厚生労働科学研究費補助金（肝炎等克服政策研究事業）
令和４年度　研究報告書
全国規模の肝炎ウイルス感染状況の把握及びウイルス性肝炎 elimination に向けた
方策の確立に資する疫学研究

ベトナムにおける住民多段階無作為抽出調査(2012 年) 保存血清を用いた HAV 抗体・HEV 抗体保有状況把握等を目的とした疫学的研究（NIID 共同）

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研究要旨

【背景】本研究は，国立感染症研究所（NIID）からの共同研究の申し出により，ベトナムにおける A 型肝炎（HAV）および E 型肝炎（HEV）の血清疫学調査を行うものである。

厚労省肝炎疫学研究班では、広島一般住民を対象に HAV 抗体保有状況に関する血清疫学調査を実施し、若い世代の抗 HAV の有病率がほぼ 0%であることを報告した。また、同研究班ではカンボジア王国全国から無作為抽出された 5-7 歳児とその母を対象とした血清疫学調査を行い、HAV 抗体陽性率は 5-7 歳児で 31.5%、母親で 91.3%と報告した。また、同国シェムリアップ州の住民を対象とした調査では、HEV IgG 陽性率は 18.4%であった

一方、2012 年にベトナムのビントン省の無作為抽出された住民 509 名を対象とした血清疫学調査では、HBsAg および HCV 抗体陽性率はそれぞれ 15.8%および 3.3%と報告した。これらの成果から、NIID より、アジアの発展途上国における HAV および HEV 肝炎ウイルス感染状況の把握を目的とした共同研究の提案が NIID からあった。そこで本研究では HAV および HEV の抗体保有率、遺伝子型および分子特性を検討した。

【方法】2012 年にベトナム・ビントン省の多段階無作為抽出された一般住民 509 人を対象に実施した HBV および HCV 感染状況把握のための血清疫学調査で得られた保存血清（広島大学　田中 純子教授保有）を用いて実施した。本研究は広島大学疫学研究倫理審査委員会（E-2714）および NIID ヒトを対象とする医学研究倫理審査委員会（No. 1298）の承認を得ている。NIID で開発された ELISA 法を用いて総 HAV 抗体および HEV IgG 抗体を検出し、すべての陽性検体は、広島大学 において特異的なユニバーサルプライマーを用いたネステッドポリメラーゼ連鎖反応ベースの増幅により HAV RNA および HEV RNA を検出し、増幅を電気泳動で可視化した。

【結果】住民 509 人のうち、血清量が十分であった 504 検体を対象とした。総 HAV IgG 抗体陽性率は 93.8%，HEV IgG 抗体陽性率は 31.9%であった．30 歳以上の住民ではほぼ 100%の HAV 抗体陽性である一方、40 歳未満の住民では HEV 抗体陽性は 25%未満であった。HAV，HEV ともに，性別，年齢層，HBsAg，HCV 抗体と有意な関連は認められなかった。

【結論】ベトナムにおける HAV 抗体および HEV 抗体の陽性率が高く、ベトナムで将来起こりうるアウトブレイクを防ぐ集団免疫としての役割が期待される。一方で、食品と水の安全、衛生、個人衛生、および健康教育の改善が急務であると考えられた。

A. 研究目的

This study was proposed by National Institute of Infectious Disease (NIID) of Japan, to conduct the sero-prevalence study of hepatitis A (HAV) and hepatitis E (HEV) in Vietnam. The Hepatitis Epidemiology Research Group led by Prof. Junko TANAKA under the Ministry of Health, Labour, and Welfare of Japan had conducted the sero-prevalence study of HAV in Hiroshima, Japan and reported that the prevalence of anti-HAV among young generation is almost 0% (1). The research group conducted seroprevalence study of HEV among randomly selected 868 residents in Siem Reap, Cambodia in 2010-2014, the prevalence of anti-HEV IgG was 18.4% (2). The research group also conducted the sero-prevalence study of HAV among 2514 children of 5-7 years old and their 2021 mothers in the whole Cambodia in 2017 using multistage cluster random sampling strategy and reported that anti-HAV positivity was 31.5% in 5-7 years old children and 91.3% in their mothers (3).

Moreover, the research group had conducted sero-prevalence study of HBV and HCV among 509 residents in Binh Thuan province of Vietnam in 2012 and reported that the prevalence of HBsAg and anti-HCV was 15.8% and 3.3% respectively (4). Based on abovementioned facts, NIID supposed that Vietnam has higher HAV and HEV positivity than Japan. Therefore, NIID proposed to examine and analyze HAV and HEV prevalence in Vietnam to understand the infection or endemic status of viral hepatitis in developing countries in Asia. Therefore, our study aimed to estimate the prevalence of anti-HAV and anti-HEV and to examine the genotypes and molecular characteristics of HAV and HEV among general population in Binh Thuan Province, Vietnam.

B. 研究方法

This study is a continuum of previously conducted cross-sectional sero-epidemiological study on HBV and HCV infection among 509 general population resided in La Gi Town of Binh Thuan Province in the Southeast coast of Vietnam in 2012 (4, 5). The subjects were recruited by multistage cluster random sampling strategy (4).

In collaboration with National Institute of Infectious Disease (NIID) of Japan in 2021, Hepatitis Epidemiology Research group examined prevalence of anti-HAV and anti-HEV among 509 stocked samples from Binth Thuan province of Vietnam. Total anti-HAV and anti-HEV IgG were measured at NIID using inhouse developed ELISA method.

All anti-HAV and anti-HEV sero-positive samples were then detected for HAV RNA and HEV RNA respectively in our laboratory. HAV RNA is planned to detect using the nested polymerase chain reaction (nested PCR) with universal primers which can detect all genotypes of HAV(6). Similarly, HEV RNA is also planned to detect using the nested PCR with universal primers specific to all genotypes of HEV open reading frame ORF 1 and/or 2 (7). All detection will be performed by pool sample testing strategy (5 samples pooling). The HAV RNA or HEV RNA will be extracted by SMI-test Ex R&D (Medical and Biological Laboratories Co. Ltd, USA). The first round of nested PCR will be performed using prime script One Step enzyme mix and the second round nested PCR will be performed using Ex Taq Hot Start. The amplified products will be visually examined by gel electrophoresis. The same protocol will be applied for the detection of both HAV and HEV. This study was conducted with approval from Hiroshima University Epidemiological Research Ethic Committee (E-2714) and the National Institute of Infectious Disease (No. 1298) following the declaration of Helsinki.

C. 研究結果

A total of 504 stocked samples out of 509 samples were included in this study after excluding 5 samples for the insufficient sample volume. 45% of subjects were male and the age ranges from 20 to 81 years with mean age of 40.79± 12.79 years old (figure 1a-c).

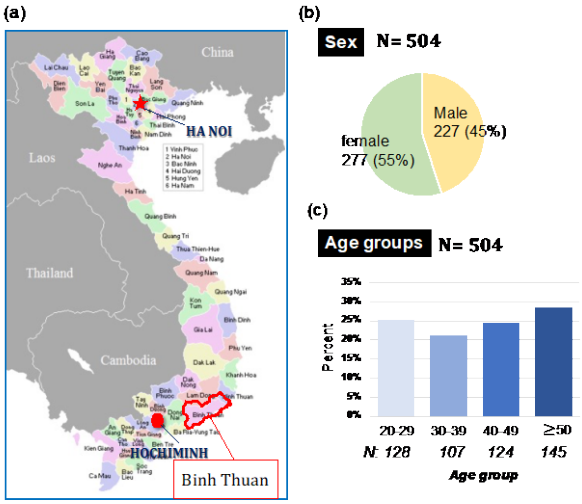

Figure 1: (a) Study area of Vietnam (b) sex distribution (c) age group distribution of study subjects (N= 504)

Total 477 samples were positive to total anti-HAV detected by in-housed ELISA. Thus, the overall prevalence of anti-HAV was 93.8% and there is no difference by sex (93.8% in male and 94.9% in female). The total anti-HAV prevalence was not significantly differed by age groups, but anti-HAV positivity was almost 100% in residents over 30s as shown in Figure 2a.

Total 162 samples were positive to anti-HEV IgG detected by in-housed ELISA. Thus, the overall prevalence of anti-HEV IgG was 31.9% and there is no significant difference by sex (32.6% in male and

31.4% in female). People under 40 years old, the prevalence of anti-HEV IgG was less than 25% whilst those over 40 years old was over 40% positive rate. (Figure 2b)

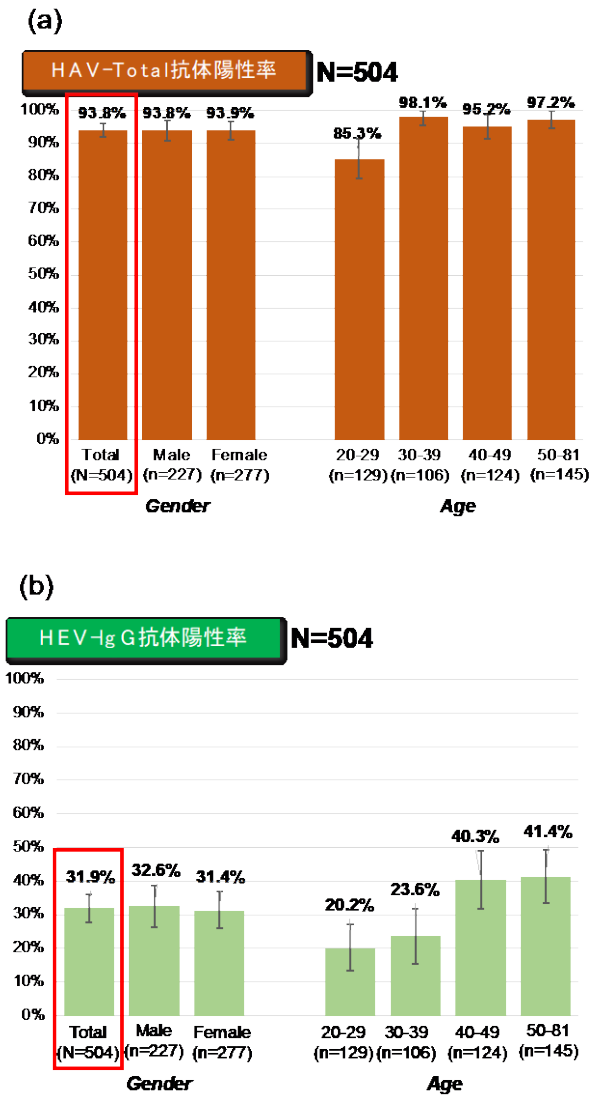

Figure 2: Overall, Sex and Age specific prevalence of (a) total anti-HAV (b) anti-HEV IgG among 504 residents in Binh Thuan Province of Vietnam

Grouping the subjects based on their HBsAg and anti-HCV positive status, there was no significant difference in anti-HAV and anti-HEV positivity among HBsAg positive and HBsAg negative status as well as anti-HCV positive and anti-HCV negative status (p>0.05). (Figure 3)

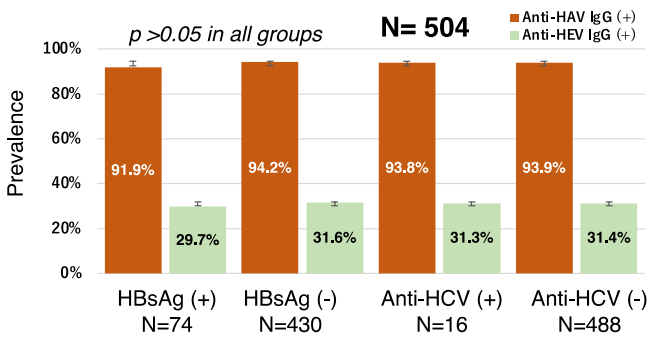

Figure 3: Prevalence of total anti-HAV and anti-HEV IgG stratified by HBsAg and anti-HCV positive status

Using 5 samples pooling to 162 anti-HEV IgG positive samples, no samples were positive for HEV RNA by mean of nested PCR based amplification (Figure 4). As for HAV RNA, the detection of HAV RNA by nested PCR based method is now proceeding in total 477 anti-HAV positive samples by pooling system.

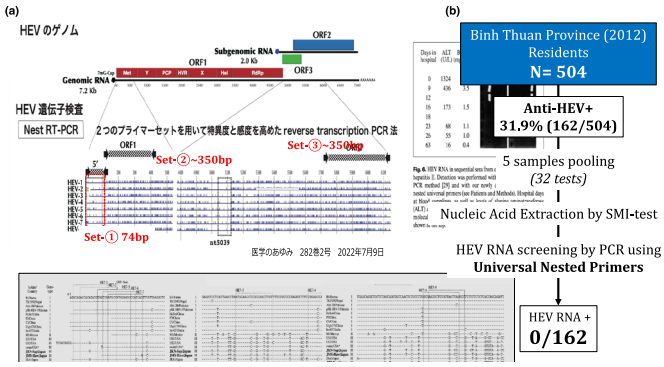

Figure 4: (a) Sketch diagram of universal primer sets used for HEV RNA amplification and (b) its result

D. 考察

Our study examined the total anti-HAV and anti-HEV IgG by means of in-housed developed ELISA method at NIID, Japan.

The high prevalence of both anti-HAV and anti-HEV was found in all age groups in both sexes in Vietnam. Almost 100% were tested positive to anti-HAV in their 30s and over. Anti-HEV prevalence was also high at 31.9%. One fourth of residents under less than 40s were tested positive to anti-HEV whilst nearly half of residents over 40s were tested positives.

Our study reported the high prevalence of anti-HAV and anti-HEV among general population which is consistent with the previous reports (8, 9). As Binh Thuan province is a rural part in Southeast coast of Vietnam, most of the residents were farmers or fisherman (4). The absence of HEV RNA indicated that all cases were past infection with the immunity to HEV. The existence of herd immunity might prevent from the episode of sporadic or large outbreak in Vietnam in the future. But it also indicated that the personal hygiene, sanitation, food, and water safety is

not adequate, and the health service and care are limited with insufficient infrastructure. Moreover, the literacy and poverty add on the high prevalence of both HAV and HEV infection.

E. 結論

The high prevalence of anti-HAV and anti-HEV in Vietnam had good advantage of herd immunity which prevent the potential sporadic or large outbreak in the future. Nevertheless, the improvement of food and water safety, sanitation, personal hygiene, and health education is urgently required in Vietnam.

F. 健康危険情報

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G. 研究発表

1. 論文発表

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2. 学会発表

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H. 知的財産権の出願・登録状況（予定を含む。）

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