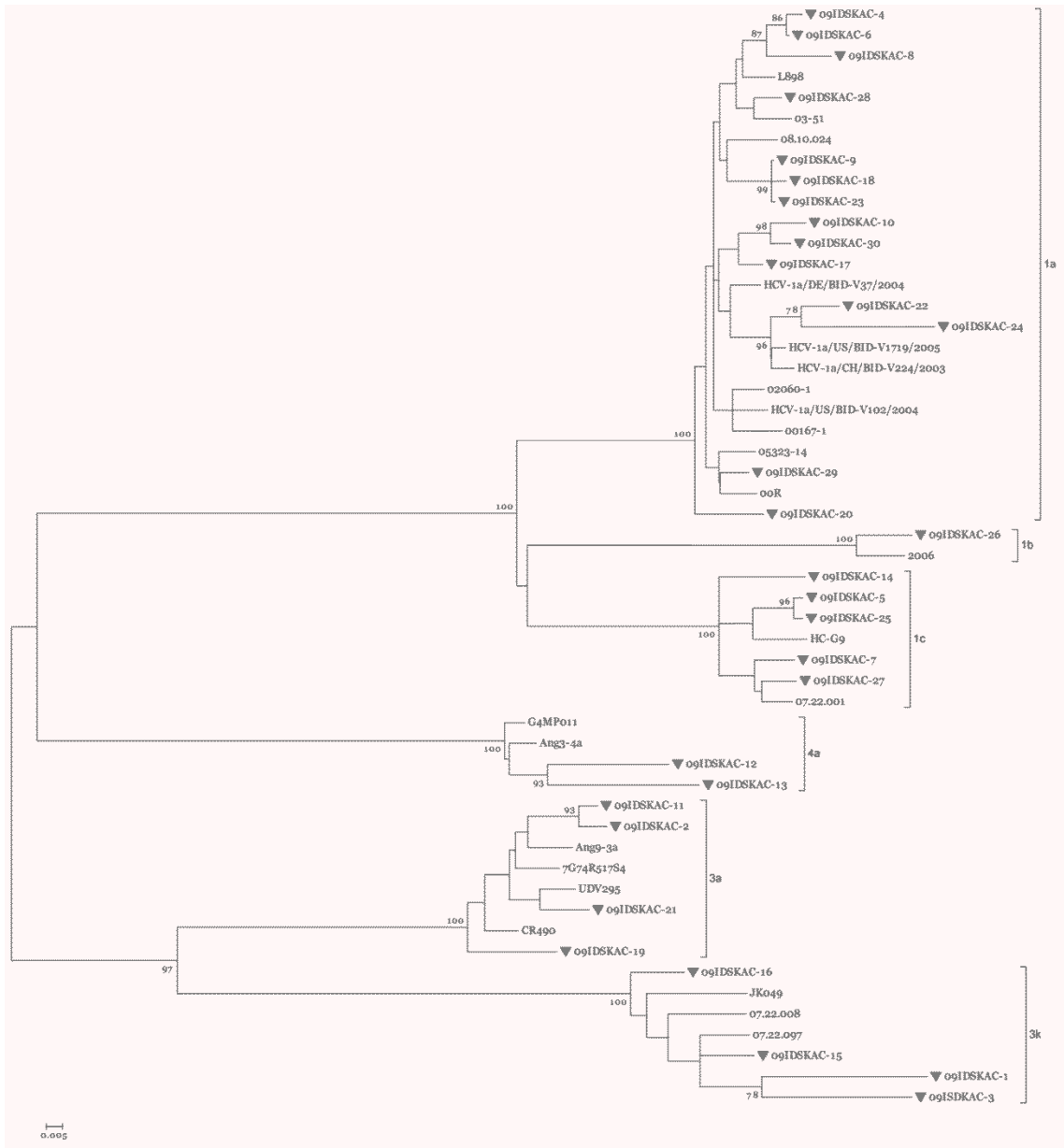
**Figure 4.** Phylogenetic analysis of HBV isolates obtained from the drug abuser inmates on the basis of HBV preS2/S nucleotide sequences (■).



GenBank Accession Numbers of the clones in the phylogenetic tree are as follows: M43, GQ924625; 1813Java, DQ141644; other06-06, FJ391884; EIH44AJava, DQ141628; NMB09010, AB554017; 1839Java, EF473972; M36, GQ924674; M31, GQ924614; HBV ADW, V00866.



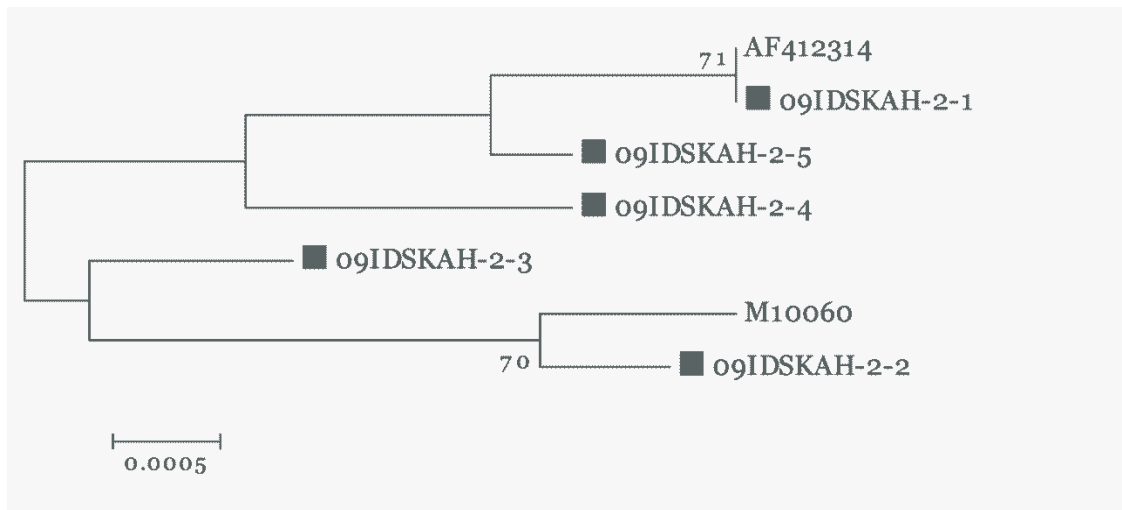
**Figure 5.** Phylogenetic analysis of HCV isolates obtained from the drug abuser inmates on the basis of HCV NS5B nucleotide sequences (▼).



GenBank Accession Numbers of the reference isolates in the phylogenetic tree are as follows: L898, EU781755; 03-51, EU781774; 08.10.024, GQ418298; HCV-1a/DE/BID-V37/2004, EU862834; HCV-1a/US/BID-V1719/2005, FJ024282; HCV-1a/CH/BID-V224/2003, EU255944; 02060-1, AY682778; HCV-1a/US/BID-V102/2004, EU255993; 00167-1, AY682748; 05323-14, AY683132; 00R, EU781748; 2006, EF407467; HC-G9, D14853; 07.22.001: GQ418337; G4MP011, AY743073; Ang3-4a, FJ872320; 07.22.097, GQ418376; 07.22.008, GQ418373; JK049, D63821; CR490, EF543245; UDV295, EF543246; 7G74R517S4, EU710458; Ang9-3a, FJ872292.



**Figure 7.** Phylogenetic analysis of HTLV-2 isolates obtained from the drug abuser inmates on the basis of partial LTR sequences (■).



All Central Javan Indonesian isolates were clustered along with the representative strains of genotype 2a (GenBank Accession Numbers AF412314, and M10060).

and most of them were IDUs. The habit of injectable drug use is more common in Jakarta than among the drug abuser community of Central Java (59.3% vs. 8.3%), and most inmates who tested positive for anti-HIV-1/2, HBsAg, anti-HCV, and anti-HTLV-1/2 living in Central Java had no IDU history. Unfortunately, there is inadequate data regarding the risk factors from each area in Indonesia, especially the risk factors influencing blood-borne virus infection among non-IDUs. The characteristics of each area may play an important role in virus transmission as Indonesia is the largest archipelagic country in the world and has considerable ethnic, linguistic, and religious diversity. The sample size allows us to obtain reliable prevalence figures for HIV, hepatitis virus, and HTLV-1/2 infections in drug abuser inmate populations of the correctional facilities studied. Nevertheless, the relatively low frequency of HBV, HIV, and HTLV-1/2 infection found in the inmates limited the study in terms of detecting associations by multivariate analysis. Taken together, further study is needed to firmly establish the prevalence and characteristics of blood-borne viruses in each area in Indonesia to better aid blood-borne virus management programs.

The prevalence of HIV antibodies among the drug abuser inmates in the studied Central Javan correctional facilities was 4.8%, which was lower than that reported in Banceuy Prison of Bandung,

Indonesia, one of the two prisons specified for drug-related offences in West Java, where 7.2% of the inmates had HIV infections [2]. However, the prevalence is higher than the rate reported among the general population [26]. The prisoners with an IDU history had a higher risk for HIV infection compared to non-IDU prisoners. In addition, the prevalence of HIV antibodies in those from Jakarta was higher than that of prisoners from other locations. These data are concordant with the higher prevalence of HIV infection in Jakarta compared with other provinces in Indonesia [27], and most of the prisoners transferred from Jakarta were IDUs. Based on our molecular analysis, all HIV isolates were CRF01\_AE. The nucleotide sequences of three HIV isolates presented higher homology to foreign strains retrieved from the Genbank/EMBL/DDBJ databases. That result suggests that foreign HIV strains are occasionally introduced into Indonesia.

With respect to HBV infection, the 3.2% prevalence rate found was based on HBsAg reactive samples and was lower than that reported in Banceuy Prison in Bandung, Indonesia (5.8%) [2], and lower than that reported previously among the Indonesian high-risk population (4.0 to 20.3%) [28]. In the present study, we could not find any association between HBV infection and the sociodemographic data; however, all inmates who were HBsAg positive were more than 25 years old. This observation agrees with those of previous reports that indicated that being aged older

than 25 years was a significant predictor for HBV infection in inmates entering a correctional facility [9]. Unfortunately, our data could not be used to draw statistical conclusions regarding the association between age and HBV infection. Moreover, our findings may reflect the impact of the HBV vaccination program started in 1987 in Indonesia. Other characteristics of the inmates reported as strongly associated with HBV marker presence, such as intravenous drug use [29-30] and duration of current imprisonment [31], did not have any association with HBV infection in our study. None of the inmates who tested positive for HBV infection had HDV antibodies. This frequency is lower than that reported in an American study [29] where researchers found that 8% of HBV-infected inmates had HDV markers. Based on a molecular analysis of a portion of the HBV preS2/S gene, most HBV isolates belonged to genotype B3. This finding supports a previous finding that B3 is the major genotype in Indonesia [32-34]. The nucleotide sequences of the seven HBV isolates had higher homology to foreign strains retrieved from the Genbank/EMBL/DDBJ database, suggesting that foreign HBV strains are occasionally introduced into Indonesia.

The HCV prevalence among the drug abuser inmates was high (34.1%) compare with the general population in Indonesia [35-40] and higher than the prevalence among general prisoners in Banceuy Prison (18.6%) [2]. However, the prevalence was lower than that in children with hematological diseases (39%) [41] and in hemophilia patients (56.9%) [42]. The anti-HCV antibody-positive rate was significantly higher among the IDUs; however, 28.8% of the anti-HCV antibody-positive samples were derived from the non-IDUs. HCV infection among non-IDUs was more common in inmates living in Central Java (72.8%, 59/81) than in inmates from other areas. Tattoos and piercings were associated with HCV infection in non-IDUs. This is an important finding because the general awareness of the risk of tattooing and piercing is very low in Indonesia, and this observation is consistent with those of a previous investigation [2]. Previous studies indicate that the majority of HCV infections in inmates occur before incarceration [12,43]. However, it was unclear whether the HCV-infected subjects in the present study acquired the HCV infection before or during incarceration, as we did not find enough evidence to draw such conclusions even though most subjects confessed to performing high-risk activities during their incarceration (*e.g.*, sharing needles for tattoos and receiving piercing in the prison) (data not

shown). The high frequency of HCV infection among IDUs found in this study agrees well with previous observations [2-3,10,43]. The high frequency of HCV infection among inmates from Jakarta compared to those from other provinces may be explained by the higher frequency of IDUs in subjects from Jakarta.

It is believed that the most prevalent subtype of HCV in Indonesia is 1b, followed by 3k, 2a, 1a, 1c, and 2e. Moreover, subtypes 1d, 2b, 2e, 2f, 3a, 3b, 3g, and 4a can be found in Indonesia [34,44-45]. However, we found that HCV genotype 1a was predominant in HCV-infected inmates, followed by genotypes 3a, 4a, 1b, 1c, and 3k. The prevalence of HCV genotype 1a found in the inmates was higher than that reported in blood donors and patients with liver disease in Indonesia [34], in whom HCV genotype 1b is predominant. Differences in this genotype prevalence might be due to differences in risk factor frequencies. Reports on HCV genotypes found in inmates are scarce, and our study is the first to report on the molecular epidemiology of HCV in correctional facilities in Indonesia, especially among drug abuser inmates. One study indicated that HCV genotypes 1a and 1b were also predominant in HCV-infected inmates in Manila [46].

There is very low seroprevalence of HTLV-1 among the healthy blood donors from Jakarta, which is also reflected in the western part of Indonesia, and no seropositive individuals were found among healthy patients from Papua [47-48]. However, we detected HTLV-1 and HTLV-2 among the drug abuser inmates examined in this study. This finding suggests circulation of HTLV-1/2 in the high-risk community, especially drug abusers, in Indonesia.

## Conclusion

We conclude that the prevalence of HIV, HBV, HCV, and HTLV-1/2 infections in drug abuser inmates in Central Java was higher than the prevalence reported in the Indonesian general population. The epidemic of HIV, hepatitis B, hepatitis C, and HTLV-1/2 infections among drug abuser prisoners constitutes a major public health problem and should be addressed to prevent further spread in the community by prison-based prevention programs. Prisons seem to be a suitable setting for counseling the inmates about blood-borne virus infections, for providing risk-reduction information, and for monitoring prevalence, as long as the prisoners' right to voluntary testing is protected. Voluntary HIV and other human blood-borne virus testing in prison may greatly improve access to adequate infection management programs in

a developing country [1-2]. In addition, the prisoners could have been infected by the infectious agents before and during incarceration and will be released into the general community after they complete their sentences. Therefore, it is important to screen the presentation of infectious agents such as blood-borne viruses in the prisoners and to gather molecular epidemiology data to strengthen infection management programs. Our results suggest that all inmates should undergo testing upon arrival at a facility, not only for the IDUs. However, performing molecular epidemiology studies in correctional facilities, especially among the drug abuser prisoner population, is useful as surrogate tool to gain molecular epidemiology data on human blood-borne viruses for a developing country such as Indonesia in which it is difficult to perform active surveillance among the general population.

### Acknowledgements

The authors would like to thank Faqihuddin Ahmad, Rochmali Zultan, Lushiana Primasari, Wisnu Iskandar, and Alexius Purwoko for technical assistance.

This work was supported partially by grants from the Indonesian Directorate of Higher Education No. 322/SP2H/PP/DP2M/VI/2009, 440/SP2H/PP/DP2M/VI/2010, 505/SP2H/PL/Dit.Litabmas/VII/2011, and a grant from Sebelas Maret University No. 2342/UN27.16/PN/2012).

### References

- Nelwan EJ, Diana A, van Crevel R, Alam NN, Alisjahbana B, Pohan HT, van der Ven A, Djaya I (2009) Indonesian prisons and HIV: part of the problem, part of the solution? *Acta Med Indones* 1: 52-56.
- Nelwan EJ, Van Crevel R, Alisjahbana B, Indrati AK, Dwiyanita RF, Nuralam N, Pohan HT, Jaya I, Meheus A, Van Der Ven A (2010) Human immunodeficiency virus, hepatitis B and hepatitis C in an Indonesian prison: prevalence, risk factors and implications of HIV screening. *Trop Med Int Health* 15: 1491-1498.
- Adjei AA, Armah HB, Gbagbo F, Ampofo WK, Quaye IK, Hesse IF, Mensah G (2006) Prevalence of human immunodeficiency virus, hepatitis B virus, hepatitis C virus and syphilis among prison inmates and officers at Nsawam and Accra, Ghana. *J Med Microbiol* 55(Pt 5): 593-597.
- Solomon L, Flynn C, Muck K, Vertefeuille J (2004) Prevalence of HIV, syphilis, hepatitis B, and hepatitis C among entrants to Maryland correctional facilities. *J Urban Health* 81: 25-37.
- Pontali E and Ferrari F (2008) Prevalence of hepatitis B virus and/or hepatitis C virus co-infections in prisoners infected with the human immunodeficiency virus. *Int J Prison Health* 4: 77-82.
- Hennessey KA, Kim AA, Griffin V, Collins NT, Weinbaum CM, Sabin K (2009) Prevalence of infection with hepatitis B and C viruses and co-infection with HIV in three jails: a case for viral hepatitis prevention in jails in the United States. *J Urban Health* 86: 93-105.
- Gaube J, Feucht HH, Laufs R, Polywka S, Fingscheidt E, Muller HE (1993) Hepatitis A, B and C as desmotic infections. *Gesundheitswesen* 55: 246-249.
- Krebs CP and Simmons M (2002) Intraprison HIV transmission: an assessment of whether it occurs, how it occurs, and who is at risk. *AIDS Educ Prev* 14: 53-64.
- Butler TG, Dolan KA, Ferson MJ, McGuinness LM, Brown PR, Robertson PW (1997) Hepatitis B and C in New South Wales prisons: prevalence and risk factors. *Med J Aust* 166: 127-130.
- Gore SM, Bird AG, Cameron SO, Hutchinson SJ, Burns SM, Goldberg DJ (1999) Prevalence of hepatitis C in prisons: WASH-C surveillance linked to self-reported risk behaviours. *QJM* 92: 25-32.
- Ford PM, White C, Kaufmann H, MacTavish J, Pearson M, Ford S, Sankar-Mistry P, Connop P (1995) Voluntary anonymous linked study of the prevalence of HIV infection and hepatitis C among inmates in a Canadian federal penitentiary for women. *Can Med Assoc J* 153: 1605-1609.
- Guimaraes T, Granato CF, Varella D, Ferraz ML, Castelo A, Kallas EG (2001) High prevalence of hepatitis C infection in a Brazilian prison: identification of risk factors for infection. *Braz J Infect Dis* 5: 111-118.
- Singh S, Prasad R, Mohanty A (1999) High prevalence of sexually transmitted and blood-borne infections amongst the inmates of a district jail in Northern India. *Int J STD AIDS* 10: 475-478.
- Tresó B, Barcsay E, Tarján A, Horváth G, Dencs A, Hettmann A, Csépai MM, Györi Z, Rusvai E, Takács M (2012) Prevalence and correlates of HCV, HVB, and HIV infection among prison inmates and staff, Hungary. *J Urban Health* 89: 108-116.
- Fialho M, Messias M, Page-Shafer K, Farre L, Schmalb M, Pedral-Sampaio D, Ramos M, Brites C (2008) Prevalence and risk of blood-borne and sexually transmitted viral infections in incarcerated youth in Salvador, Brazil: opportunity and obligation for intervention. *AIDS Behav* 12(4 Suppl): S17-24.
- Christensen PB, Krarup HB, Niesters HG, Norder H, Georgsen J (2000) Prevalence and incidence of bloodborne viral infections among Danish prisoners. *Eur J Epidemiol* 16: 1043-1049.
- Catalan-Soares BC, Almeida RT, Carneiro-Proietti AB (2000) Prevalence of HIV-1/2, HTLV-I/II, hepatitis B virus (HBV), hepatitis C virus (HCV), *Treponema pallidum* and *Trypanosoma cruzi* among prison inmates at Manhuaçu, Minas Gerais State, Brazil. *Rev Soc Bras Med Trop* 33: 27-30.
- Miura T, Sakuragi J, Kawamura M, Fukasawa M, Moriyama EN, Gojobori T, Ishikawa K, Mingle JA, Nettey VB, Akari H, Enami M, Tsujimoto H, Hayami M (1990) Establishment of a phylogenetic survey system for AIDS-related lentiviruses and demonstration of a new HIV-2 subgroup. *AIDS* 4: 1257-1261.
- Tonetto PA, Gonzales NSL, Fais VC, Vigani AG, Gonzales ESL, Feltrin A, Gonzales Jr. FL (2009) Hepatitis B virus: molecular genotypes and HBeAg serological status among HBV-infected patients in the southeast of Brazil. *BMC Infect Dis* 9: 149. DOI:10.1186/1471-2334-9-149.
- White PA, Zhai X, Carter I, Zhao Y, Rawlinson WD (2000) Simplified hepatitis C virus genotyping by heteroduplex mobility analysis. *J Clin Microbiol* 38: 477-482.



21. Zhang L, Chen Z, Cao Y, Yu J, Li G, Yu W, Yin N, Mei S, Li L, Balfe P, He T, Ba L, Zhang F, Lin HH, Yuen MF, Lai CL, Ho DD (2004) Molecular characterization of human immunodeficiency virus type I and hepatitis C virus in paid blood donors and injecting drug users in China. *J Virol* 78: 13591-13599.
22. Flodgren E, Bengtsson S, Knutsson M, Strebkova EA, Kidd AH, Alexeyev OA, Kidd-Ljunggren K (2000) Recent high incidence of fulminant hepatitis in Samara, Russia: Molecular analysis of prevailing hepatitis B and D virus strains. *J Clin Microbiol* 38: 3311-3316.
23. Morimoto HK, Morimoto AA, Reiche EMV, Ueda LT, Matsuo T, Reiche FL, Caterine-de-Araujo A (2007) Difficulties in the diagnosis of HTLV-2 infection in HIV/AIDS patients from Brazil: comparative performances of serologic and molecular assays, and detection of HTLV-2b subtype. *Rev Inst Med trop S Paulo* 49: 225-230.
24. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22: 4673-4680.
25. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetic Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24: 1596-1599.
26. Mathers BM, Degenhardt L, Phillips B, Wiessing L, Hickman M, Strathdee SA, Wodak A, Panda S, Tyndall M, Toufik A, Mattick RP (2008) Global epidemiology of injecting drug use and HIV among people who inject drugs: a systematic review. *Lancet* 372: 1733-1745.
27. Ministry of Health of Indonesia (2011) Report on HIV/AIDS, Jakarta.
28. Khan M, Dong JJ, Acharya SK, Dhagwahdorj Y, Abbas Z, Jafri SMW, Mulyono DH, Tozun N, Sarin SK (2004) Hepatology issues in Asia: Perspectives from regional leaders. *J Gastroenterol Hepatol* 19: S419-30.
29. Barry MA, Gleavy D, Herd K, Schwingl PJ, Werner BG (1990) Prevalence of markers for hepatitis B and hepatitis D in a municipal house of correction. *Am J Public Health* 80: 471-473.
30. Tucker RM, Gaffey MJ, Fisch MJ, Kaiser DL, Guerrant RL, Normansell DE (1987) Seroepidemiology of hepatitis D (delta agent) and hepatitis B among Virginia State prisoners. *Clin Ther* 9: 622-628.
31. Decker MD, Vaughn WK, Brodie JS, Hutcheson Jr. RH, Schaffner W (1984) Seroepidemiology of hepatitis B in Tennessee prisoners. *J Infect Dis* 150: 450-459.
32. Mulyanto, Depamede SN, Surayah K, Tsuda F, Ichiyama K, Takahashi M, Okamoto H (2009) A nationwide molecular epidemiological study on hepatitis B virus in Indonesia: identification of two novel subgenotypes, B8 and C7. *Arch Virol* 154: 1047-1059.
33. Mulyanto, Depamede SN, Surayah K, Tjahyono AA, Jirintai, Nagashima S, Takahashi M, Okamoto H. Identification and characterization of novel hepatitis B virus subgenotype C10 in Nusa Tenggara, Indonesia. *Arch Virol* 155: 705-715.
34. Utama A, Budiarto BR, Monasari D, Octavia TI, Chandra IS, Gani RA, Hasan I, Sanityoso A, Miskad UA, Yusuf I, Lesmana LA, Sulaiman A, Tai S. Hepatitis C virus genotype in blood donors and associated liver disease in Indonesia. *Intervirology* 51: 410-416.
35. Surya IG, Kornia K, Suwardewa TG, Mulyanto, Truda F, Mishiro S (2005) Serological markers of hepatitis B, C, and E viruses and human immunodeficiency virus type-1 infections in pregnant women in Bali, Indonesia. *J Med Virol* 75: 499-503.
36. Achwan WA, Muttaqin Z, Zakaria E, Depamede SA, Mulyanto, Sumoharjo S, Tsuda F, Takahashi K, Abe N, Mishiro S (2007) Epidemiology of hepatitis B, C, and E viruses and human immunodeficiency virus infections in Tahuna, Sangihe-Talaud Archipelago, Indonesia. *Intervirology* 50: 408-411.
37. Akbar N, Basuki B, Mulyanto, Garabrant DH, Sulaiman A, Noer HM (1997) Ethnicity, socioeconomic status, transfusions and risk of hepatitis B and hepatitis C infection. *J Gastroenterol Hepatol* 12: 752-757.
38. Handajani R, Soetjipto, Lusida MI, Suryohudoyo P, Adi P, Setiawan PB, Nidom CA, Soemarto R, Katayama Y, Fujii M, Hotta H (2000) Prevalence of GB virus C/Hepatitis G virus infection among various populations in Surabaya, Indonesia, and identification of novel groups of sequence variants. *J Clin Microbiol* 38: 662-668.
39. Soetjipto, Handajani R, Lusida MI, Darmadi S, Adi P, Soemarto, Ishido S, Katayama Y, Hotta H (1996) Differential prevalence of hepatitis C virus subtypes in healthy blood donors, patients on maintenance hemodialysis, and patients with hepatocellular carcinoma in Surabaya, Indonesia. *J Clin Microbiol* 34: 2875-2880.
40. Sulaiman HA, Julitasari Sie A, Rustam M, Melani W, Corwin A, Jennings GB (1995) Prevalence of hepatitis B and C viruses in healthy Indonesian blood donors. *Trans R Soc Trop Med Hyg* 89: 167-170.
41. Chanpong GF, Laras K, Sulaiman HA, Soeprapto W, Purnamawati S, Sukri N, Sie A, Tan R, Campbell JR, Corwin AL (2002) Hepatitis C among child transfusion and adult renal dialysis patients in Indonesia. *Am J Trop Med Hyg* 66: 317-320.
42. Timan IS, Aulia D, Atmakusma D, Sudoyo A, Windiastuti E, Kosasih A (2002) Some hematological problems in Indonesia. *Int J Hematol* 76: 286-290.
43. Reindollar RW (1999) Hepatitis C and the correctional population. *Am J Med* 107: 100S-103S.
44. Hotta H, Kemapunmanus M, Apichartpiyakul C, Soetjipto, Handajani R, Barzaga NG (1997) Differential distribution of hepatitis C virus subtypes in Asia: comparative study among Thailand, Indonesia, the Philippines and Japan. *Southeast Asian J Trop Med Public Health* 28: 23-31.
45. Inoue Y, Sulaiman HA, Matsubayashi K, Julitasari, Inuma, K, Ansari A, Laras K, Corwin AL (2000) Genotypic analysis of hepatitis C virus in blood donors in Indonesia. *Am J Trop Med Hyg* 62: 92-98.
46. Katayama Y, Barzaga NG, Alipio A, Soetjipto, Doi H, Ishido S, Hotta H (1996) Genotype analysis of hepatitis C virus among blood donors and inmates in Metro Manila, The Philippines. *Microbiol Immunol* 40: 525-529.
47. Tango Y, Gultom SP, Simanjuntak T, Sibuea WH, Matsuzaki H, Yamaguchi K (2000) Human T lymphotropic virus I in Indonesia. Very low seroprevalence in the Jakarta area: antibodies in healthy blood donors and in various nonhematological diseases. *Intervirology* 43: 77-99.
48. Takao S, Ishida T, Bhatia KK, Saha N, Soemantri A, Kayame OW (2000) Seroprevalence of human T-lymphotropic virus type 1 in Papua New Guinea and Papua measured using different western blot criteria. *J Clin Virol* 16: 129-133.

**Corresponding author**

Afiono Agung Prasetyo

Department of Microbiology Faculty of Medicine

Sebelas Maret University, Jl. Ir. Sutami no 36A

Surakarta, Indonesia

Telephone: 62-271-632489; Fax: 62-271-632489

Email: aficagp@yahoo.com; afie.agp.la@gmail.com

**Conflict of interests:** No conflict of interests is declared.