【参考資料】カンボジア王国シェムリアップ州 1,565 人の妊婦を対象にした In-house 二重抗原サンドイッチ ELISA 法を用いた E 型肝炎ウイルス感染の疫学的評価

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※ 本稿では、厚生労働科学研究費補助金（肝炎等克服政策研究事業）以外で実施したウイルス肝炎の疫学研究を参考資料として記載する。

研究要旨

【背景】E 型肝炎は世界で、毎年推定で 2,000 万人が感染し、推定 330 万人が E 型肝炎を発症し、そして、56,600 人が E 型肝炎の関連で死亡している。その病原体である E 型肝炎ウイルス（HEV）の有病率は、途上国を中心に高く、カンボジアは HEV 高侵淫地域として知られているが、途上国で使用可能な安価・簡便な検査方法がなかったため、HEV 感染状況の実態は明らかにされていなかった。また、HEV 感染の致死率は通常 1〜2%であるが、妊婦は 20%と高いことが知られている。以上の背景から、本研究は、安価・簡便な ELISA 法による HEV 抗体測定法を開発し、カンボジア妊婦集団の HEV 抗体陽性率を評価することを目的とした。

【方法】In-house 二重抗原サンドイッチ ELISA 法による HEV 抗体検査法を開発した。主要なコーティング抗原としては、C 末端にマウス Fc タグが付加されたリコンビナント HEV カプシドタンパク質（ORF2）を使用した。二次抗原には、His タグ付きのリコンビナント HEV 抗原タンパク質を使用し、これをビオチンで標識して、多価ストレプトアビジン HRP を用いた化学発光検出システムを構築した。
HEV 抗体測定法を評価するため、カンボジアのシェムリアップ州で 2020 年に田中 純子研究室が実施した B 型肝炎ウイルス母子感染実態調査※で得られた保存血清検体 1,565 人のうち検体 ID に基づき無作為に選択された 262 検体を使用し In-house ELISA 法の評価を行った。
※2020 年 2 〜 9 月に同州の 3 医療機関で妊婦健診を受けた全妊婦のうち同意の得られた 1,565 人が調査の対象である。この調査では、5 項目の社会人口学的項目(出生年、民族、最終学歴、児の数、世帯主の職業)および 2 項目の既往歴情報項目(輸血歴、手術歴)のアンケート調査を同時に実施している。

2 種類の HEV IgG 測定商用キット（特殊免疫研究所の IgG anti-HEV EIA、および Mikrogen GmbH の recomLine HEV IgG/IgM Line immunoassay）を基準として、感度、特異性、ROC-AUC、一致率、コーエンの κ 係数を算出し、また費用を比較した。
次に、全 1,565 検体の HEV 抗体を本手法で測定し、HEV 抗体陽性とそれに関連する因子の多変量解析を実施した。

【結果】262 例を対象に In-house ELISA 法を評価した結果、本手法は、特殊免疫研究所のキットを基準とした時、感度 76%、特異度 94.1%、一致度 92.4%、κ =0.61 であった。Mikrogen の recomLine LIA を基準と

した時、感度 71.4%、特異度 98.6%、一致度 94.3%、κ=0.76 であった。ROC-AUC はいずれも 0.85 で、良好な識別力を示した。一方、1 回あたりの検査費用は本手法 133 円、Mikrogen 社製キット 4,333 円、特殊免疫研究所製キット 1,680 円と本手法は最も安価であった。次に、本手法を用いて全保存検体の HEV 抗体を評価したところ、全 1,565 人の妊婦における抗 HEV 抗体陽性率は 11.6%（181/1,565）であり、年齢が高い集団で高値を示す傾向を示した。
HEV 抗体陽性者の中で 22.7%（41 人）が IgM 抗体陽性であり、主に若年女性で最近または進行中の HEV 感染を示していた。一方、IgM 抗体陽性の検体からは HEV RNA は検出されなかった。多変量解析の結果から、高年齢、妊婦の世帯主職業が公務員である、が HEV 抗体陽性と関連があることが示され、生涯にわたる暴露によるリスク増加が示唆されている。
【結論】カンボジア王国シェムリアップ州の妊婦集団では、HEV 抗体陽性率が 11.6%であり、南アジアの妊婦集団の HEV 陽性率（Mirzaev and Tanaka et al. BMC Infect Dis. 24:525, 2024）よりは低いものの、HEV 抗体陽性者のうちの 22.7%が IgM 陽性であり、妊娠中の急性肝不全のリスクがあることを示した。本研究で開発した In-house ELISA 法は、既存の商用キットと同程度の診断精度を示し、安価であることから、途上国等の資源が限られた状況での HEV 検出や HEV 流行状況の把握に役立つと考えられた。

A. 研究目的

According to recently accepted meta-analysis from our department (Mirzaev U, Tanaka J et al., BMC Inf.Dis., accepted, 2024) the seroprevalence of Hepatitis E in Cambodia is 19.6%, which means that Cambodia is endemic for HEV. However, there is no information about the HEV seroprevalence among pregnant women, which are at risk of development of severe clinical course with serious outcome [1].
The principal goal of this study was to develop a new in-house ELISA method that is user-friendly, cost-effective, and less prone to errors by laboratory personnel. Because pregnant women are at an increased risk of experiencing severe HEV infections, especially in highly endemic areas including Cambodia, we then estimated the prevalence of HEV among this specific population

B. 研究方法

The in-house ELISA was designed for large-scale screening in resource-limited settings. Its performance was benchmarked against two commercial tests: the Anti-HEV IgG EIA (Institute of Immunology, Co. Ltd) and the Anti-HEV IgG RecomLine LIA (Mikrogen). This study builds upon a previous research project on the investigation of mother-to-child transmission of hepatitis B virus infection undertaken in Cambodia in 2020, which involved 1565 pregnant women from three hospitals in the Siem Reap region using a convenient sampling strategy [2]. The blood samples were collected from all participants and stored at −25°C for later analysis, and a well-structured questionnaire in the local Khmer language was used to gather sociodemographic information.
After accuracy assessment, we estimated the prevalence of total anti-HEV using the in-house ELISA across all 1565 participants. Next, we conducted the detecting IgM among those who tested positive for total anti-HEV immunoglobulins, using an anti-HEV IgM RecomLine LIA, Mikrogen GmbH, Germany. For the final phase, the positive anti-HEV IgM cases were tested by RT-PCR to confirm HEV RNA presence.
Additionally, in the study, we estimated an epidemiological trend of HEV transmission and association between HEV infection prevalence and various socio-demographic factors based on previously developed questionnaire (Figure 1).

C. 研究結果

The newly developed In-house ELISA showed a sensitivity of 76% and specificity of 94.1% against the Institute of Immunology kit, with a Cohen's kappa of 0.61. Against the RecomLine LIA by Mikrogen, it demonstrated a sensitivity of 71.4% and specificity of 98.6%, with a Cohen's kappa of 0.76 (Table 1). Both tests had an area under the curve (AUC) of 0.85, indicating good diagnostic accuracy (Figures 2 and 3).
The prevalence of total anti-HEV among 1565 pregnant women was found to be 11.6% (181/1565). The prevalence of anti-HEV IgM among 181 total anti-HEV was 22.7% (41 cases), indicating recent or ongoing infection.
The prevalence of total anti-HEV varied significantly across age groups, with higher rates observed in older women. Multivariate analysis revealed no significant association between total anti-HEV immunoglobulins positivity and socio-demographic factors such as education level, occupation, family size, or history of blood transfusion and surgical operations, except age and occupation of head of household as public officer.
The anti-HEV IgM presence was not associated with any of those factors.

HEV RNA was not detected in any of the 41 anti-HEV IgM positive samples, suggesting the absence of active viral replication among the participants.

D. 考察

The new in-house ELISA assay showed strong agreement with established commercial tests, achieving agreement scores of 0.76 and 0.61 with RecomLine, Mikrogen, Germany, and the Institute of Immunology, respectively. Despite variations in sensitivity and specificity among commercial systems and the absence of a universally accepted standard for HEV antibody detection, this assay is a promising tool for widespread screening in HEV-endemic, resource-limited settings [3, 4].

Research on stored sera from pregnant women indicated an 11.6% positivity for anti-HEV antibodies, with seroprevalence increasing with age, suggesting lifelong HEV exposure. Additionally, 22.7% of these positive cases had IgM antibodies, predominantly in younger women, indicating recent or ongoing infections.

Given the severe risks HEV poses during pregnancy, especially in the third trimester, our study underscores the urgency of HEV screening and preventive measures, such as maintaining hygiene and avoiding undercooked meat. The strengths of this study include its large sample size, the use of a novel ELISA method, and comprehensive analysis of HEV immunoglobulin prevalence.

E. 結論

Our study on the prevalence of Hepatitis E Virus (HEV) among pregnant women in Siem Reap, Cambodia, found that tested positive for total anti-HEV antibodies 11.6%, and 22.7% among last were IgM positives , highlighting the significant risk of severe outcomes such as acute liver failure during pregnancy. The newly developed in-house double antigen sandwich ELISA method proved to be an effective diagnostic tool, particularly suitable for use in areas with limited resources, and it aids in better understanding the epidemiological trends of HEV.

The absence of HEV RNA in samples positive for anti-HEV IgM could indicate either false positive results or transient viremia. The study also identified factors such as older age and occupation as a public officer as being linked to higher rates of HEV seropositivity, suggesting increased risk due to prolonged exposure.

These findings underscore the importance of implementing stronger preventive strategies, including enhanced hygiene practices and food safety measures.

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

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H. 知的財産権の出願・登録状況（予定を含む。）

なし

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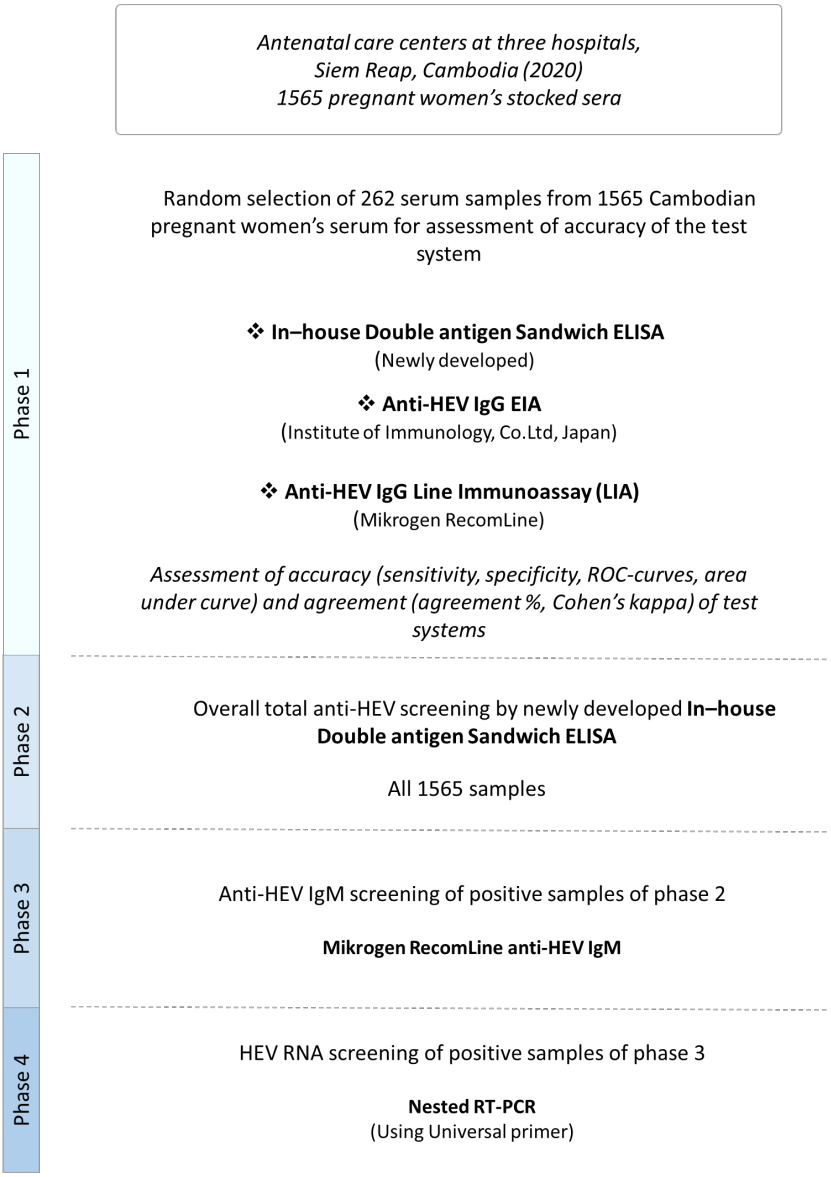

Figure 1. The outline and the phases of the study

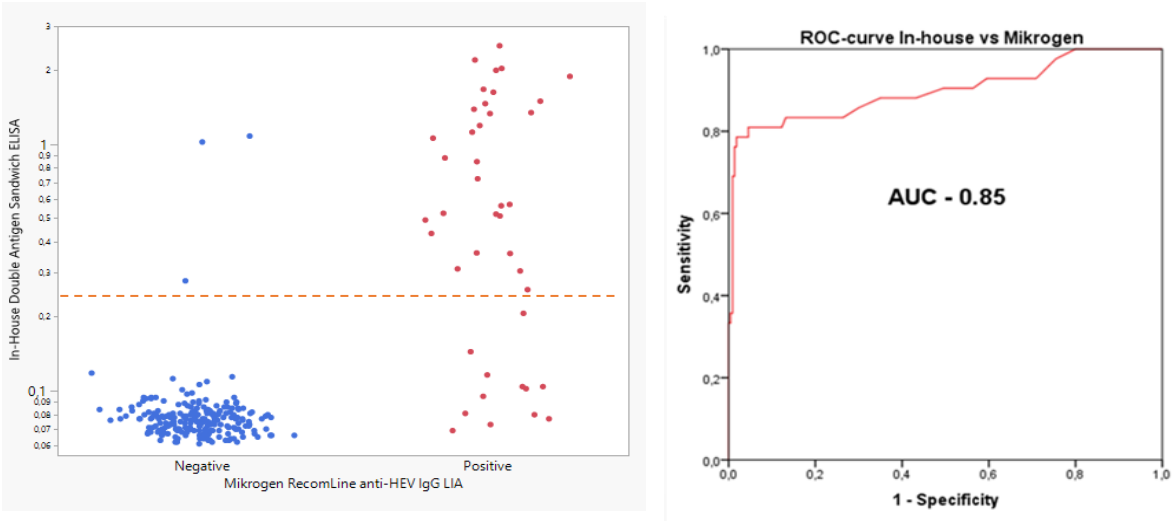

Figure 2. Comparison of commercial test system "RecomLine anti-HEV IgG", Mikrogen, Germany, and newly developed In-house Sandwich ELISA method

(Horizontal interrupted line – 0.24, OD cut-off value of In-house double antigen Sandwich ELISA; RecomLine anti-HEV IgM/IgG is line immunoassay (strips) is qualitative method, the positivity of the assay is measured by the number of lines appearance on the strip following the manufacturer's instructions).

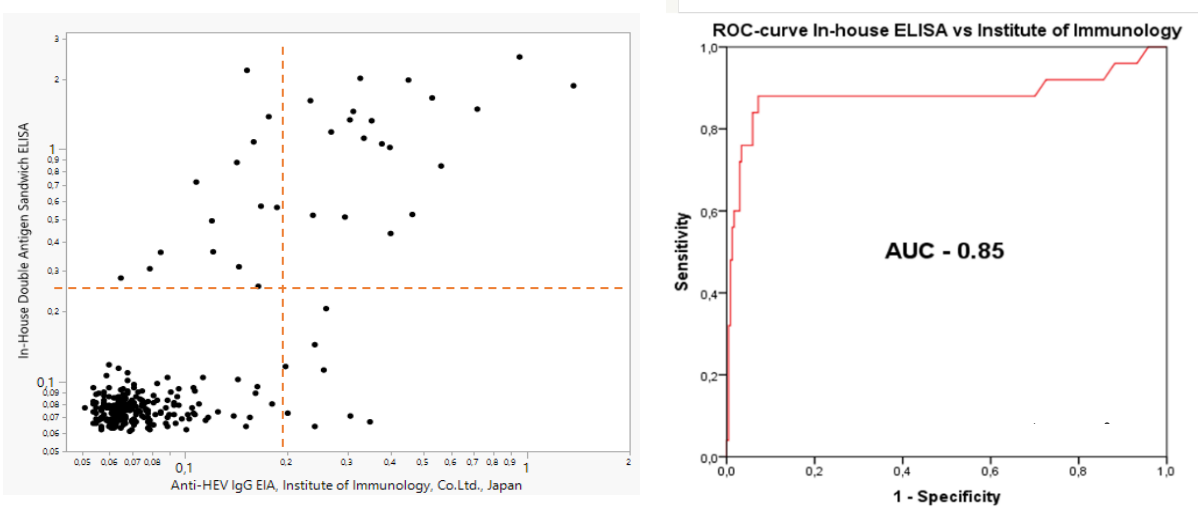

Figure 3. Comparison of commercial test system "anti-HEV IgG EIA", Institute of Immunology, Co. Ltd, Japan, and our newly developed In-house Sandwich ELISA method.
(Vertical red interrupted line – 0.198, OD cut-off value of Anti-HEV IgG EIA, Institute of Immunology, Co. Ltd, Japan; Horizontal interrupted line – 0.24, OD cut-off value of In-house double antigen Sandwich ELISA).

Table 1. Accuracy assessment of newly developed in-house double antigen sandwich enzyme-linked immunosorbent assay (ELISA) against two commercial test systems.

	Anti-HEV IgG EIA (Institute of Immunology)[a]		Anti-HEV IgG LIA (RecomLine; Mikrogen)[a]	
Test system	**Positive**	**Negative**	**Positive**	**Negative**
In-house double-antigen sandwich ELISA				
Positive	19	14	30	3
Negative	6	223	12	217
Total	25	237	42	220
Accuracy and agreement levels of the newly developed in-house double sandwich ELISA with each commercial test system as a reference method				
Sensitivity (%)	76.00		71.40	
Specificity (%)	94.10		98.60	
Agreement (%)	92.40		94.30	
Cohen's kappa	0.61		0.76	

Abbreviations: EIA, enzyme immunoassay; HEV, hepatitis E virus; IgG, immunoglobulin G; LIA, line immunoassay.

[a]The method was set as a reference ("gold standard") for the assessment of sensitivity and specificity.