HIV-1 Incidence Estimates among Young Thai Men Using IgG-Capture BED-Enzyme Immunoassay (BED-CEIA) during 2005-2006

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Background: Knowledge about the most recent HIV epidemic among young generation in Thailand is crucial for improving the prevention programs. It is important to distinguish between recent and long-term HIV-1 infections among the sero-surveillance populations to estimate the HIV-1 incidence.

Objective: To obtain the HIV-1 incidence estimates in young Thai men from the HIV-1 sero-surveillance among the Royal Thai Army (RTA) conscripts inducted between November 2005 and November 2006.

Material and Method: The confirmed HIV-1 positive serum samples obtained from the November 2005, May 2006, and November 2006 rounds of RTA conscripts induction were selected to be included in the study. The recent HIV-1 infections were detected among the confirmed HIV-1 positive serum samples using an HIV-1 BED incidence EIA Kit (Calypte HIV-1 BED Incidence EIA, Calypte Biomedical Corporation, Maryland, USA. The incidence estimates were obtained in each round of the induction using a consensus formula was agreed upon at the US Centers for Disease Controls and Preventions (CDC).

Results: Eighty seven thousand one hundred seventy eight RTA conscripts were tested for HIV-1 infection between November 2005 and November 2006. The prevalence of HIV-1 infection was 0.51%, 0.60%, and 0.50% for the period of November 2005, May 2006, and November 2006, respectively. The HIV-1 incidence estimates were 0.14%/year (95% CI, 0.09-0.20), 0.20% year (95% CI, 0.13-0.28), and 0.17%/year (95% CI, 0.10-0.29) in November 2005, May 2006, and November 2006, respectively.

Conclusion: We reported the HIV-1 incidence estimates obtained from the IgG-capture BED-enzyme immunoassay (BED-CEIA) method in the RTA conscripts sero-surveillance population. The incidence estimates were ranging from 0.14% - 0.20%/year between November 2005 and November 2006. The estimates could serve as the recent baseline information for future HIV prevention interventions in Thailand.

Keywords: HIV-1, Immunoenzyme techniques, Immunoglobulin G, Incidence

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The course of HIV-1 epidemic in Thailand has been decreased dramatically since its peak in the early 1990s(1-3). Although the successful of the HIV-1 prevention and control program in Thailand has been demonstrated, the occurrence of new infections is still present in general populations. Knowledge about the most recent HIV epidemic in the young generation in Thailand is crucial for improving the prevention programs. In Thailand, several high-risk groups for HIV-1 infection has been evaluated for HIV-1 prevalence under the National HIV-1 Sero-Surveillance Program since 1989. One of the surveillance populations is the military conscripts. The surveillance has been carried out on approximately 60,000 21-year-old military conscripts of the Royal Thai Army (RTA) annually. It is believed to be the nationally representative sample of young Thai men. HIV-1 prevalence data has been
served as the a fundamental information for the HIV-1 epidemic patterns. National HIV/AIDS surveillance has been limited to monitor HIV prevalence and does not allow us to calculate or track HIV incidence. Therefore, it is important to distinguish between recent and long-term HIV-1 infections in the population. The more accurate measures, HIV-1 incidence estimates, are needed to explain the clearer recent picture of the epidemic dynamics and to demonstrate impact of HIV prevention programs over time. However, identification of recently infected persons (generally within 6 months of infection) and accurate estimation of incidence are difficult and have traditionally relied on the prospective testing and longitudinal follow-up of people at risk.

A number of methods have been proposed to estimate HIV incidence data from cross-sectional surveys. The methods included HIV-1 p24 antigen test and HIV-1 RNA testing, which has been used to identify recent HIV-1 infection. However, those methods require testing for all HIV-1 sero-negative specimens to identify the recent infections. In 2002, Parekh and colleagues described a new assay, the BED-CEIA (HIV-1 subtypes B, E, and D, IgG-Capture enzyme immunoassay), which was shown to have similar sensitivity to multiple HIV-1 subtypes. The objectives of this study were to use the BED-CEIA to detect recent HIV-1 infections among HIV-1-sero-positive Royal Thai Army RTA conscripts, and to estimate incidence during 2005-2006.

Material and Method
Recruitment of Thai men (mostly 21-year-olds) occurs in April every year. The total number of the RTA conscripts recruited is around 60,000 men annually. Placement takes place twice a year in May and November. Since November 1989, the Royal Thai Army Institute of Pathology (AIP) and the Armed Forces Institute of Medical Sciences (AFRIMS) in cooperation with 37 Royal Thai Army hospitals have conducted HIV screening among RTA conscripts to assess the prevalence of HIV infection in young Thai men. HIV screening was performed among ~30,000 newly inducted RTA conscripts bi-annually in May and November. HIV-1 positive test results were defined as positive samples for anti HIV-1 antibody test (enzyme-linked immunosorbent assay) and the positive sample was confirmed by Western Blot using licensed commercial reagents.

The confirmed HIV-1 positive serum samples obtained from the November 2005, May 2006, and November 2006 rounds were randomly selected to be included in the current study. The recent HIV-1 infections were detected among the confirmed HIV-1 positive serum samples using an HIV-1 BED Incidence EIA Kit (Calypte HIV-1 BED Incidence EIA, Calypte Biomedical Corporation, Maryland, USA). The Calypte HIV-1 BED Incidence EIA is an Ig-capture enzyme immunoassay. In this assay, the wells of a microplate were coated with goat anti-human IgG. When serum or plasma is added to the wells, anti-HIV-IgG and non-anti-HIV-IgG are captured on the goat-anti-human IgG coated wells. The relative amounts of anti-HIV-IgG and non-anti-HIV-IgG captured represent IgG antibody populations found in the serum. Indirectly, the test measures the proportion of HIV-1 specific IgG in a given specimen with respect to total IgG. Early seroconverters have a lower proportion of HIV-specific IgG in the serum than those with long-term infection. Previous studies have indicated that HIV-specific-IgG may continue to increase for more than 2 years after seroconversion when tested by this assay. By this test, an optimal normalized optical density (ODn = specimen-OD/Calibrator-OD) cutoff of 0.8 and a seroconversion period of 153 days offered the best combination of sensitivity and specificity for distinguishing between incident and long-term infections.

Statistical analysis
Minor variations of the formula for calculating incidence have been used in earlier studies. Recently, a consensus formula was agreed upon at the US Centers for Disease Controls and Preventions (CDC) for calculating incidence. Annual HIV-1 incidence is calculated using the following consensus formula:

\[ I = \frac{(365/w)N_{inc}}{N_{neg} + (365/w)N_{inc}/2} \times 100 \]

\( I \) = calculated incidence
\( w \) = window period
\( N_{inc} \) = number recent HIV infection
\( N_{neg} \) = number HIV seronegative

The total number of people tested, number seronegative, number seropositive, and number recently infected must be known for calculating incidence in a given cross-sectional population. Note that Calculated incidence does not differ significantly when different formulae are used. However, a consistent
approach is recommended for rational comparisons among populations and trend analysis.

The 95% confidence interval (CI) for the incidence estimate is:

\[
95\%CI = I +/- 1.96 \frac{I}{\sqrt{N_{inc}}}
\]

Where:
- \( I \) = calculated incidence
- \( \sqrt{N_{inc}} \) = square root of number of recent HIV infections

This new formula for calculating the 95% CI best fits the observed CI in statistical modeling and is dependent on the calculated incidence and the number found to be recently infected.

**Results**

Eighty seven thousand one hundred seventy eight RTA conscripts were tested for HIV-1 infection between November 2005 and November 2006. The prevalences of HIV-1 infection was 0.51%, 0.60%, and 0.50% for the period of November 2005, May 2006, and November 2006, respectively.

Of the 29,614 conscripts screened for HIV-1 in November 2005, 151 (0.51%) conscripts were sero-positive. Of the sero-positive specimens, 25 (16.6%) were classified as recent infections by reactivity on the BED-CEIA. Overall, estimated BED incidence among conscripts screened was 0.14% per year (95% confidence interval 0.09-0.20% per years).

Of the 29,858 conscripts screened for HIV-1 in May 2006, 161 (0.60%) conscripts were sero-positive. Of the sero-positive specimens, 29 (18.0%) were classified as recent infections by reactivity on the BED-CEIA. Overall, estimated BED incidence among conscripts screened was 0.20% per year (95% confidence interval 0.13 - 0.28% per years).

Of the 27,706 conscripts screened for HIV-1 in November 2007, 125 (0.50%) conscripts were sero-positive. Of the sero-positive specimens, 22 (17.6%) were classified as recent infections by reactivity on the BED-CEIA. Overall, estimated BED incidence among conscripts screened was 0.17% per year (95% confidence interval 0.10 - 0.29% per years).

**Discussion**

We reported for the first time the HIV-1 incidences among young Thai men using BED-CEIA methods. The HIV-1 incidences among these young Thai men were 0.14, 0.20, and 0.17% per year during November 2005, May 2006, and November 2006 rounds of conscript inductions, respectively. The HIV-1 incidence in this population of young Thai men has been studied a number of times, especially during the 1990s. The peak incidence of the HIV epidemic was demonstrated in 1991-1993. Nopkesorn et al., Celentano et al., and Carr et al. reported the HIV incidences among young Thai military conscripts from the upper northern provinces of 2.0, 2.5 and 3.2 per 100 person-years, respectively. After that peak of the epidemic, the decline of HIV-1 incidence rate had been observed. In 1993-1995, the incidence among this group had declined to 0.55 per 100 person-years. Later on, the incidence rate of the former military conscripts after discharge from the Royal Thai Army RTA in 1995-1999 was 0.31 per 100 person-years. The declining of the incidence among these groups reflected the situation on the general Thai populations, which was believed to be the success of national comprehensive multisectoral programs, especially 100% condom campaign.

There were many surveillance populations that used BED-CEIA including men who have sex with men (MSM) in Beijing, injecting drug users in Bangkok, those who attended out-patient clinic in Germany, and several high risk surveillance populations in Cambodia. The proportion of recent HIV-1 infection among the HIV-1 positive (16.6, 18.0, and 17.6% in November 2005, May 2006, and November 2006, respectively) from our study is comparable with the recent HIV-1 infection among injecting drug users in Bangkok in 1996, which was 19%. While the proportion of recent HIV-1 infection in MSM were

<table>
<thead>
<tr>
<th>Round of induction</th>
<th>No. conscripts</th>
<th>No. HIV-1 positives</th>
<th>Prevalence (%)</th>
<th>No. BED-CEIA [% per year, (95% CI)]</th>
<th>Estimated incidence recent infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov-05</td>
<td>29,614</td>
<td>151</td>
<td>0.51</td>
<td>29</td>
<td>0.14 (0.09-0.20)</td>
</tr>
<tr>
<td>May-06</td>
<td>29,858</td>
<td>161</td>
<td>0.60</td>
<td>29</td>
<td>0.20 (0.13-0.28)</td>
</tr>
<tr>
<td>Nov-06</td>
<td>27,706</td>
<td>125</td>
<td>0.50</td>
<td>22</td>
<td>0.17 (0.10-0.29)</td>
</tr>
</tbody>
</table>

Table 1. The HIV-1 incidence estimates and 95% CI of the RTA conscripts during 2005-2006
relatively higher, which was 41.2% in Beijing in 2005-2006 and 54% in MSM subpopulation who attended the out-patients clinic in Germany in 2005-2007. When compared with the Cambodia recent HIV-1 infection surveillance data in 1999-2002, we found that the young Thai men had lower incidence. The recent HIV-1 infection incidences among polices aged 20-29 years old polices in Cambodia were 2.0, 0.91, and 1.16 per 100 person-years, in 1999, 2000, and 2002, respectively.

The BED-CEIA can be used to estimate HIV-1 incidence and appropriate adjustments can be made in sentinel or population-based sero-surveys. Only specimens with confirmed HIV-1 infection should be tested with the BED-CEIA. Individuals with HIV-2 infection but without HIV-1 infection should be excluded from all studies to avoid the potential for even larger misclassification of results. Specimens should be collected, processed, and stored optimally and cold chain should be maintained if transported to another laboratory for testing. Strict quality control and quality assurance measures should be adapted to ensure valid and accurate results. When possible, serosurveys and studies should measure factors that may affect the interpretation of BED-CEIA results. These factors include long-term history of known HIV infection, symptoms of immunosuppression, and use of antiretroviral therapy (ARV).

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References
การประมาณค่าอุบัติการณ์ของการติดเชื้อ HIV-1 ในชายไทยวัยหนุ่ม ระหว่างปี พ.ศ. 2548-2549

คุณากร คณา, สุชชนา แทบประสิทธิ์, ทิพวรรณ ชื่นจิตร, ณรงคฤทธิ์ สิริโสภณา, รามรังสินธุ์

ภูมิหลัง: องค์ความรู้เกี่ยวกับลักษณะการระบาดของ HIV ในกลุ่มประชากรวัยรุ่นของประเทศไทยมีประโยชน์อย่างยิ่งในการพัฒนาการรณรงค์การป้องกันโรค การจำแนกการติดเชื้อว่าเป็นการติดเชื้อที่เพิ่งเกิดขึ้น จากการติดเชื้อในระหว่างการติดเชื้อ HIV-1 ในกลุ่มประชากรต่าง ๆ ทำให้สามารถประเมินการควบคุมการติดเชื้อจากข้อมูลดังกล่าวได้อย่างถูกต้อง

วัตถุประสงค์: เพื่อทำการประมาณการอุบัติการณ์ของการติดเชื้อ HIV-1 ในประชากรชายไทยวัยหนุ่มที่เข้ารับราชการเป็นทหารกองประจำการในช่วงระหว่างพฤศจิกายน พ.ศ. 2548 ถึงพฤศจิกายน พ.ศ. 2549

วิสัยทัศน์: เพื่อต่อยอดช่วงที่ได้จากการตรวจป้องกันการติดเชื้อ HIV-1 ของการติดเชื้อใน群体ในช่วงเวลาที่ทำการศึกษาทั้งหมด

วิธีการ: นำตัวอย่างซีรัมที่ได้รับการตรวจยืนยันการติดเชื้อ HIV-1 ทหารกองประจำการผลัดพฤศจิกายน พ.ศ. 2548, พฤศจิกายน พ.ศ. 2549 และพฤศจิกายน พ.ศ. 2549 มาทำการตรวจด้วยวิธี IgG-capture BED-enzyme immunoassay (BED-CEIA) การคำนวณค่าประมาณการอุบัติการณ์การติดเชื้อใช้สูตรการคำนวณมาตรฐานของ Centers for Disease Controls and Preventions (CDC) ประเทศสหรัฐอเมริกา

ผลการศึกษา: จากจำนวนทหารกองประจำการในช่วงเวลาที่ทำการศึกษาทั้งหมด 87,178 นาย พบว่ามีความชุกของการติดเชื้อ HIV-1 ทหารกองประจำการผลัดพฤศจิกายน พ.ศ. 2548, พฤศจิกายน พ.ศ. 2549 และพฤศจิกายน พ.ศ. 2549 เท่ากับ ร้อยละ 0.51, 0.60 และ 0.50 ตามลำดับ ค่าประมาณการของการอุบัติการณ์ของการติดเชื้อ HIV-1 เท่ากับ ร้อยละ 0.14 (95%CI, 0.09 - 0.20), 0.20 (95%CI, 0.13 - 0.28) และ 0.17 ต่อปี ในทหารกองประจำการผลัดพฤศจิกายน พ.ศ. 2548, พฤศจิกายน พ.ศ. 2549 และพฤศจิกายน พ.ศ. 2549 ตามลำดับ

สรุป: อุบัติการณ์ของการติดเชื้อ HIV-1 ในช่วงระยะเวลาของ 0.14 - 0.20 ต่อปีในช่วงระยะเวลาที่มีการศึกษา การติดเชื้อในช่วงระยะเวลาดังกล่าวต่อปีสามารถประเมินได้ด้วยการใช้เครื่องมือการวิเคราะห์ข้อมูลที่แม่นยำและมีประสิทธิภาพอย่างมีประสิทธิภาพในประเทศไทย