HEPATITIS A VIRUS ANTIBODY PREVALENCE AND ENVIRONMENTAL SANITATION CONDITIONS AMONG AKHA-HILL TRIBE YOUTH, CHIANGRAI PROVINCE, THAILAND

SUPRANEE TONGPRADIT

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SUPRANEE TONGPRADIT: HEPATITIS A VIRUS ANTIBODY PREVALENCE AND ENVIRONMENTAL SANITATION CONDITIONS AMONG AKHA-HILL TRIBE YOUTH, CHIANGRAI PROVINCE, THAILAND. THESIS ADVISORS: PIPAT LUKSAMIJARULKUL, M.Sc. PISIT VATANASOMBOON, M.Sc. FUANGFA UTRARACHKIJ, M.Sc. 111 P. ISBN 974-664-045-3

Among the hill-tribe people in the rural northern part of Thailand, reliable data of hepatitis A virus (HAV) infection, one of the food and water-borne diseases, are not known. A cross-sectional analytic study was conducted for determining HAV antibody prevalence and assessing the environmental sanitation conditions among Akha-hill tribe youth at Ban-Doi-Chang, Amphoe Mae-Saui, Chiangrai Province, Thailand, between July and December 1999. Serum specimens, obtained from 190 Akha-hill tribe youth, were tested for antibody to HAV (anti-HAV) by an ELISA. The environmental sanitation conditions and the hygienic behaviors of the studied households were obtained by observation and interview. The household's drinking water was screened for coliform contamination by using SI-2 media. The results showed very high rate of HAV antibody prevalence among the Akha-hill tribe youth (87.89%). Most studied households had unsanitary environmental sanitation conditions and poor personal hygiene and child health care behaviors. The coliform contamination in studied households' drinking water was 73.53% (125/170). From the anti-HAV results, the studied Akha-hill tribe youth were divided into 2 groups: individuals with and without anti-HAV. The studied variables of 2 groups were analyzed to identify some factors associated with anti-HAV positivity. It was found that monthly income and numbers of household members were associated with anti-HAV positivity ($p=0.040$ and 0.007, respectively). Personal hygiene factors, including not washing hand with soap after using the toilet ($p=0.013$), and environmental sanitation conditions, including use of latrine ($p=0.008$), latrine emptying method ($p=0.003$), household refuse management ($p<0.001$), and control of insects and rodents ($p<0.001$), were also associated with anti-HAV positivity. Based on these findings, improving the socio-economic situation, personal hygiene, and environmental sanitation management should be done to reduce hepatitis A infection in Akha-hill tribe people.

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สุปราณี ทองประดิษฐ์: ความชุกของแอนติบอดีต่อไวรัสตับอักเสบชนิดเอและสภาวะทางสุขาภิบาลสิ่งแวดล้อมในเยาวชนชาวเขาเผ่าอาข่า จังหวัดเชียงราย ประเทศไทย (HEPATITIS A VIRUS ANTIBODY PREVALENCE AND ENVIRONMENTAL SANITATION CONDITIONS AMONG AKHA-HILL TRIBE YOUTH, CHIANGRAI PROVINCE, THAILAND) คณะกรรมการควบคุมวิทยานิพนธ์: พิพัฒน์ ลักษมีจรัลกุล, วท.ม., พิศิษฐ์ วัฒนสมบูรณ์, วท.ม., เฟื่องฟ้า อุตตรารัชต์กิจ, วท.ม. 111 หน้า ISBN 974-664-045-3

ชาวเขาในชนบททางภาคเหนือของประเทศไทย ที่ซึ่งยังไม่มีข้อมูลที่น่าเชื่อถือของการติดเชื้อไวรัสตับอักเสบชนิดเอ หนึ่งในโรคติดเชื้อที่มีอาหารและน้ำเป็นสื่อ การศึกษาภาคตัดขวางเชิงวิเคราะห์นี้จัดทำขึ้นเพื่อหาความชุกของแอนติบอดีต่อไวรัสตับอักเสบชนิดเอและประเมินสภาวะทางสุขาภิบาลสิ่งแวดล้อมของเยาวชนชาวเขาเผ่าอาข่าบ้านดอยช้าง อำเภอแม่สรวย จังหวัดเชียงราย ในระหว่างเดือนกรกฎาคมถึงธันวาคม พุทธศักราช 2542 เก็บตัวอย่างซีรั่มจากเยาวชนชาวเขาเผ่าอาข่า จำนวน 190 ราย ทำการตรวจหาแอนติบอดีต่อไวรัสตับอักเสบชนิดเอด้วยวิธีอีไลซ่า สังเกตและสัมภาษณ์สภาวะทางสุขาภิบาลสิ่งแวดล้อม พฤติกรรมอนามัยส่วนบุคคลและตรวจกรองการปนเปื้อนโคลิฟอร์มในตัวอย่างน้ำดื่มของครัวเรือนตัวอย่างด้วยอาหารเลี้ยงเชื้อ SI-2 ผลการศึกษาพบอัตราความชุกของแอนติบอดีต่อเชื้อไวรัสตับอักเสบชนิดเอสูงร้อยละ 87.89 ส่วนใหญ่มีสภาวะทางสุขาภิบาลสิ่งแวดล้อมไม่ถูกสุขลักษณะ พฤติกรรมอนามัยส่วนบุคคลและพฤติกรรมอนามัยการเลี้ยงดูเด็กที่ไม่ดี อัตราการปนเปื้อนของโคลิฟอร์มในตัวอย่างน้ำดื่มร้อยละ 73.53 (125/170) จากผลการตรวจแอนติบอดีต่อไวรัสตับอักเสบชนิดเอ แบ่งกลุ่มตัวอย่างออกเป็นกลุ่มที่มีและไม่มีแอนติบอดี นำตัวแปรในการศึกษามาวิเคราะห์หาปัจจัยที่มีความสัมพันธ์กับการมีแอนติบอดี พบว่าปัจจัยที่มีความสัมพันธ์กับการมีแอนติบอดีคือ ปัจจัยทางเศรษฐสถานะทางสังคมได้แก่ รายได้และจำนวนสมาชิกในครัวเรือน ($p=0.040$ และ 0.007 ตามลำดับ) ปัจจัยด้านอนามัยส่วนบุคคลได้แก่การไม่ล้างมือด้วยสบู่หลังการขับถ่ายอุจจาระ ($p=0.013$) และปัจจัยทางสภาวะสุขาภิบาลสิ่งแวดล้อมได้แก่ การใช้ส้วม ($p=0.008$) การจัดการเมื่อส้วมเต็ม ($p=0.003$) การกำจัดขยะในครัวเรือน ($p<0.001$) และการควบคุมแมลงพาหะนำโรคและหนู ($p<0.001$) ดังนั้นการปรับปรุงระดับเศรษฐสถานะทางสังคม อนามัยส่วนบุคคล และการจัดการด้านสภาวะทางสุขาภิบาลสิ่งแวดล้อม ควรกระทำเพื่อลดการติดเชื้อไวรัสตับอักเสบชนิดเอในกลุ่มชาวเขานี้

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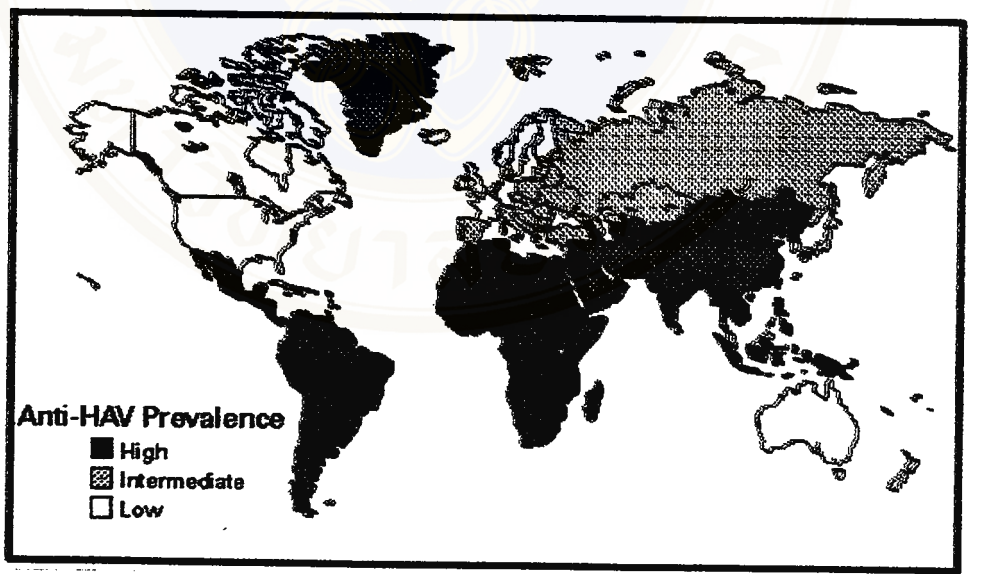
CHAPTER I

INTRODUCTION

1.1 Rationale and Justification

Hepatitis A is an acute, necro-inflammatory infection of the liver caused by the hepatitis A virus (HAV). The virus classified within the genus hepatovirus of the picornavirus family has a worldwide distribution. Hepatitis A infection has been one of public health problems, it is a significant cause of morbidity and attendant economic losses in many parts of the world.



*Low, intermediate, and high.
†This map generalizes available data, and patterns may vary within countries.

Figure 1 Endemicity patterns* of hepatitis A virus infection worldwide†

From: MMWR 1996; 45(RR15): 1-30.

World Health Organization estimated mortality rate of viral hepatitis in 1998 about 0.2% for both sexes (1). Fulminant hepatitis A leads to about 100 deaths per year in the U.S. The case-fatality rate among reported cases of all ages is approximately 0.3% (2). Hepatitis A results in substantial morbidity with associated costs caused by medical care and work loss. Hospitalization rates for hepatitis A are 11%-22%. Adults who become ill lose an average of 27 work-days per illness and health departments incur the costs of postexposure prophylaxis for 11 contacts per case. Average direct and indirect costs of hepatitis A range from $1,817 to $2,459 per adult case and $433 to $1,492 per pediatric case. In 1989, the estimated annual total cost of hepatitis A was more than $200 million in the United States alone (3).

HAV infection is transmitted from person to person by the fecal-oral route or ingestion of contaminated food or water. Improvements in public hygiene and higher socio-economic level are associated with declining prevalence of this infection. In communities that have intermediate rates of hepatitis A, the disease occurs among children, adolescents, and young adults, in contrast to communities that have high rates of hepatitis A, in which the majority of cases occur among children less than 15 years of age (2). In Thailand, hepatitis A infection caused 69% of acute viral hepatitis in children aged less than 15 years (4,5). In the previous two decades, a large amount of Thai children were seropositive for anti-HAV (6). For the last ten years, the socio-economic situation and sanitary condition have been much improved as a newly industrialized country. The seroprevalence of hepatitis A virus infection has changed from high endemic into intermediate endemic status (7,8). Increasingly susceptible adolescents and young adults contribute to sporadic symptomatic hepatitis A disease, particularly in high school children, communities and among factory workers. In

young children, hepatitis A is usually asymptomatic in 75% of cases. Malaise, nausea, fever, and diarrhea occur in approximately 50% of the cases; joint pain, abdominal pain, or vomiting occurs in 20% of the cases and jaundice in 10% (9). Presumably many more children have unrecognized asymptomatic infection and can be a source of infection for others (10). The true incidence and prevalence of hepatitis A infection are still unknown because most infections are generally subclinical, particularly in younger people, and many cases are not reported. The more reliable figures for HAV infection can only be achieved from seroepidemiological surveys.

Most of the Akha-hill tribes are found in Chiangrai Province and their culture of wearing silver helmet and wearing suit are attractive the travellers to come to their community. It is known that their personal hygiene and environmental sanitation are poor. The travellers from low endemicity areas are at risk for acquiring hepatitis A (11). This study attempted to determine the hepatitis A antibody prevalence among youth of the Akha-hill tribes, Chiangrai Province, and to assess the environmental sanitation conditions as well as personal hygiene associated with the infection among them. The results are valuable for preventing and controlling the hepatitis A virus infection and for improving their personal hygiene and environmental sanitation in the studied community.

1.2 Objectives

General Objective

To study prevalence rate of hepatitis A virus antibody (anti-HAV) and the environmental sanitation conditions associated with the infection among youth of

Akha-hill tribe at Amphoe Mae-Suai, Chiangrai Province, Thailand.

Specific Objectives

1. To describe general characteristics of studied Akha-hill tribe community at Amphoe Mae-Suai, Chiangrai Province, Thailand, emphasizing the environmental sanitation conditions.

2. To determine anti-HAV prevalence rate in youth of Akha-hill tribe at Amphoe Mae-Suai, Chiangrai Province, Thailand.

3. To analyze the socio-economic factors, environmental sanitation conditions, and personal hygienes and child health care behavior factors associated with anti-HAV positivity in youth of Akha-hill tribe at Amphoe Mae-Suai, Chiangrai Province, Thailand.

1.3 Research Hypotheses

1. Some factors including low socio-economic status, poor personal hygienes and poor child health care behaviors were associated with anti-HAV positivity of the youth of Akha-hill tribe, Ban-Doi-Chang, Amphoe Mae-Suai, Chiangrai Province, Thailand.

2. Some conditions of environmental sanitation were associated with anti-HAV positivity of the youth Akha-hill tribe, Ban-Doi-Chang, Amphoe Mae-Suai, Chiangrai Province, Thailand.

1.4 Scope of Research

Between July and September 1999, 190 participants from youth of Akha-hill tribe at Amphoe Mae-Suai, Chiangrai Province, Thailand, both genders were interviewed by using the structured questionnaires including socio-economic factors, environmental and sanitary conditions, personal hygiene and child care behavior factors. Blood specimens were collected from the studied subjects for detecting anti-HAV and studied households' drinking water samples were collected for screening coliform bacteria. From the laboratory results, the studied subjects were divided into two groups, the first was anti-HAV positive and the second was anti-HAV negative. The variables of these two groups were compared and analyzed for determining some factors associated with anti-HAV positivity.

1.5 Definition of Terms

1. **Studied subjects:**

The youth Akha-hill tribe subjects' age ranged from 15 to 24 years old. Youth has been defined by the World Health Organization as being between the ages of 15 and 24 years (12).

2. **Studied household:**

The house or residence that the studied subjects lived in.

3. **Anti-HAV positive person:**

A person who has anti-HAV positive detected by ELISA, excluded a person who has anti-HAV positive by immunization.

4. Anti-HAV prevalence rate:

$$= \frac{\text{Number of subjects with anti-HAV}}{\text{Number of all studied subjects in this study}} \times 100$$

5. Studied factors:

The factors included:

5.1 Socio-economic factors

The factors included subjects' age, gender, marital status, education, and income.

5.2 Environmental sanitation conditions

These factors included numbers of family members, conditions of household environment and level of sanitation of the subject as well as disposal of human excreta, wastewater disposal, household refuse disposal, domestic water supply, housing, food sanitation, vectors and rodents control.

5.3 personal hygiene and child health care behavior factors

These factors were composed of the subject's hygiene which included hand-washing after using toilet, hand-washing before preparing food, eating or drinking something contaminated by feces of an infected person and child health care behaviors (especially subjects who had babies).

6. Drinking water:

The water that consumed by the people in family, it was stored in a jar, a bottle or a pot.

1.6 Variables of the Study

Independent Variables

The independent variables of the study were included:

1. Socio-economic factors as well as:
   - Age
   - Gender
   - Marital status
   - Education
   - Income
2. Environmental sanitation conditions as well as:
   - Conditions of household environment and level of sanitation including:
     - excreta disposal
     - wastewater disposal
     - household refuse disposal
     - domestic water supply
     - housing
     - food sanitation
     - vectors and rodent control
   - Numbers of household members
3. Personal hygienes and child health care behaviors
   - hand-washing

- Eating or drinking something contaminated by feces of an infected person.

Dependent Variable

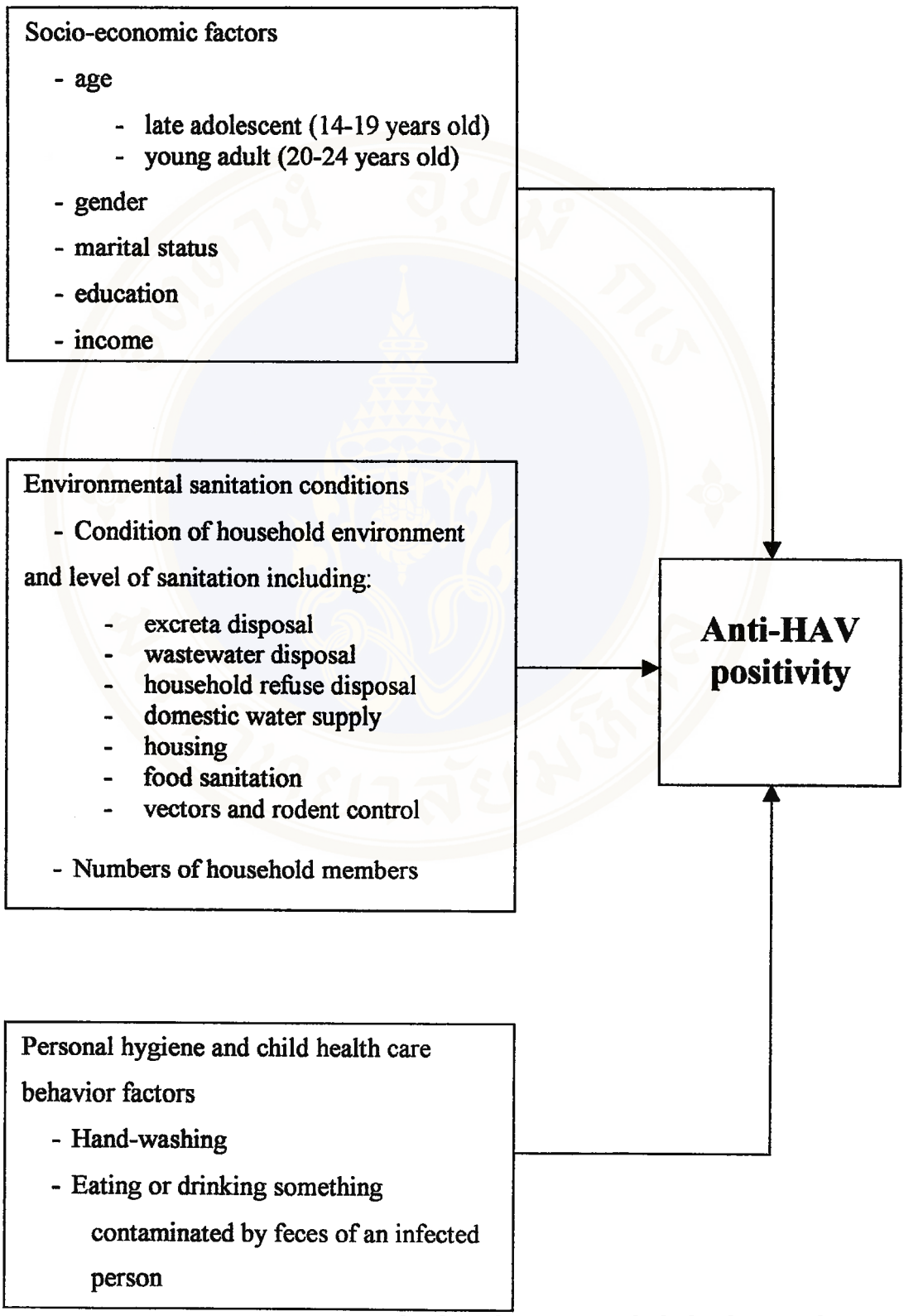
The dependent variable of the study was anti-HAV positivity.

1.7 Limitation

1. The difference between languages from interviewing through the translator may cause the missing data and some data translation bias may occur.

2. The coliform bacteria screening in food was not done because most HAV outbreaks in Thailand were associated with drinking water (13, 14) and the limited budget of the study.

1.8 Conceptual Framework



CHAPTER II

LITERATURE REVIEW

2.1 Akha-Hill Tribe

The smallest populations of hill tribe peoples - about ten times less than the Karen - the Akha have in some ways coming to symbolize the hill tribes in the popular imagination. One of the main reasons for this is the spectacular appearance of Akha girls and women, with their heavily ornamented peaked headdress and mini – skirt. Made from materials as diverse as beaten silver, gibbon fur, beads, seeds and feathered tassels, the Akha headdress appears like a helmet from some elaborate mythology, and is undoubtedly the crowning glory of hill tribes finery.

The current 'homeland' of the Akha is Yunnan, particularly in the region of Xishuangbanna, but they have migrated over the centuries into eastern Burma and Laos. Most of the Thai Akha have arrived from the Shan States, beginning at the turn of century. The Thai name for them is quite derogatory: 'Eekaw', which broadly means 'low slave'. Not surprisingly, the Akha themselves resent this term. Most of Thailand's Akha are to be found in Chiangrai Province, the north of the Mae-Kok River. There were about 56,616 Akha persons in Thailand, 47,207 persons lived in Chiangrai Province and 13,408 persons lived in Amphoe Mae-Suai, Chiangrai Province (15).

Although there are many more in Burma and in Yunnan, three distinctive Akha sub-groups are found in Thailand, identifiable mainly by the women's

headdress. The most numerous are the U Lo-Akha, who wear the conical headdress and are the longest settled in the country. The second most common are the Loimi-Akha, named after a mountain in the Shan States ('Bear Mountain'); their style is characterized by a tapering flat plate of silver projection upwards at the back, and by quantities of hollow silver balls. The third group is naved after their principal settlement – Phami-Akha near Mae Sai in the far north of Chiangrai Province – and wear a heavy, silver-covered helmet that is flat at the back with side-panes of overlapping silver coins (16).

2.2 History of Hepatitis A Virus

Infectious jaundice is a disease described by Hippocrates (17). In previous centuries it was frequently epidemic during periods of war. Most of these cases can be assumed to have been hepatitis A infection. Large epidemics were noted during World Wars I and II and the Korean and Vietnamese conflicts. These epidemics stimulated extensive research on the epidemiology, clinical features, and pathology of hepatitis virus in Great Britain, United States, Germany, and West Africa. In 1972 MacCallum had summarized several results from these studies, that one type of hepatitis, called "serum hepatitis," had a long incubation period of 60 to 160 days and was parenterally transmitted by blood. This was clearly distinguished from so-called "infectious hepatitis" which manifested a shorter incubation period and was transmitted orally by infectious stool (18). By recommendation of the World Health Organization (WHO), serum hepatitis was later renamed hepatitis B, and infectious hepatitis renamed hepatitis A (19).

The characteristics of hepatitis A that could be confirmed in later studies were: incubation period between 15 and 40 days, presence of virus in feces and blood, but not in nasopharyngeal washings; oral and parenteral mode of infection; homologous immunity after infection; protection by prophylactic administration of normal immune serum globulin (20 - 22).

Further studies in the 1960s in an institution for mentally retarded children led to the isolation of the first internationally used reference strain (MS-1) of HAV (23). At about the same time marmoset monkeys were found to be a suitable experimental animal for HAV infection (24). However, HAV was not visualized until 1973, when Feinstone, Kapikian, and Purcell detected the virus by immune electron microscopy particles approximately 27 nm in diameter in acute phase stool specimens from two of four adult volunteers infected with the MS-1 strain (25). This led to the development of sensitive methods to determine hepatitis A antigen (HAAg) and anti-HAV. Extensive seroepidemiologic studies were performed in the late 1970s and culminated in the first International Workshop on Hepatitis A Virus Infection held in November 1980 in Athens. Biochemical and biophysical properties of HAV were studied more closely and the virus was classified as a picornavirus (26).

In 1979 two groups independently, Frösner G, et al. (27) and Provost PJ, et al. (28), succeeded in cultivation HAV in different cell culture systems. They demonstrated that infection could be transmitted to marmosets and chimpanzees led to a new era of research on hepatitis which culminated in the propagation in cell culture, molecular cloning and sequencing of the viral genome, and the development of effective vaccines.

2.3 Structure and Biology of the Agent

Hepatitis A virus (HAV), member of the *Picornaviridae* family, had originally been classified as a picornavirus genus, *Enterovirus* type 72 (2). Biochemical, biophysical and molecular biological differences indicate that it should be in its own genus within the Picornaviridae family, which has now been named *Heparnavirus*, containing only one species member, hepatitis A virus (29).

HAV is spherical, RNA-containing particle 27 to 28 nm in diameter without an envelope (Figure 2) having a sedimentation coefficient of 156 to 160S and a density of 1.33 to 1.34 g/mL in cesium chloride (30 - 32). The biologic and physical characteristics of HAV were summarized in the report of the International Workshop on Hepatitis A virus Infection shown in Table 1. The capsid has cubical symmetry and consists of 32 capsomers. In addition to the mature virion, dense particles (1.38-1.44 g/mL) and immature particles (1.32-1.33 g/mL) can be purified from fecal material of infected man and chimpanzee and from tissue culture-grown viral suspension.

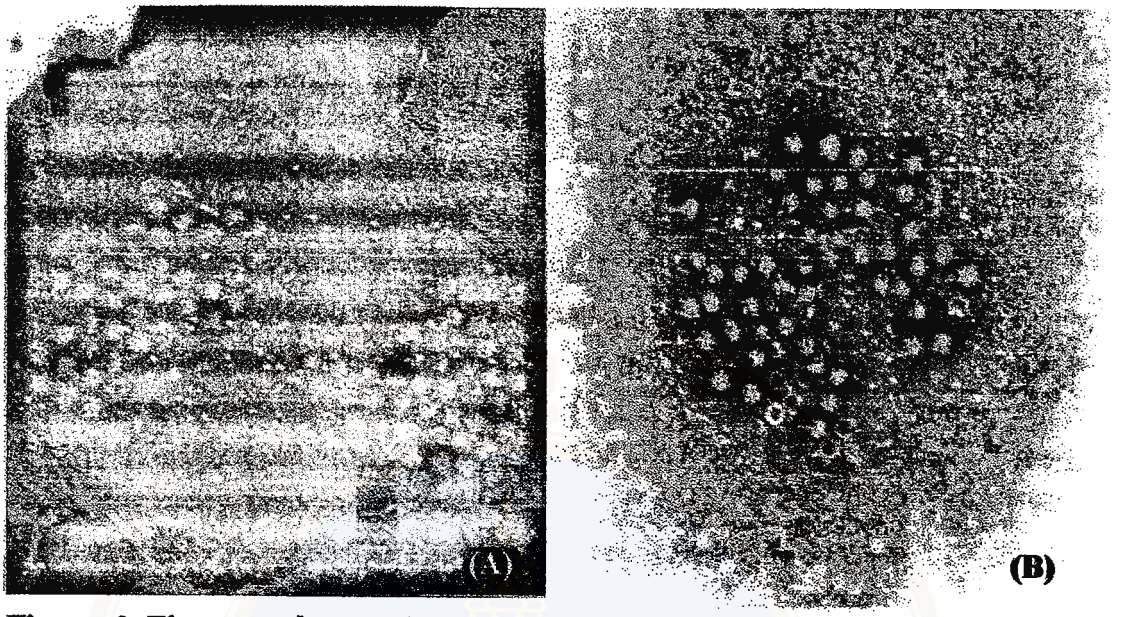


Figure 2 Electron micrographs of (A) hepatitis A virus particles aggregated by antibody, 27-28 nm in diameter, and (B) highly concentrated, purified hepatitis A virus from human feces.

From: Battergay M, Feinstone S. Hepatitis A virus, In: Willson R. editor. Viral hepatitis. New York: Mercel Dekker; 1997. p.37.

The coding region of picornaviruses has been arbitrarily divided into three parts. The three regions are termed P1, P2, and P3, and the peptide that are ultimately cleaved from the translation products of these regions are referred to as 1A, 1B, etc., and 2A, 2B, etc., in order of translation from the 5' to the 3' end of the genome (33). The mature virion contains 60 copies each of four structural polypeptides (VP_{1-4}). These structure polypeptides are coded within P1 region and are referred to as virion proteins (VP), VP1 = peptide 1D, VP2 = 1B, VP3 = 1C and VP4 = 1A, each has a molecular weight between 2,500 and 33,200 daltons (34). Immature particles additionally exhibit a precursor protein (VP_0 that is cleaved to VP_2 and VP_4 during maturation. Measurement of the relative susceptibility of the protein of the virion to

iodination suggests that VP_2 and VP_4 appear to be located inside the particle (35). In contrast with other picornaviruses, there is a protein consisting of VP_1 and 2A region of VP_2 in the mature particle (36).

HAV possess a single-stranded linear RNA genome of 7478 nucleotides with a sedimentation coefficient of 32.5S under nondenaturating conditions. Like the genome of other picornaviruses, it can be functionally divided into 5′ -terminal untranslated region composed of 734 nucleotides, a single open reading frame of 6681 nucleotides, and a short untranslated segment of 63 nucleotides at the 3′ end (Figure3). The molecular weight of the genome was calculated to be 2.25×10^6 on the basis of sedimentation under nondenaturating conditions and 2.8×10^6 from the electrophoretic mobility of completely denatured molecules.

Table 1 Properties of Hepatitis A virus

Virion			
Naked, spherical particles; capsid probably consisting of 32 capsomers arranged according to the symmetric requirements of a rhombic triacontahedron.			
Diameter		27-28 nm	
Sedimentation coefficient		156-160S	
Density in CsCl (g/mL)	Dense-particle	Mature-virion	Immature-particles★†
	1.38-1.44	1.33-1.34	1.32-1.33
Proteins (daltons) †			
VP0		precursor to VP_2 and VP_4	
VP1		33,200	
VP2		24,800	
VP3		27,800	
VP4		2,500 (17 amino acids)	
Nucleic acid			
Type		RNA	
Configuration		Single-stranded, linear	
Sedimentation coefficient ‡		32.5S	
Mol wt (daltons)		2.25×10^6 § – 2.8×10^6 §§	
Number of nucleotides		8,000-8,100	
Polyadenylic acid		40-80 nucleotides	
Polarity		Positive	
Translation strategy		Monocistronic	

★ Immature particles (empty viral capsids or defective particles) also contain a precursor protein VP_0 having the sequences of VP_2 and VP_4

† In preliminary experiments the structural disposition of these polypeptides in the capsid was studied by measurement of their relative susceptibility to iodination. It was concluded that the VP_1 and VP_3 polypeptides are partially exposed. Other polypeptides, VP_2 and VP_4 appear to be located inside.

‡ Under nondenaturing conditions.

§ Calculated on the basis of sedimentation under nondenaturing conditions.

§§ From electrophoretic mobility of completely denatured molecules.

From : Frösner G. Hepatitis A virus, In: Belsho R, editor. Textbook of human virology. Baltimore: Mosby Year Book; 1991. p.500.

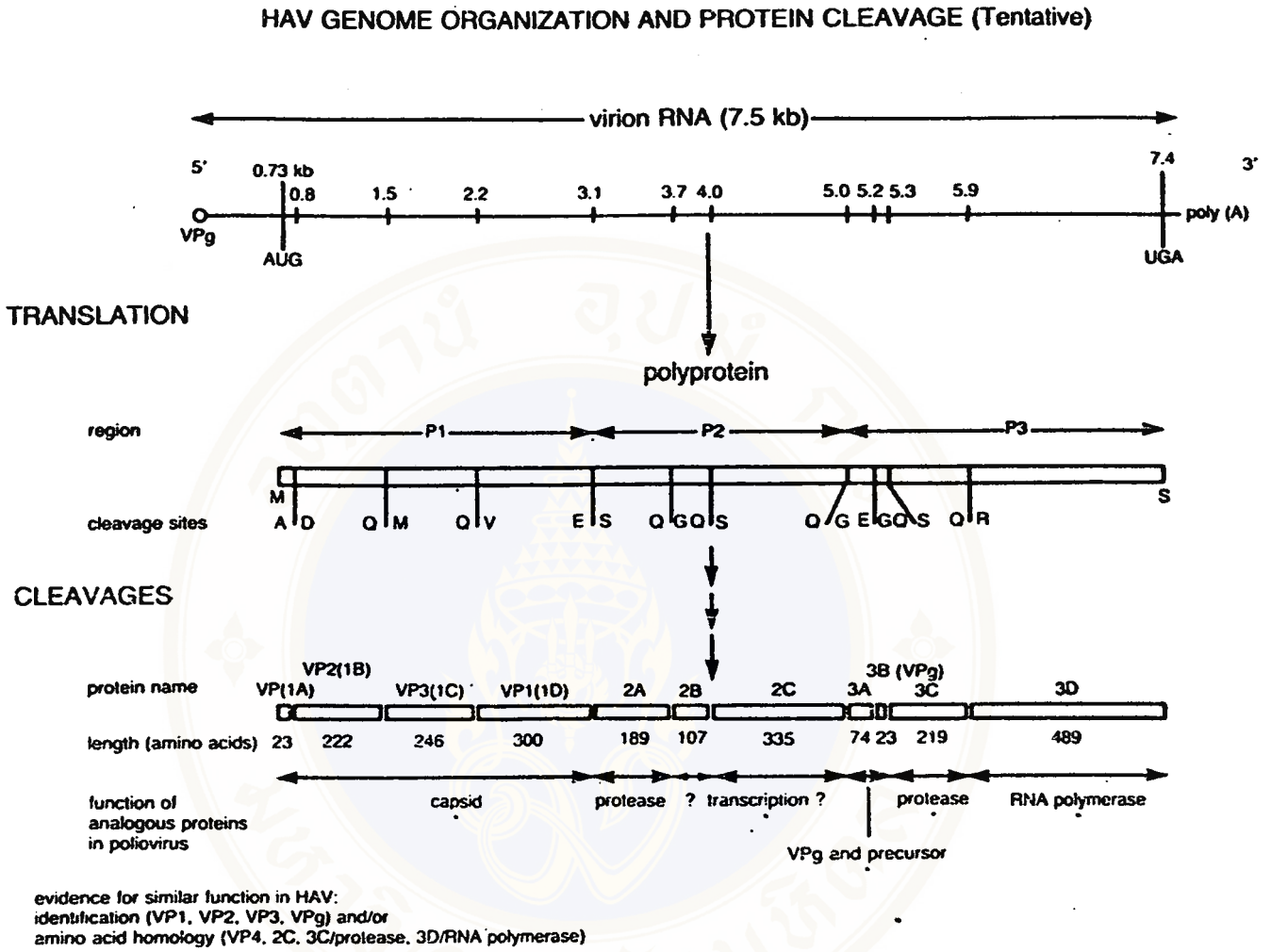


Figure 3 Organization of the RNA genome of hepatitis A virus and protein cleavage. Covalent attached Vg protein (5′ end: (top) start codons at 0.73 kb, gene start/end positions (small vertical marks and stop codons at 7.4 kb), and poly (A) at the 3′ terminus. HAV RNA is probably translated (large vertical arrow) into a precusor polyprotein (middle) that is cleaved by viral proteases in several steps (small vertical arrows) to generate mature proteins. Regions of the polyprotein are indicated according to standard nomenclature, as are predicted residues at the amino and carboxy termini of mature proteins (M = methionine; A = alanine; D = aspatate; O = glutamine; V = valine; F = glutamate; S = serine; G = glycine; R = arginine). Names, lengths, and proposed functions in poliovirus are indicated in the lower diagram along with HAV proteins. HAV is composed of four capsid polypeptides which are coded within the P1 region. These proteins are referred to as virion proteins (VP_1, VP_2, VP_3 and VP_4). Nonstructural proteins are coded within the P2 and P3 region.

From: Battergay M, Feinstone S. Hepatitis A virus, In: Willson R. editor. Viral hepatitis. New York: Mercel Dekker; 1997. p.40.

In comparison with the four established genera of the picornavirus family, the genome of HAV has a low GC content. Nucleotide sequence exhibits less homology with other picornaviruses than non-HAV picornaviruses which show with one another. The closest amino acid homology is found for the RNA transcribing portions 2C and 3C (28% and 25%, respectively) of HAV and encephalomyocarditis virus (35). Although the genome size and organization is similar to those of enteroviruses, the amino acid sequence of HAV proteins is distinct.

HAV is stable at pH 3 and in organic solvents such as ether, chloroform, or Freon. Temperatures above 60°C are necessary to destroy infectivity within a short time. At room temperature or lower, the virus maintains its infectivity in the environment for several weeks, even in a dried form. A less than 100-fold reduction in infectious HAV is found after 56 days at 25°C in ground water, coralla sand, cecil clay, and marine sediment, and isolation of virus usually is possible even after 86 days (37). The stability of HAV was shown in Table 2.

Chlorine concentrations of 2 to 2.5 mg/L can be reliably inactivated the virus (38) and purified HAV is inactivated by formalin treatment as applied in the preparation of a killed virus vaccine (1:4,000, 37°C, 72 hours). For the complete inactivation of unpurified HAV present in tissue culture supernatant, much higher formalin concentrations (1:100, 37°C, 72 hours) are necessary, and this also completely destroys the antigenicity of HAAg. Peracetic acid is an effective disinfectant to inactivate HAV and is used in hospitals. Blood and blood products may be sterilized by a combined UV-β-propiolactone treatment in countries where this procedure is licensed (39).

Table 2 Stability of Hepatitis A Virus

Exposure to	Treatment Conditions	Effect on Infectivity
Acid	pH3, room temp	Stable
Organic solvents	Ether, chloroform, Freon	Stable
Temperature	-20°C or -70°C for 6 wk	No reduction in infectivity
	Room temp, 1 wk	No measurable Reduction in infectivity
	Room temp, 8 wk	Infectivity destroyed
	25°C for 30 d (in dried feces)	Stable
	56°C for 30 min	No reduction in infectivity
	60°C for 4 h	Partial inactivation
	60.6°C for 19 min	Partial inactivation
	85°C for 1 min	Complete inactivation
	98°C for 1 min	Complete inactivation
Chlorine	0.5-1.0 mg HOCl/L for 60 min	Partial inactivation
	1.5-2.5 mg HOCl/L for 30 min	Complete inactivation
Formalin	1:350, room temp, 60 min	Partial inactivation
	1:4000, 37°C, 72 h	Inactivation
	About 1:1000 (0.055% - formaldehyde) 37°C, 72 h	Nearly inactivated (tissue culture supernatant)
	About 1:100 (0.35% - formaldehyde) 72 h	Complete inactivation (tissue culture supernatant)
Peracetic acid	1% room temp, 1 h	Not inactivated (tissue culture extract)
	2% room temp, 4 h	Complete inactivation
	1% room temp, 1 h	Complete inactivation
β-propiolactone	0.25%, 5°C, 1 h, pH 7.2	Complete inactivation
UV irradiation	2 mW/cm²/min	Complete inactivation

From : Frösner G. Hepatitis A virus, In: Belsho R, editor. Textbook of human virology. Baltimore: Mosby Year Book; 1991. p.501.

Although a variety of genotypes of HAV have been identified, there appears to be only one serotype throughout the world (40, 41). Polyclonal and monoclonal antibodies directed against the major antigenic determinant appear to be capable of detecting strains of HAV isolated in different parts of the world, suggesting that there is only one major serotype (41, 42). The immunodominant neutralization site of HAV is conformationally determined. Antibodies against HAV polypeptides do not neutralize the virus. Neutralization escape mutants have been mapped to exhibit mutations only in a few areas of the βB and βC loops of VP_3 and VP_1, VP_3 and VP_1 loops form a single functional site (43).

HAV is the first human hepatitis virus to be propagated in tussue culture. Provost and Hillemand (27) used primary marmoset liver cells and fetal rhesus kidney cells (FRhK6) whereas Frösner, *et al.* (28) succeeded in propagation in an easier-to-handle continuously growing hepatoma cell line (PLC/PRF/5 cells). The described cultivation in human embryo fibroblasts (44) is of special interest for vaccine production. HAV grows very slowly in tissue culture and usually does not induce a cytopathic effect (CPE) in infected cells (27, 34). The virus requires several days to weeks to reach maximal titers in cell cultures, and usually requires immunohistochemical or hybridization techniques to detect because of the lack of virus-induced cytopathic effect (45, 46).

2.4 Epidemiology

1. Incidence and prevalence in different geographic areas

The incidence of HAV infection cannot be deduced from mortality data because death is extremely infrequent, especially in children. Morbidity data, which are not available from many developing and even some developed countries, are biased by several facts: only clinical but not the frequently occurring subclinical cases are registered; because of limited epidemiological and laboratory services in most parts of the world, it is not possible to differentiate among hepatitis A, B and hepatitis C, and cases of hepatitis are usually registered as a single nosologic entity; there is a considerable underreporting of clinical cases of hepatitis (47). The most appropriate means to obtain information on the incidence of HAV infection in different geographic areas and epidemiologic setting is by a calculation from the prevalence of anti-HAV in the sera of appropriate population groups (48). Three different patterns of anti-HAV prevalence have been described from different areas of the world.

1.1 Pattern Ia found in developing countries of Asia, Africa, and the Americas virtually all individuals become anti-HAV positive during childhood (49) and remain positive during adulthood (50, 51). Pattern Ib showed a decline in proportion of anti-HAV-positive individuals, which may be found in older age groups (52, 53), and this is probably due to a decrease in antibody concentration below the detection limit of the test systems used. In these countries, where sanitary and hygienic conditions are relatively primitive, HAV infection is highly endemic and usually acquired in early childhood. In endemic countries with a lower incidence in infection, the same situation type of age-dependent prevalence curve is found (pattern

Ic). However, the increase of the proportion of anti-HAV-positive individuals is not as fast as in pattern Ia, reaching high levels only in adulthood (52, 54).

1.2 In highly developed countries of Europe and in the United States a sigmoidal type of age-dependent prevalence curve is noted (54, 55). The percentage of antibody-positive individuals is low during childhood, increases sharply during adolescence or early adulthood, and reaches a high (pattern IIa) or medium (pattern IIb) level during late adulthood. This pattern is caused by a cohort effect in countries where the incidence of HAV infection has decreased substantially during recent decades. The relatively high prevalence of anti-HAV in adults is due to a more universal exposure of these adults when they were children at a time when there was a higher incidence of infection. An extreme of this pattern is the complete or nearly complete disappearance of hepatitis A infection in younger individuals of isolated populations and a virtually universal exposure of adults during previous large-scale epidemics, as documented in Greenland (56) and in the South Pacific Islands (48). In the future the low incidence of HAV infection in these countries will lead to a mostly susceptible population which is endangered during travel to endemic areas.

1.3 A third type of antibody pattern may be found in populations in which hepatitis A is no longer an endemic disease but is introduced regularly from endemic areas and transmitted to certain groups of the population. This type III pattern, at present found in Germans living in West Berlin, is characterized by an increase to 25% anti-HAV-positive children aged 2 to 5 years, a stable prevalence of 25% to 30% up to 20 years of age, followed by an increase to 85% in those older than 50 years. The relatively high prevalence of infection in German children may be due to close contact with children of emigrant workers from Mediterranean countries in

the kindergarten. The population of some districts of Berlin consists of 20% to 29% emigrant workers whose children usually acquire HAV infection during a visit in their home countries (57).

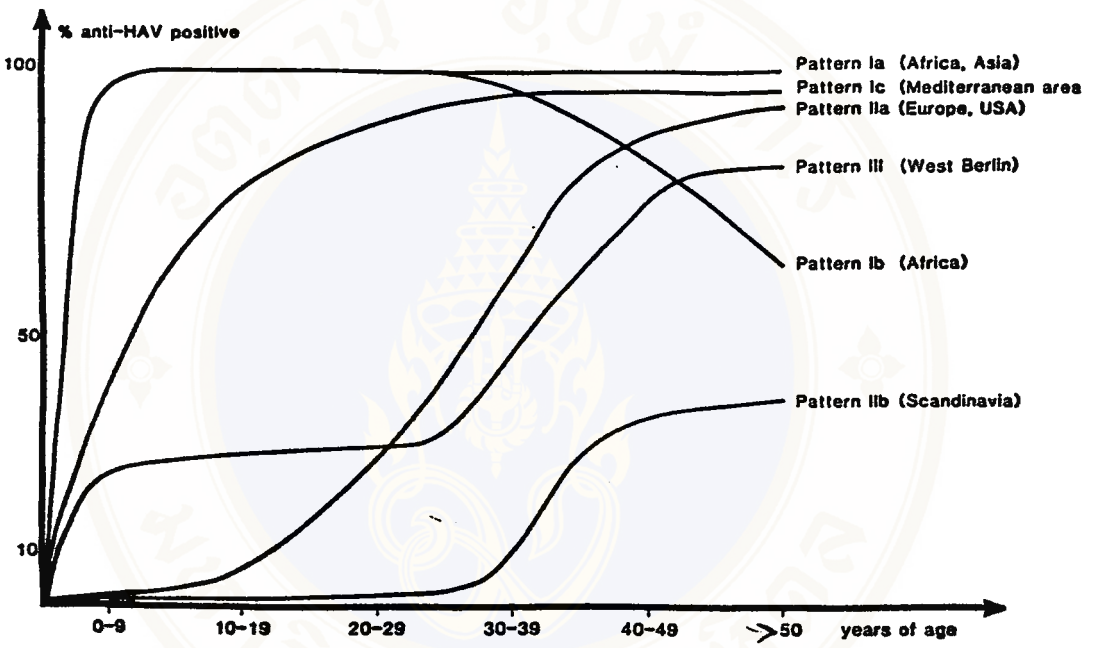


Figure 4 Different patterns of age-specific prevalence of anti-hepatitis A virus in different parts of the world.

From: Frösner G. Hepatitis A virus, In: Belsho R, editor. Textbook of human virology. Baltimore: Mosby Year Book; 1991. p.503.

2. Age, Sex, Social, Economic, and other Factors

Age-related HAV seroprevalence patterns change in poor countries as they undergo socio-economic development which indicated that HAV seroprevalence decline markedly in children, although a substantial proportion of the adult population

remains seroconverted as a result of previous infection. The proportion of seroconverted adults gradually declines as uninfected children become adults, and the overall population seroprevalence declines. The curves present in Figure 5 show that the countries undergoing development have curves which shift to the right and downwards as fewer children, and eventually adults, have seroconverted (58).

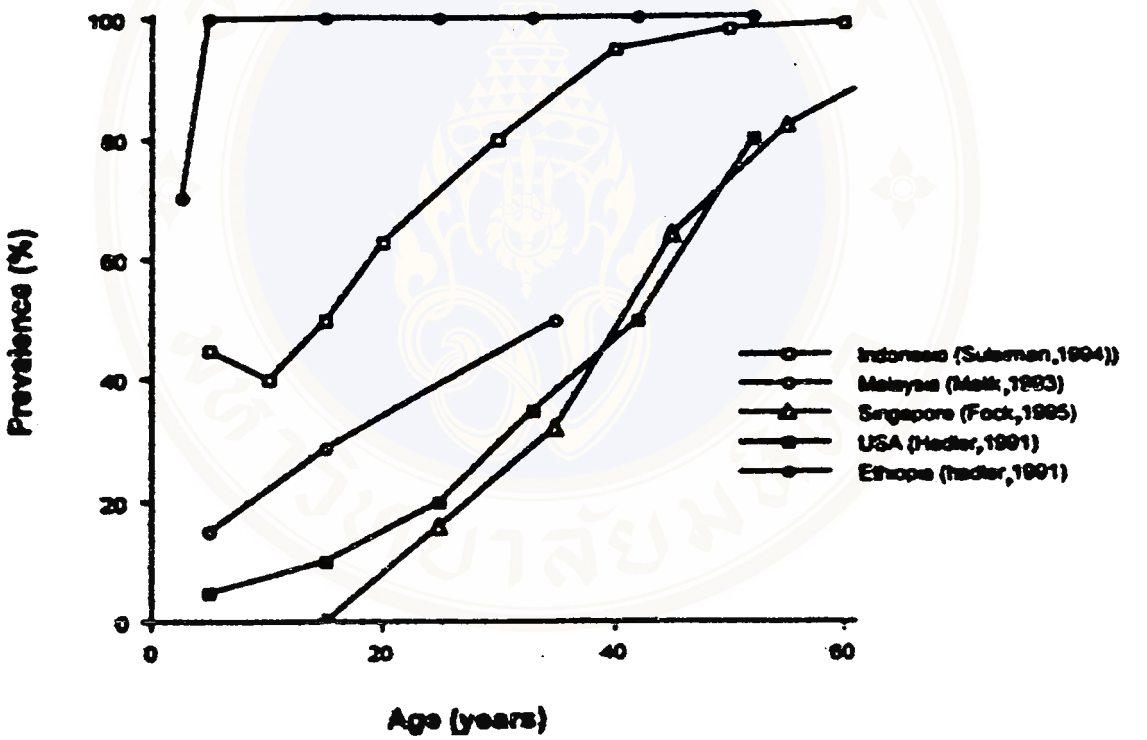


Figure 5 Age-related prevalence patterns in SE Asia, USA and Ethiopia

Sex: In the United States, the sex ratio among reported hepatitis A cases has been 1.3 to 1.5: 1 (male: female) in recent years (59). This modest male predominance in part represents unequal distribution in high risk behaviors including homosexual activity, international travel, and drug abuse.

Race: Hepatitis A occurs in all racial groups, and race is not believed to predispose to HAV infection except where related to socio-economic status or origin. In the United States, prevalence of HAV is higher in blacks and Hispanics than whites, but differences largely disappear when controlled for socio-economic status (60).

Occupation: specific occupational risk factors for HAV are not well documented. Some studies of sewage workers in Europe have suggested 2 to 3 fold elevated risk of infection. Staff institution for the mentally retarded and workers in day-care centers with diapered children are likely to be at elevated risk, as are those persons who work for extended periods in less developed countries in which hepatitis A is highly endemic (10, 61).

Other factors favoring the transmission of hepatitis A are large-sized families, overcrowded living conditions, and poor hygienic and sanitary conditions (62). In developed countries the decrease in incidence of hepatitis A infection during the last decades was associated with a considerable improvement of living conditions as measured by the percentage of families with an indoor toilet, by the quality of community water supply, and by the method of wastewater disposal. A higher prevalence of anti-HAV was demonstrated in rural compared to urban areas of Greece, Switzerland and Australia (54, 62).

Homosexual men exhibited an increased prevalence of anti-HAV, and in a prospective study the annual incidence of hepatitis A in susceptible homosexuals was high as 22% (63). A positive correlation was found between the prevalence of anti-HAV and the number of episodes of syphilis (64).

Drug addicts are not only exposed to hepatitis B and hepatitis C, but also to hepatitis A (65). In Norway, prevalence of anti-HAV in drug addicts was 43% in 1983, versus about 5% in corresponding age group of the general population and 1% in Norwegian (United Nations) soldiers sent to Lebanon. Epidemics of hepatitis A were seen among drug addict in 1975 and 1979, coinciding with epidemics in Malmo, Sweden.

Medical personnel not especially exposed to hepatitis A patients do not seem to be at a special risk of contraction hepatitis A. No increased prevalence of anti-HAV was detected in staff, nurses, and patients of dialysis centers (66).

2.5 Pathogenesis

1. Incubation period

The incubation period is defined as the time between exposure to virus and clinical illness, average 28 days (range, about 15 to 50 days) (11). Early symptoms of hepatitis are often vague and nonspecific include fever, malaise, anorexia, nausea, diarrhea, vomiting and right upper quadrant pain, and usually precede signs of liver injury, including jaundice and dark urine. In the Shanghai outbreak, mean incubation period was 21 days (12 to 36 days) (67).

2. Viral replication

HAV is generally transmitted by the fecal-oral route. Because the virus is acid-resistant, it probably passes the stomach and transverses the intestine, after which the virus is transported to the liver by an undetermined mechanism. The virus replicates in hepatocytes and is released into the bloodstream (viremia). At the same time, the virus is present in the bile and shed in the feces. Fecal shedding and viremia

are maximal just before or shortly after the onset of liver function abnormalities and terminate at about the time humoral immunity to HAV is detected (68).

3. Pathogenesis

HAV is generally not cytopathic in cell culture, liver cell damage is due to a cell-mediated immune response, whereas circulating antibodies are probably more important in limiting spread of virus to uninfected liver cells and other organs. Viral induced T cells target infected liver cells and thereby mediate immunopathology (43). In human studies, in 1986 Vallbracht (69) found that lymphocytes from convalescent patients produced cytotoxic effects against autologous epidermal cell lines infected with HAV and CD8+ T-cell clones demonstrated cytotoxic activity against autologous fibroblasts infected with hepatitis A, which strongly support the hypothesis that CD8+ T lymphocytes mediate liver cell damage. Furthermore, natural killer cells have been demonstrated to be capable of lysing HAV-infected tissue culture cells.

4. Clinical features

Hepatitis A is an acute or subacute, often subclinical infection of the liver with an icteric or anicteric course. The ratio of anicteric to icteric cases has been reported to vary from 12:1 to 1:3.5 in different epidemiological investigations (2). Young children usually experience a mild, anicteric disease, whereas in adults the illness is generally more severe and more prolonged. Between 70 and 80% of adult patients were icteric (70, 71). Symptomatic cases typically have a 4-10 day prodrome with anorexia, malaise, weight loss, pyrexia, vomiting, disturbance of bowel habit, often associated with upper respiratory symptoms. Passage of dark urine is usually the first hepatitis symptom, followed a day or two later by jaundice and pale stools; earlier

symptoms and pyrexia then usually resolve, but pruritus, localized hepatic pain and moderate hepatomegaly often develop. (72)

2.6 Immune Response and Immunity

Anti-HAV usually becomes positive in serum before the first rise in transaminases. It is virtually always detectable at onset of disease (73). During the first days of illness, most and sometimes all of the detectable anti-HAV belongs to the IgM class (anti-HAV IgM) (74). In clinical cases a peak in anti-HAV IgM is reached about 8 to 16 days after hospitalization, then the titer decreases gradually, becoming undetectable by currently used techniques between 3 and 6 months after onset of illness (75). Anti-HAV of the IgG class (anti-HAV IgG), which may not be detectable at onset of illness, slowly increases in titer during the next weeks and reaches a maximal titer after about 6 to 12 months (Figure 6). By immune adherence hemagglutination (IAHA), a method which mainly detects antibody of the IgG and not of the IgM class, a negative result for anti-HAV may be found up to 49 days after the first transaminase elevation (76) or up to 4 weeks after onset of illness (77). Using a test system that does not differentiate between anti-HAV of the IgG and IgM classes, a transient decrease in total anti-HAV titer can be found about 1 month after onset of illness. From the high prevalence of antibody in older individuals of developed countries with decreasing incidence of hepatitis A, it can be assumed that anti-HAV IgG usually persists for life. Persons infected during an epidemic outbreak still exhibited high antibody titers when tested 12 years later (78).

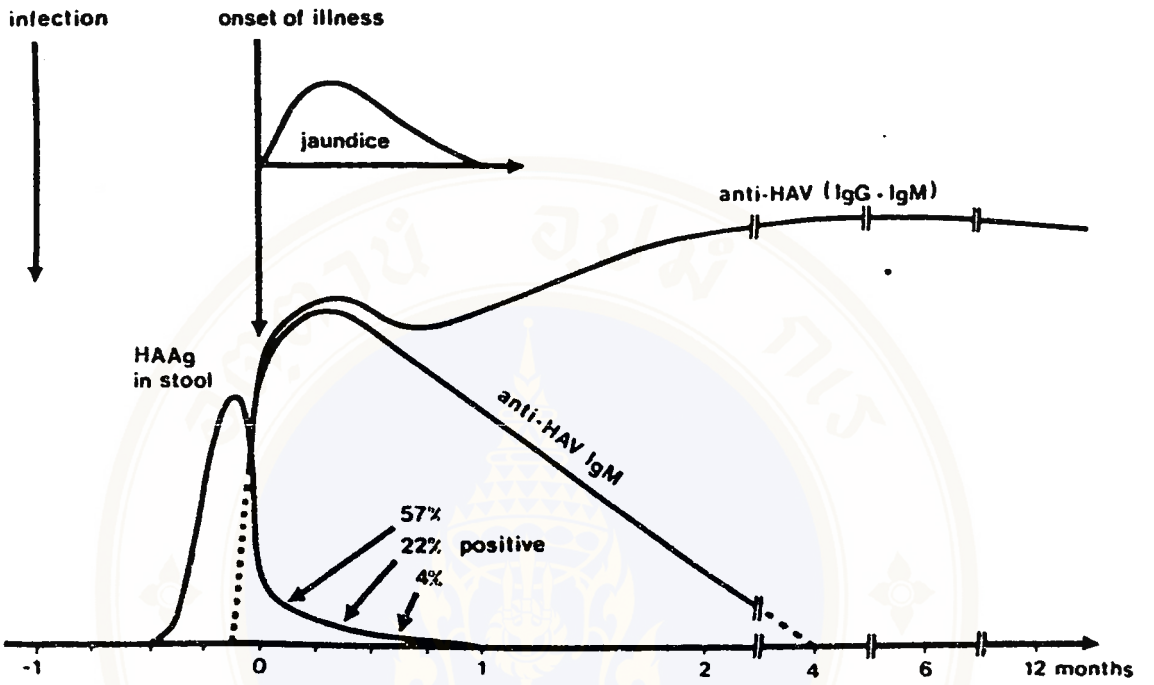


Figure 6 Model of the serology of clinical hepatitis A infection. The percentages of patients with HAAg in stool during the first, second, and third week after onset of illness are from an earlier study.

From Frösner G. Hepatitis A virus, In: Belsho R, editor. Textbook of human virology. Baltimore: Mosby Year Book; 1991. p.508.

2.7 Laboratory Diagnosis

The specific diagnosis of acute hepatitis A is most commonly confirmed by detection of IgM-class antibody specific for HAV, which indicates ongoing or recent infection (75). The advantage of the IgM test is that the diagnosis can be established on a single acute-phase serum sample, as IgM antibody is usually present at the time of clinical presentation and may be detectable by the time of the first rise in alanine

aminotransferase (ALT) (21). While the typical radioimmunoassay (RIA) or enzyme immunoassay (EIA) measures total antibody (IgM and IgG) to the virus, this is of little diagnosis value because IgG persists for many years and it is impossible to determine if it is due to a past or a present infection (68). Nevertheless, a fourfold rise in titer in two consecutive sera indicates an acute hepatitis A infection (79). The most widely used are capture radioimmune or enzyme immunoassays. Highly specific antiserum to human IgM is bound to the solid phase. The test serum is then placed in contact with the solid phase, which binds any IgM that may be present in the serum. HAV is added, followed by labeled anti-HAV IgG. The quantity of label that is bound is an indirect measure of the amount of the specific anti-HAV IgM present in the serum. These assays are highly specific and so sensitive that the test serum must be diluted so that the assay will reflect only recent infections. IgM anti-HAV can be detected in nearly 100% of patients with acute hepatitis A at their first clinical presentation, and it then remains positive for 3 to 6 months; it is detected in as many as 25% of patients 12 months after the onset of illness (80). False-positive tests are rare and should be suspected when IgM anti-HAV is found to persist for more than a year.

Detection of virus or viral antigen in the stools of patients is possible beginning 1 to 2 weeks before symptoms develop. HAV excretion in stool may be the first marker of infection, because it begins up to 2 weeks before seroconversion to anti-HAV, elevation of liver enzymes, of first symptoms. The presence of HAAg in stool correlates with infectivity as measured by animal inoculations and isolation in tissue culture. HAAg can be detected in stool by solid-phase RIA or EIA as well as by immune electron microscopy (81, 82).

Routine cell culture of HAV is difficult for most clinical isolates and would take too long to be useful for clinical diagnosis (27, 28). After primary inoculation of HAV in tissue culture, replication cannot be demonstrated for 4 to 6 weeks. Not only patient specimens (stool, blood, and liver biopsy material) but also possibly contaminated food or wastewater can be investigated for the presence of the virus.

Molecular hybridization to detect the RNA genome of HAV with complementary DNA (cDNA) cloned HAV is more sensitive than immune electron microscopy or RIA for HAAg (83). Hepatitis A genomic sequences can be detected by in situ hybridization in liver biopsies using complementary single-stranded RNA (84). As a little 10^3 $TCID_{50}$ of HAV, or approximately 0.1 pg of viral RNA can be detected. PCR detection of HAV in clinical samples is possible and as little as one genome copy can in theory be detected, which would provide the sensitivity required for a virologic diagnosis in most cases.

2.8 Routes of Infection

Person

The most important means of transmission is from person to person via the fecal-oral route. Transmission is generally limited to close contacts, espacially those within families (71). Young children are frequently involved in the spread of infection in households, as infections in this group are often silent and standards of hygiene are lower among children than among adults (85, 86). Most household cases occur one incubation period or more after the index case, indicating that the patient is most infectious just before the onset of symptoms. Hepatitis A is rarely spread by casual contact, and play contacts are more important than contacts in the classroom.

Outbreaks have also been reported in neonatal intensive care units (87). The major features of these outbreaks are a low incidence of clinically apparent disease in the children but a high rate of infection among adult contacts, including family members. A single episode of vertical transmission was reported associated with and outbreak in a neonatal intensive care unit (10). In this study, prenatal transmission might have occurred during the viremic episode when maternal blood containing HAV entered the fecal circulation because of reported placental abruption.

Food and water-borne transmission

Epidemics of hepatitis A due to the consumption of contaminated food have been reported on numerous occasions. In many countries, an important mechanism of transmission appears to be consumption of raw or partially cooked shellfish. Shellfish are particularly likely to transmit hepatitis A because they filter large quantities of water and may serve as reservoirs of infection by concentration virus. Shellfish are often eaten raw or after gentle steaming, which is sufficient to cause the shell to open but inadequate to inactivate the virus. The epidemic of hepatitis A in 1988 in Shanghai, with 310,746 reported cases, could be attributed to the ingestion of raw clams (88).

Despite the fact that in the United States an estimated 1,000 food handlers are infected with hepatitis A each year, the number of food handlers associated outbreaks reported to the Centers for Disease Control is no more than four or five per year. Vehicles that have been involved in outbreaks of hepatitis A including beverages such as raw or cold milk, orange juice, salads, hamburgers, oysters, spaghetti, cream, pastries, strawberries, and lettuce (89 - 93). On each occasion uncooked food or food handled after cooking was the presumed vehicle of infection. In most outbreaks,

transmission of infection can be traced to a food handler who failed to observe hand-washing procedures after defecation.

The first waterborne epidemic of hepatitis A was described in 1920 (94). However, waterborne outbreaks of hepatitis A are uncommon in developed countries. In the United States, waterborne outbreaks account for less than 1% of all cases seen in this country. Although it may be possible to acquire hepatitis A by swimming in contaminated water, there is no data indicating that this is relevant risk.

2.9 Prevention and Control

Hepatitis A is usually transmitted by fecal-oral route, the most effective methods of control are improvements in standards of hygiene and environmental sanitation, especially by the provision of clean water. Good hygienic practices with particular emphasis on hand-washing, restriction of activities of any workers who are ill is of primary importance in the food preparation industry.

1. Prophylaxis with immune serum globulin (IG)

The efficacy of IG was first demonstrated in an outbreak at a summer camp in 1944 (95) and confirmed in large-scale studies among American soldiers serving in Europe during World War II (96). It is assumed that passive immunization alone can reduce the incidence of hepatitis A by up to 90% (97). During World War II, 1732 soldiers receiving IG were evaluated. Compared to nonimmunized controls, the incidence of jaundice fell by more than 80% at 7 weeks and more than 70% by 12 weeks (98). Postexposure prophylaxis with IG is also effective if administered within 2 weeks of exposure and will usually eliminate or reduce the severity of disease (99).

Recommendations for administration of IG for prevention of hepatitis A (3)

The levels of anti-HAV achieved following IM administration of IG are below the level of detection of most commercially available diagnostic tests. When administered for preexposure prophylaxis, a dose of 0.02 mL/kg IM confers protection for less than 3 months, and a dose of 0.06 ml/kg IM confers protection for less than or equal to 5 months. When administered within 2 weeks following an exposure to HAV, IG is greater than 85% effective in preventing hepatitis A. Serious adverse events from IG are rare. Anaphylaxis has been reported after repeated administration to persons who have known IgA deficiency; thus, IG should not be administered to these persons. Pregnancy or lactation is not a contraindication to IG administration. IG does not interfere with the immune response to oral poliovirus vaccine or yellow fever vaccine, or, in general, to inactivated vaccines. However, IG can interfere with the response to live, attenuated vaccines (e.g., measles, mumps, rubella, and varicella) when vaccines are administered either individually or as combination vaccines. Administration of these vaccines should be delayed for at least 5 months after administration of IG for hepatitis A prophylaxis. IG should not be administered within 2 weeks after the administration of live, attenuated vaccines (or within 3 weeks after varicella vaccine) unless the benefits of IG administration exceed the benefits of vaccination. If IG is administered within 2 weeks after administration of these vaccines (or within 3 weeks after administration of varicella vaccine), the person should be revaccinated, but not sooner than 5 months after the administration of IG.

2. Hepatitis A vaccine

Several inactivated and attenuated hepatitis A vaccines have been developed and evaluated in human clinical trials and in primate models of HAV infection. In 1978, Provost and Hilleman (27) described the successful immunization of marmosets with a killed hepatitis A vaccine prepared from liver of a marmoset infected with the Costa Rica HAV isolate CR326 and in 1986, an excellent immunogenicity in mice and marmosets of a formalin-inactivated vaccine produced from CR326-infected, continuous monkey kidney cells (LLC-MK2) had been reported (100). Three subcutaneous doses of 1 ng each induced anti-HAV in 50% of marmosets and three doses of 10 ng induced antibody in 100% of animals. All marmosets that developed detectable antibody were protected from infection after experimental challenge with virulent virus. Protection was also shown after immunization of owl monkeys with a formalin-inactivated vaccine produced from BS-C-1 cells infected with the Australian isolate HM175 (101).

Several large scale evaluations of killed hepatitis A vaccine were presented to the 1990 International Symposium on Viral Hepatitis and Liver Disease, April 4 through 8, 1990 in Houston. Inactivated vaccines have been shown to be safe and immunogenic (102). Development of live attenuated vaccine has been done in parallel with killed vaccine. The vaccines currently licensed in United States are HAVRIX (manufactured by SmithKline Beecham Biologicals) and VAQTA (manufactured by Merck & Company, Inc). Both are inactivated vaccines.

Route of administration, vaccination schedule, and dosage

The vaccine should be administered intramuscularly into the deltoid muscle. A needle length appropriate for the vaccinee's age and size should be used (103).

HAVRIX is currently licensed in three formulations, and the formulation and number of doses differ according to the vaccinee's age: for persons 2-18 years of age, 360 EL.U. per dose in a three-dose schedule and 720 EL.U. per dose in a two-dose schedule; for persons greater than 18 years of age, 1,440 EL.U. per dose in a two-dose schedule. VAQTA is licensed in two formulations, and the formulation and number of doses differ according to the person's age: for persons 2-17 years of age, 25 U in a two-dose schedule; for persons greater than 17 years of age, 50 U per dose in a two-dose schedule.

HAV isolated from China (H_2 strain), was attenuated by serial passage in newborn monkey kidney cells at 35° C and 32° C, and human lung diploid cells at 32° C (104). Live virus vaccine was given subcutaneously to 12 volunteers without local or systemic reactions, and without liver enzyme elevation. All vacinated subjects developed anti-HAV antibody at a mean time of 3 weeks after inoculations.

Clinical trials indicate that formalin-inactivated vaccine derived from HM175 strain are safe and highly immunogenic, providing in over 40,000 children in Thailand was found to be safe and highly effective. There were no serious adverse reactions in this group (102).

CHAPTER III

MATERIALS AND METHODS

3.1 Study Design

This study was a cross-sectional study conducted in youth participants, whose ages ranged from 15 to 24 years, both gender of the Akha-hill tribe at Amphoe Mae-Soui, Chiangrai Province, Thailand, between July and September 1999.

3.2 Sample Size

The sample size was calculated by using the formula (105) as follow:

$$n = \frac{N Z^2_{\alpha/2} P(1-P)}{\left[d^2(N-1)\right] + \left[Z^2_{\alpha/2} P(1-P)\right]}$$

N = Akha-hill tribe population whose ages ranged from 15 to 24 years old at Ban Doi-Chang, Amphoe Mae-Soui, Chiangrai Province, Thailand. There were 375 persons based on the survey of Doi-Chang Health Center.

$Z_{\alpha/2}$ = Standard normal deviation, it was chosen to be 1.96 which corresponds to the 95 % confidence interval

P = Estimated proportion of antibody to hepatitis A virus in population. It was 39.4 per cent in mental retard children age ≥ 16 years from the previous study in 1994 (106), and then P was given to 0.394.

d = The error for acceptation set at 0.05

n = The desired sample size

The estimation of sample size for this study was:

$$n = \frac{375 \times (1.96)^2 \times 0.394 \times (1-0.394)}{[(0.05)^2 \times (375-1)] + [(1.96)^2 \times 0.394 \times (1-0.394)]}$$

= 186 persons

3.3 Studied Subjects

The subjects were 190 Akha-hill tribe persons whose ages ranged from 15 to 24 years who participated in the study. They received an explanation of the study and the written informed consents were signed by whom agreed to participate. All participants were enrolled to the study and the participants who had previous immunization with hepatitis A vaccine were excluded. The studied subjects were interviewed about socio-economic factors and personal hygiene by using the structured questionnaire and their environmental sanitation conditions were observed. Studied households' drinking water samples were collected for screening the coliform bacteria contamination by using sanitation index 2 (SI-2) media, which sensitivity, specificity and efficiency of the test were 81%, 100% and 91% respectively (107), and their blood specimens were collected for anti-HAV detection. From laboratory results, the studied subjects were divided into two groups, the first group was the subjects with anti-HAV positive and the second was the subjects without anti-HAV. The studied variables including socio-economic factors, personal hygiene and environmental sanitation conditions of these two groups were compared and analyzed for searching some factors associated with anti-HAV positivity.

3.4 Steps of Data Collection

The data and specimens were collected by following steps below:

1. The questionnaires and the observation form of the environmental sanitation were designed and tested content validity by advisors.

2. The research details were approached to the Heads of Provincial Public Health Office, Chiangrai Province for permission to collect data between July and September 1999.

3. All participants, youth male and female were interviewed through translator and environmental sanitation conditions were observed.

4. Their blood specimens were collected by venepuncture about 2 to 4 ml per person.

5. Blood specimens were standed at room temperature until clotting, and then sera were separated and stored at -20°C until tested for antibody.

6. The serum specimens were tested for anti-HAV by Enzyme Immunoassay (EIA) Commercial Kits (HEPAVASE A-96, General Biologicals Corp., Taiwan). The sensitivity and specificity of the test were approximately 100 per cent. The laboratory test for anti-HAV was performed at the Department of Microbiology, Faculty of Public Health, Mahidol University.

7. Five milliliters of studied household's drinking water sample each were collected for screening the coliform bacteria contamination by using SI-2 media.

3.5 Determination of Anti-HAV Antibody

The determination of anti-HAV in serum specimens was performed by using reagents and procedure as follow:

Reagents

1. Antibody: Mouse monoclonal anti-HAV conjugated with HRP was used for detection the absence of antibody in ELISA test.

2. Antigen: Hepatitis A antigen (HA Ag) coated on the microplate wells.

3. Human serum containing diluted anti-HAV dissolved in a buffer solution was used as anti-HAV positive control.

4. Normal human serum free from HAV markers was used as hepatitis A negative control.

5. Tetramethylbenzidine (TMB) solution A and TMB solution B were the substrate for peroxidase conjugated antibody. TMB solution was prepared by mixing TMB solution A and B in equal volume.

6. Phosphate buffered saline solution (PBS) with Tween-20 (20X) was used as a washing buffer. Buffer solution was prepared as 1:20 dilution with purified water.

7. 2N sulfuric acid was used for stopping reaction.

Principle of test

HEPAVASE A-96 was an ELISA based on the serum antibody competition test immunoassay principle. Specifically, microelisa wells were coated with hepatitis A antigen (HA Ag). Anti-HAV coupled to horseradish peroxidase (HRP) served as competitive antibody and TMB solution (TMB/H_2O_2) served as the substrate. The test sample, an appropriate control, and anti-HAV labeled with HRP were collectively incubated in a well. Available anti-HAV in the sample competed with anti-HAV labeled with HRP to bind the solid-phase HA Ag. Following an incubation, the samples were aspirated and the wells were washed with buffer. In the sample of a

positive anti-HAV, less anti-HAV labeled with HRP bound to the solid-phase HA Ag because it was competitive bound by available anti-HAV in the sample. After incubation with TMB solution, blue color was produced which turned yellow when the reaction was stopped with sulfuric acid. Upon completion of the test, the intense color development suggested the absent of anti-HAV in the sample while pale color suggested the available anti-HAV in the sample.

Procedure of serum antibody competition immunoassay

Step 1 Test serum and conjugate

Ten microlitres of normal human serum free from HAV markers were added into the three assigned wells for negative control and 10 µl of human serum containing diluted anti-HAV were added into the two assigned wells for positive control. Two wells were reserved for blanks and PBS (10 µl) was added into the wells. Ten microlitres of serum specimen were added into the assigned wells, respectively. After serum specimens, negative and positive control, were applied to the assigned wells, 100 µl of anti-HAV HRP conjugate were added into each well except the blanks. Ensure through mixing by gently tapping the side of the plate. The plate was incubated at 39°$\pm$ 2°C for 60 minutes.

Step 2 Washing

The samples and controls were discarded and the plates were washed for 6 cycles with 0.5 ml per well of washing buffer.

Step 3 Substrate

After washing, the plates were dried by tapping on blotting paper and 100 µl of

freshly prepared substrate were added to each well. The plates were kept in dark box at room temperature for 30 minutes.

Step 4 Stop reaction and read the result

The reaction was stopped by adding 100 µl of 2N sulfuric acid in each well. The optical density (OD) of the color reaction was measured by an EL 312e Bio-Kinetics reader at 450 nm.

Step 5 Interpretation of result

The cut-off value was taken by calculating the mean OD value of negative control (NC $\bar{x}$) and positive control (PC $\bar{x}$) minus two standard deviation as the following formula.

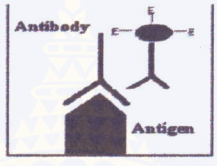
$$\text{Cut-off OD} = (\text{NC } \bar{x} + \text{PC } \bar{x})/2 - 2\,\text{SD}$$

The samples with the greater OD value than cut-off value were considered negative for anti-HAV. For positive anti-HAV consideration, the OD value was less than the cut-off value. (The cut-off value was equal to 0.51).

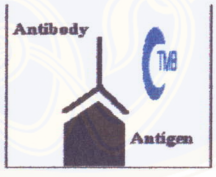
(A) Serum antibody competition test

1. Antigen adsorbed to plate

2. Participant's serum and enzyme labeled antibody are added; specific antibody in serum competes with enzyme labeled antibody to attach antigen.



3. Enzyme's substrate is added, the reaction produces pale or invisible color.



(B)

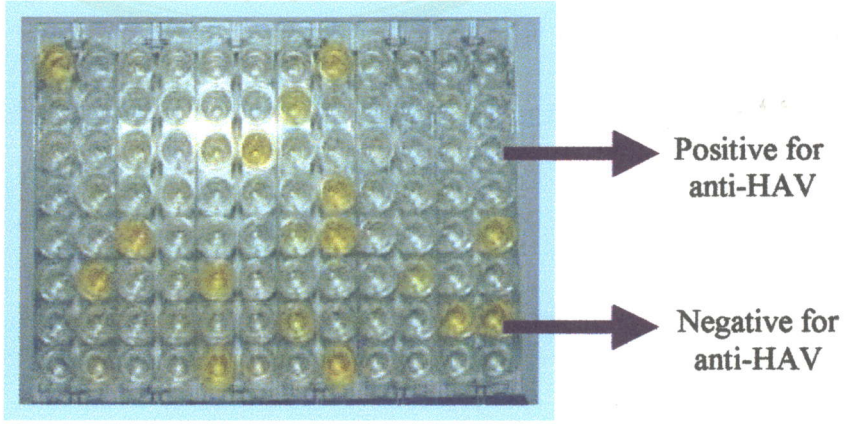


Figure 7 The steps of the serum antibody competition ELISA method detects antibodies (A) and interpretation of test results (B).

3.6 Screening of Coliform Bacteria Contamination

1. SI-2 media preparation (107, 108)

1.1 SI-2 media was prepared by warming distilled water in water bath at temperature of 60°C then add each chemical reagent (Appendix C) into the warmed distilled water and stir until the chemical reagents were dissolved. The pH of the media was adjusted to 7.0 to 7.2 with HCl or NaOH.

1.2 The SI-2 media was divided into aliquots. Each of 10 ml of SI-2 media was pipetted into sterile screw cap tube (20 ml volume size) and loosen replace cap. The media were autoclaved at 121°C for 15 minutes and let it cool in room temperature then seal the junction between tube and the cap with paraffin film and store in room temperature until use.

2. Screening coliform contamination in drinking water:

The steps of screening coliform contamination were described as follow:

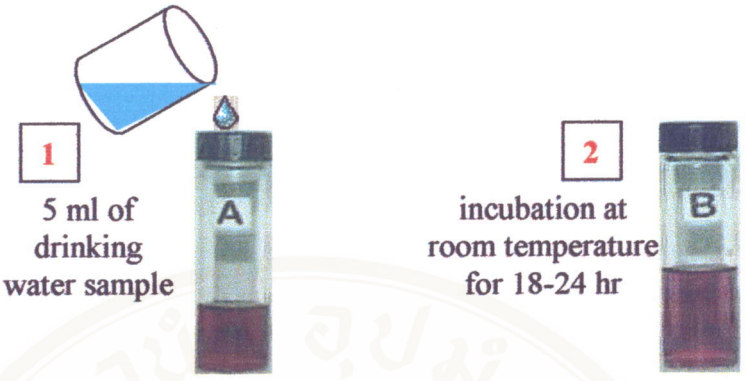
2.1 The paraffin film was removed and loosen the cap for flame the lip of tube by passing it through the upper cone of the flame.

2.2 The pH in drinking water sample was tested by using pH paper and recorded. Five milliliters of the drinking water sample were poured or rinsed into medium tube and gently mixed. The tube was flamed again before replacing cap.

2.3 The result of screening test was interpreted after the tested tube was incubated at room temperature for 18 to 24 hours.

2.4 Positive result for screening coliform contamination in drinking water samples was the change in SI-2 media's color from purple to yellow after incubation. Negative result for screening coliform contamination was considered when the media's color did not change.

A) SI-2 media for screening coliform bacteria contamination in drinking water sample



B) Interpretation of screening results

Figure 8 SI-2 media screening test for coliform bacteria contamination. Procedures of the SI-2 media screening test (A) and interpretation of test results (B).

3.7 Data Analysis

The statistic analysis was performed by using SPSS/PC (version 7.5) as described below.

1. The descriptive information on age, gender, marital status, education, and income were presented in terms of percentage, mean, median and standard deviation.

2. The studied factors including the socio-economic factors, environmental sanitation conditions and personal hygiene and child health care behavior factors between anti-HAV positive group and negative group were compared and analyzed by using Chi-square test or Fisher's Exact test. The statistically significant difference was expressed as p-value. The critical level of α equal to 0.05 was used for statistical significance.

CHAPTER IV

RESULTS

The results of this study were presented into 3 parts:

4.1 General characteristics of studied community,

4.1.1 Studied subjects

4.1.2 Studied households

4.1.3 Environmental sanitation conditions and bacterial quality of drinking water

4.1.4 Personal hygiene and child health care behaviors

4.2 Prevalence of antibody to hepatitis A virus in Akha-hill tribes youth

4.3 Socio-economic factors, personal hygiene behaviors, child health care behaviors, and environmental sanitation conditions associated with the antibody to hepatitis A virus.

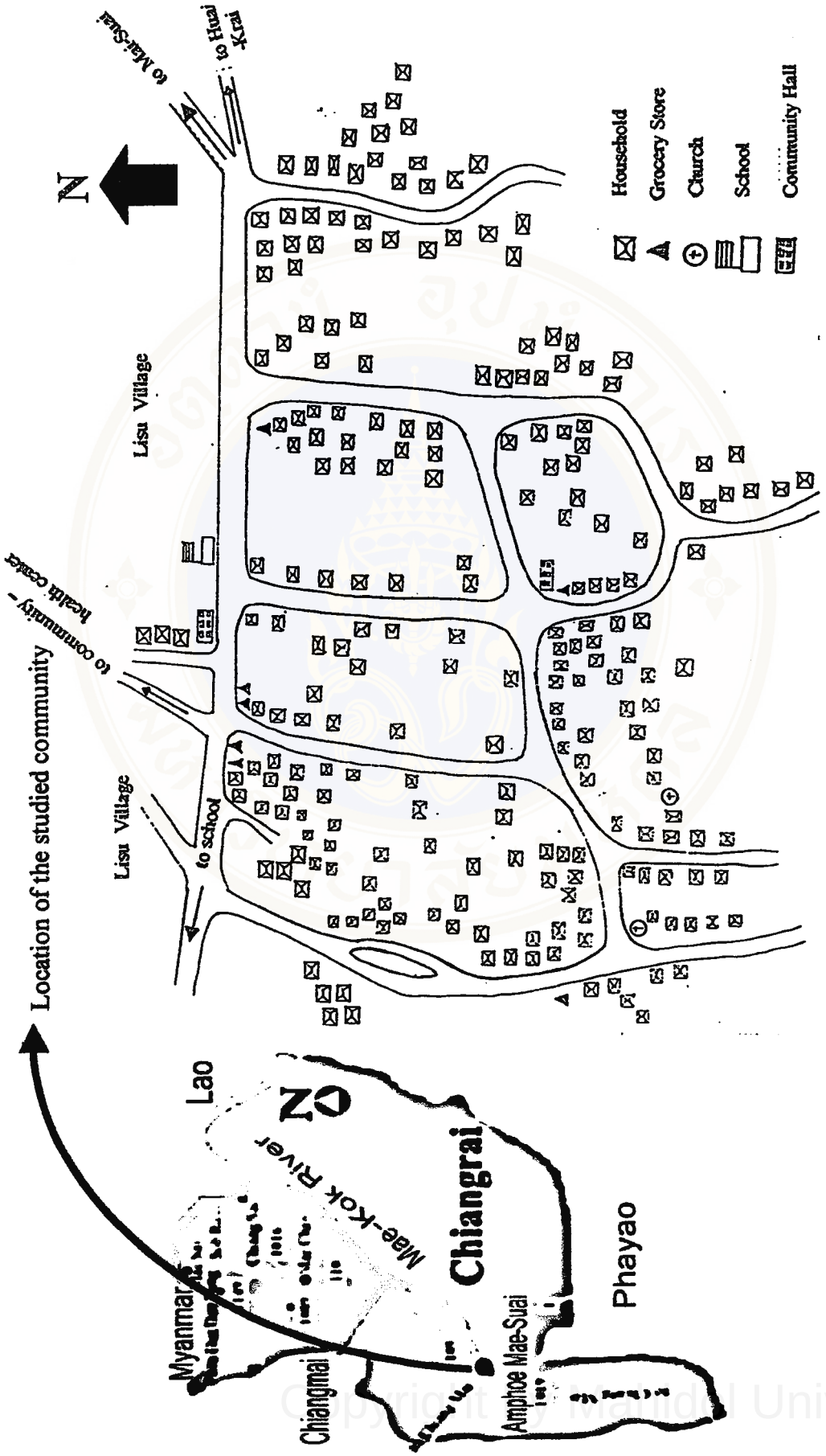


Figure 9 Map of the Akha village, Ban-Doi-Chang, Amphoe Mae-Suai, Chiangrai Province, Thailand

4.1 General Characteristics of the Studied Community

4.1.1 Studied subjects

The studied community was the Akha-hill tribes at Ban-Doi-Chang, Amphoe Mae-Suai, Chiangrai Province, Thailand. The data were obtained from one hundred and ninety youth participants by collecting blood specimens and interviewing the socio-economic factors, personal hygienes and child care behaviors. Among these participants, there were one hundred and seventy households. Each household was screened the bacterial quality of drinking water by using SI-2 media and observed the environmental sanitation conditions and sanitary factors.

There were seventy-five households with children under 5 years old, and only forty caretakers of households could give child health care information whereas the rest could not give the information because they were not caretakers of the children.

The distribution of the age, gender, family status, marital status, educational status, occupation, monthly income, economic status, numbers of household members, illness treatment and accessibility to HAV infection of the Akha-hill tribe youth are shown in Table 3.

Age of the Akha-hilltribe youth ranged from 15 to 24 years. The mean of age was 21 years with standard deviation of 3.33 years. Nearly 43% of them were 15 to 19 years and about 57% were young adults with age of 20 to 24 years. The male subjects had higher percentage than female subjects. Regarding the family status, 67.89% were sons or daughters, 14.21% were head of families, 11.58% were housewives and only 6.32% were tenants or cousins. A little more than half were single. Based on the educational status, almost 50% did not attend the school and 36.32% finished primary

school education. The small of them had higher education in secondary school, high school or university education.

It was found that the majority of the Akha-hill tribe youth were agriculturist and labourer, only 8.95% have been studying. The range of monthly income was 200-35,000 baht, 41.05% earned less than 2,000 baht a month, 22.11% had monthly income in the range of 2,001-4,500 baht, 24.73% earned more than 4,500 baht a month, and 12.11% did not know their family income. A little more than half of them thought that they earned enough for expenses, whereas nearly half of them thought that their earning were not enough.

The numbers of household members ranged from 2 to 20 persons with mean of 6.43 persons and standard deviation of 2.87 persons. About 57% had 1 to 6 persons in household and the rest had more than 7 persons in household.

Regarding the pattern of health seeking behaviors of the studied Akha-hill tribe youth, most of them went to the nearest hospital or community health care center when they were ill. Almost 48% used to receive the information about hepatitis A infection, only 5.26% had a history of jaundice and 3.16% had a history of having jaundiced patients in their families.

Table 3 General characteristics of studied subjects

	General characteristics	Numbers (n=190)	Percentage
Age			
	15-19	81	42.63
	20-24	109	57.37
Gender			
	Male	117	61.58
	Female	73	38.42
Family status			
	Head of family	27	14.21
	Housewife	22	11.58
	Son or daughter	129	67.89
	Tenant or cousin	12	6.32
Marital status			
	Single	104	54.74
	Married	80	42.11
	Separated	6	3.15
Education			
	No education	94	49.47
	Primary school	69	36.32
	Secondary school and high school	26	13.68
	University	1	0.53
Occupation			
	Labourer	43	22.63
	Agriculturist	130	68.42
	Studying	17	8.95

Table 3 General characteristics of studied subjects (Continued)

General characteristics	Numbers (n=190)	Percentage
Monthly income (Baht)		
≤ 2,000	78	41.05
2,001 - 4,500	42	21.11
> 4,500	47	24.73
Did not know	23	12.11
Economic status		
Not enough and must raise a loan	43	22.63
Not enough	49	25.79
Enough for expenditure	78	41.05
Enough with saving	20	10.53
Numbers of household members		
≤6 persons	109	57.37
7-11 persons	72	37.89
>11 persons	9	4.74
Illness treatment		
Go to the nearest hospital	81	42.63
Go to the nearest community health center	96	50.53
Go to private clinic	8	4.21
Self medication treatment	5	2.63
Accessibility to HAV infection		
Yes	90	47.37
No	100	52.63
Previous jaundice history		
Yes	10	5.26
No	180	94.74
History of having jaundiced patients in family		
Yes	6	3.16
No	184	96.84

4.1.2 Studied households

Among these studied subjects, there were 170 households. Mountain pipe water was the main source of drinking and using water (100%). It was found that studied households having own latrine were 55.29%, 18.24% shared a latrine with neighbor, and 11.18% shared with their extended family. However, 15.29% of participants went to the forest for defecation. When the latrines were full with human excreta, the most common practice for sludge removal was done by municipal or independence disposal service (33.53%), 14.12% replied that they had broken the tank, and 14.70% their latrine had not been emptied. Most of studied households (90%) drained their daily life wastewater directly through the yard.

With respect to household refuse management, 52.94% burned their refuse in open field, 1.18% did not manage their refuse, and 22.94% left it in a pit. Almost 92% had insects and rodent. An effort to get rid of these insects and rodent was done in 48.24% by using chemical or trap.

For children under 5 years in each household, there were 75 households with 1 to 4 children. The distribution of 1 child and 2 children were nearly equal, only a small number of households had more than 2 children. Details of general characteristics of studied households are shown in Table 4.

Table 4 General characteristics of the studied households

General characteristics	Numbers (n=170)	Percentage
Drinking water source of studied households		
Mountain pipe water	170	100.00
Using water source of studied households		
Mountain pipe water	170	100.00
Use of latrine		
Owner	94	55.29
Sharing with their extended family	19	11.18
Sharing with neighbor	31	18.24
Defecation in the forest	26	15.29
Latrine emptying method		
Municipal or independent disposal service	67	33.53
Breaking the tank	24	14.12
The latrine had not been emptied	25	14.70
Others:		
Sharing with neighbor	31	18.24
Defecation in the forest	26	15.29
Made new one	7	4.12
Wastewater management		
Drained to wastewater drainage canal or pond	17	10.00
Drained through the yard	153	90.00

Table 4 General characteristics of the studied households (Continued)

Conditions of environmental sanitation	Numbers (n=170)	Percentage
Household refuses management		
Leave in a pit	39	22.94
Leave on the ground and occasionally burned	90	52.94
Leave on the ground or outside the village	39	22.94
Not management	2	1.18
Insect and rodent in the house		
No	14	8.24
Yes	156	91.76
Insect and rodent control method		
Chemical or trap	82	48.24
No control	74	43.53
Have no insect and rodent	14	8.23
Under 5 years children in household		
Household with children under 5 years	75	44.12
Household without children under 5 years	95	55.88
Number of children under 5 years in household (n=75)		
1 child	33	44.00
2 children	35	46.67
3 children	6	8.00
4 children	1	1.33

4.1.3 Environmental sanitation conditions and bacterial quality of drinking water

4.1.3.1 Studied households' environmental sanitation

The studied households were observed on their environmental sanitation conditions, and it was found that about 58% of their surrounded dwelling had no any manure, 35.29% had some manure and created a little bit odor nuisance, only 7.06% had plenty of manure and created too much foul odor nuisance. Most of studied households (74.71%) kept their residential surrounding cleanliness in fair level. Regarding raising domestic animal, about 72% kept their domestic animals in premises, about 13% kept them in the house but did not create odor nuisance and 15.29% kept them in the house and made foul odor nuisance. A lot of refuse around the residence had been observed in about half of studied households (49.41%), 27.65 % had no refuse around the residence but had been observed on the street, and about 23% had neither been observed any refuse around the residence nor on the street.

For sanitary latrine available, 55.29% of the studied households had sanitary latrine facility, 44.71% had no household latrine. About 54% of studied households had no wastewater remained, 42.94% had some wastewater remained, and 2.94% had too much wastewater remained in residence and created offensive smell. Most of them (61.76%) used insanitary container for refuse disposal, less than 1% had sanitary container and 37.65% did not have refuse container. For their habit of refuse disposal, most of them (48.24%) occasionally disposed the refuse, only 15.88% always disposed it, and 35.88% of youth participants had done nothing.

A majority of the studied households' housing (52.94%) were strong firm house, 25.29% were fair firm house, and 21.77 % were temporary house. It was

found that most of studied households (44.12%) were fair level in house space utilization, about 41 % were poor in house space utilization and only 15.29% were good in house space utilization. For their house cleanliness, most of them (51.18%) were fair clean, 37.06% were poor clean and 11.76% had the clean house. Almost 69% had poor indoors lighting and ventilation, 17.06% had good indoors lighting and ventilation, and 14.12% of fair indoors lighting and ventilation.

Concerning food sanitation, most of them (82.94%) did not have food storage cabinet, 12.94% stored their cooked food in food storage cabinet or covered with sanitary shield, only 4.12% uncovered their cooked food in open air. Most of studied households' kitchens (51.76%) were unclean and untidy whereas about 41% were clean but untidy, only 7.65% were clean and tidy kitchen. A majority of studied households' dining place (82.35%) were fair clean, 12.35% were dirty dining place, and only 5.30% had the clean dining place. Most of the studied households' utensil (64.70%) were clean but not good keeping whereas only 7.65% were clean and good keeping, 27.65% were dirty utensil. About 63% attempted to control the households' insect and rodent. The details of the environmental sanitation conditions are shown in Table 5.

Table 5 Environmental sanitation conditions of the studied households

Condition of environmental sanitation	Numbers (n=170)	Percentage
Residential environmental surrounding		
1. Manure from raising domestic animal surrounded the residence		
Not any manure	98	57.65
Some and created a little bit odor nuisance	60	35.29
Plenty and created too much foul odor nuisance	12	7.06
2. Cleanliness of surrounded residence		
Clean	26	15.29
Fair	127	74.71
Dirty	17	10.00
2. Place to kept domestic animal		
Kept in premises	122	71.76
Kept in the house but did not create odor nuisance	22	12.95
Kept in the house and created odor nuisance	26	15.29
3. Refuse leave around residence.		
Neither around nor on the street	39	22.94
Not around but leave on the street	47	27.65
Lots of refuse	84	49.41
Available sanitary latrine		
No latrine	76	44.71
Own	94	55.29
Wastewater around residence		
No wastewater remained	92	54.12
Some wastewater remained	73	42.94
Too much wastewater remained and offensive smell	5	2.94

Table 5 Environmental sanitation conditions of the studied households (Continued)

Condition of environmental sanitation	Numbers (n=170)	Percentage
Refuse disposal		
1. Sanitary container in the residence		
Used Sanitary container	1	0.59
No garbage container	64	37.65
Insanitary container	105	61.76
2. Habit of refuse disposal		
Always	27	15.88
Occasionally	82	48.24
None	61	35.88
Housing		
1. House firmness		
Good	90	52.94
Fair	43	25.29
Poor	37	21.77
2. Utilization of house space		
Good	26	15.29
Fair	75	44.12
Poor	69	40.59
3. House cleanliness		
Good	20	11.76
Fair	87	51.18
Poor	63	37.06
4. Indoors lighting and ventilation		
Good lighting that can read or write and fresh air flow	29	17.06
Fair lighting that can read or write but no air flow	24	14.12
Poor lighting that can not read or write and no air flow with musty smell	117	68.82

Table 5 Environmental sanitation conditions of the studied households (Continued)

Condition of environmental sanitation	Numbers (n=170)	Percentage
Food sanitation		
1. Food storage or protection		
Sanitary food storage cabinet or shield	22	12.94
Insanitary food storage cabinet or shield	7	4.12
No food storage cabinet or shield	141	82.94
2. Kitchen cleanliness		
Clean and tidy	13	7.65
Clean but untidy	69	40.59
Unclean and untidy	88	51.76
3. Dining place		
Clean	9	5.30
Fair	140	82.35
Dirty	21	12.35
4. Utensil cleanliness and good keeping		
Clean and good keeping	13	7.65
Clean but not good keeping	110	64.70
Dirty utensil	47	27.65
Insect and rodent control		
Control	106	62.35
No control	64	37.65

The scoring results of observed environmental sanitation conditions were followed by Uytrakul, *et al.* (109). They were as following.

1. Residential environmental surrounding, wastewater around residence, garbage disposal, and housing, each item had three choices, good environmental sanitation was given a score of 5, fair environmental sanitation was given a score of 3, and poor environmental sanitation was given a score of 1.

2. Available sanitary latrine had four choices, defecation in the forest was given a score of 0, sharing was given a score of 1, insanitary latrine was given a score of 3, and owner of latrine was given a score of 5.

3. Food sanitation had four items and three choices in each item

3.1 Food storage or protection, sanitary food storage cabinet or shield was given a score of 5, insanitary food storage cabinet or shield was given a score of 3, and no food storage cabinet or shield was given a score of 0.

3.2 Kitchen cleanliness, clean and tidy was given a score of 5, clean but untidy was given a score of 4, and unclean and untidy was given a score of 0.

3.3 Dining place, and utensil cleanliness and good keeping, clean was given a score of 5, fair was given a score of 3, and dirty was given a score of 1.

4. Insect and rodent control, control was given a score of 5, and no control was given a score of 1.

The scores were calculated and divided into three levels of environmental sanitation conditions as well as good, fair, and poor. The mean of environmental sanitation conditions score was 47 with standard deviation of 12, and then good conditions were set at scores more than $\bar{x}$ + SD, fair conditions were set at scores equal to $\bar{x} \pm$ SD, and poor conditions were set at scores less than $\bar{x}$ – SD.

Regarding to the studied households' environmental sanitation conditions observation, most of the environmental sanitation conditions (70.00%) could be classified into fair conditions. Summary of environmental sanitation conditions score are shown in Table 6.

Table 6 Summary of studied household environmental sanitation conditions

Conditions of environmental sanitation	Numbers	Percentage
Good environmental sanitation conditions	26	15.29
Fair environmental sanitation conditions	119	70.00
Poor environmental sanitation conditions	25	14.71
Total	170	100.00

4.1.3.2 Bacterial quality of drinking water

For bacterial quality of studied households' drinking water, total of 170 studied households' drinking water samples were screened for coliform contamination by using SI-2 media, and it was found that the coliform contamination was 73.53%. It was found that most of studied's household with positive for coliform contamination in drinking water samples were the fair level group of environmental sanitation conditions (71.20%) and the poor level group (16.00%) were found more positive screening results than the good level group (12.80%). The results of coliform contamination in drinking water samples and studied households' environmental sanitation conditions with coliform contamination are presented in Table 7.

Table 7 SI-2 media screening results and environmental sanitation conditions of studied households

SI-2 media result and environmental sanitation conditions	Numbers of tested (n=170)	Positivity (%)
Positive for coliform contamination	**125**	**73.53**
Good environmental sanitation conditions	16	12.80
Fair environmental sanitation conditions	89	71.20
Poor environmental sanitation conditions	20	16.00
Negative for coliform contamination	**45**	**26.47**
Good environmental sanitation conditions	10	22.22
Fair environmental sanitation conditions	30	66.67
Poor environmental sanitation conditions	5	11.11

4.1.4 Personal hygiene and child health care behaviors

4.1.4.1 Personal hygiene

A majority of the studied subjects (79.47%) did not improve their drinking water. Most of the studied subjects (46.32%) stored their drinking water in earthen pot, 18.42% stored in the bottle, 26.32% stored in the gallon, only about 9% stored in the plastic icebox. It was found that 46.32% of the studied subjects cleaned their drinking water containers with water and brush only, 31.58% used detergent or dishwashing liquid, about 18% rinsed off with water alone, 2.63% shook with sand and water, and 1.58% never cleaned the container. For frequency of cleaning the drinking water container, about 41% reported that they cleaned it twice or three times a week, 33.16% cleaned it everyday, about a quarter cleaned it every week, and only 1.58% never cleaned. Most of them (82.10%) used the drinking cup to take the water from the container to drink, 16.32% poured from the container, only 1.58% directly drank from the container. Most studied subjects (95.26%) shared their drinking cup.

For hand-washing behavior, 53.16% of the subjects washed the hands after using toilet with water, 45.79% washed the hands with soap, and only about 1% did not washed the hands after using toilet. For food preparation, about 63% washed the hands with water, 38.42% washed with soap and water, and 1.58% did not wash. About 61% washed the hands with water before eating meal, whereas 38.42% washed with soap and water, and less than 1% did not wash their hands before eating meal.

Regarding food characteristics, 56.84% of them ate freshly cooked food, 14.21% reheated cooked food before eating, and 28.95% occasionally reheated cooked food before eating. Almost 69% protected their cooked food by covering with

dish or pot's lid, 6.84% kept in food cabinet, 10% used shield cover, and 14.74% did not protect. More than half of the studied subjects (51.58%) used chopstick and spoon for eating, one third always used spoon for eating, and 13.16% sometimes used hands directly to pick up the food. For habit to use shared spoon, 73.16% never used it. A majority of the respondents (78.42%) had meal on the table or Kun-toak, about 22% had meal on the ground floor or not fixed. About 81% used dishwashing or detergent and water for cleaning the dishes and utensil, 18.95% used water only.

Concerning eating raw food habit, most of the respondents (46.84%) occasionally ate it, whereas 42.63% never ate, and 10.53% always ate it. For eating raw clams habit, 92.63% never ate raw clams, and only 1.58% have eaten raw clams. Most of the participants (97.37%) never ate unclean strawberry, only 2.10% occasionally ate it and less than 1% always ate unclean strawberry habit. The details of personal hygiene behaviors of the studied subjects are shown in Table 8.

Table 8 Personal hygiene behaviors of studied subjects

Hygiene behaviors	Numbers (n=190)	Percentage
Method of improving households' drinking water		
Boiled	21	11.05
Filter	1	0.53
Not done	151	79.47
Others (occasionally boiled)	17	8.95
Drinking water container		
Earthen pot	88	46.32
Plastic icebox	17	8.95
Bottle	35	18.42
Gallon or others	50	26.32
Cleaning method of drinking water container		
Detergent or dishwashing liquid	60	31.58
Brushing and pouring with water only	88	46.32
Pouring without brushing	34	17.89
Others (cleaning with sand)	5	2.63
Not done	3	1.58
Frequency of cleaning drinking water container		
Everyday	63	33.16
Every week	47	24.74
Not done	3	1.58
Others (twice or three times a week)	77	40.52
Utensil used for taking drinking water from the drinking water container		
Pouring from the container	31	16.32
Dipper or drinking cup	156	82.10
Directly drink from the container	3	1.58

Table 8 Personal hygiene behaviors of studied subjects (Continued)

Hygiene behaviors	Numbers (n=190)	Percentage
Use of drinking cup		
Separate	6	3.16
Sharing	181	95.26
Directly drink from the container	3	1.58
Hand-washing after using toilet		
With soap	87	45.79
With only water	101	53.16
No hand-washing	2	1.05
Hand-washing before preparing food		
With soap	68	35.79
With only water	119	62.63
No hand-washing	3	1.58
Hand-washing before eating meal		
With soap	73	38.42
With only water	116	61.05
No hand-washing	1	0.53
Food characteristic		
Freshly prepared	108	56.84
Cooked food and reheat before eating	27	14.22
Cooked food and occasionally reheat before eating	55	28.93
Cooked food storage		
Food cabinet	13	6.84
Store without protect	28	14.74
Shield cover	19	10.00
Covered with dish or pot's lid	130	68.42

Table 8 Personal hygiene behaviors of studied subjects (Continued)

Hygiene behaviors	Numbers (n=190)	Percentage
Eating method		
Always used spoon	67	35.26
Hand and spoon	25	13.16
Chopsticks and spoon	98	51.58
Usage of shared spoon		
Yes	51	26.84
No	139	73.16
Substance used for cleaning the dishes		
Dishwashing or detergent and water	154	81.05
Water only	36	18.95
Place to have meal		
On the table or Kun-toak	149	78.42
On the ground floor	41	21.58
Raw food eating behavior		
Never	81	42.63
Occasionally	89	46.84
Always	20	10.53
Raw clam eating behavior		
Never	176	92.63
Occasionally	11	5.79
Always	3	1.58
Unclean strawberry eating behavior		
Never	185	97.37
Occasionally	4	2.10
Always	1	0.5

4.1.4.2 Child health care behaviors

Among seventy-five households with children under 5 years, there were 40 caretakers from these households who could give the child health care behaviors data. It was found that most of children's drinking water (72.50%) was unimproved mountain pipe water, others (27.50%) used boiled water. About 77.50% disposed child's feces in the yard or wastewater canal, only 22.50% disposed it in the latrine. Children's clothes contaminated with feces, 70% separately washed from the others. For rinsed water with feces, 97.50% poured down through the yard. The details of child health care behaviors are shown in Table 9.

Table 9 Child health care behaviors of studied households

Child health care behaviors	Numbers (n=40)	Percentage
Children's drinking water		
Boiled water	11	27.50
Mountain pipe water	29	72.50
Disposal management of child's feces		
Disposal in the latrine	9	22.50
Leave on the ground or throw outside the yard	31	77.50
Washing management of child's contaminated feces clothes		
Separated washing	28	70.00
Pool washing with others household member's clothes	12	30.00
Disposal management of child's contaminated feces washing water		
Disposal in the latrine	1	2.50
Poured down through the yard	39	97.50

4.2 Prevalence of Antibody to Hepatitis A Virus in the Akha-Hill Tribe Youth

One hundred and ninety serum specimens obtained from the Akha-hill tribe youth volunteers were determined for anti-HAV by serum antibody competitive test immunoassay. The results revealed that prevalence of anti-HAV in studied Akha-hill tribe youth was 87.89 %. When the prevalence was analyzed by age group and by sex, it was found that there was no statistically significant difference between age group and gender of the studied subjects (p = 0.46 and 0.16 respectively), as shown in Table 10. The distribution of anti-HAV antibody by age and gender are shown in Figure 10.

Table 10 Prevalence of anti-HAV among studied Akha-hill tribe youth by age group and gender

Variable	No. of tested	No. of positive	Prevalence of anti-HAV (%)
Age			
15-19	81	71	87.65 [a]
20-24	109	96	88.07 [a]
Gender			
Male	117	105	89.74 [b]
Female	73	62	84.93 [b]
Total	190	167	87.89

[a] No significant difference by proportion Z test, p = 0.46

[b] No significant difference by proportion Z test, p = 0.16

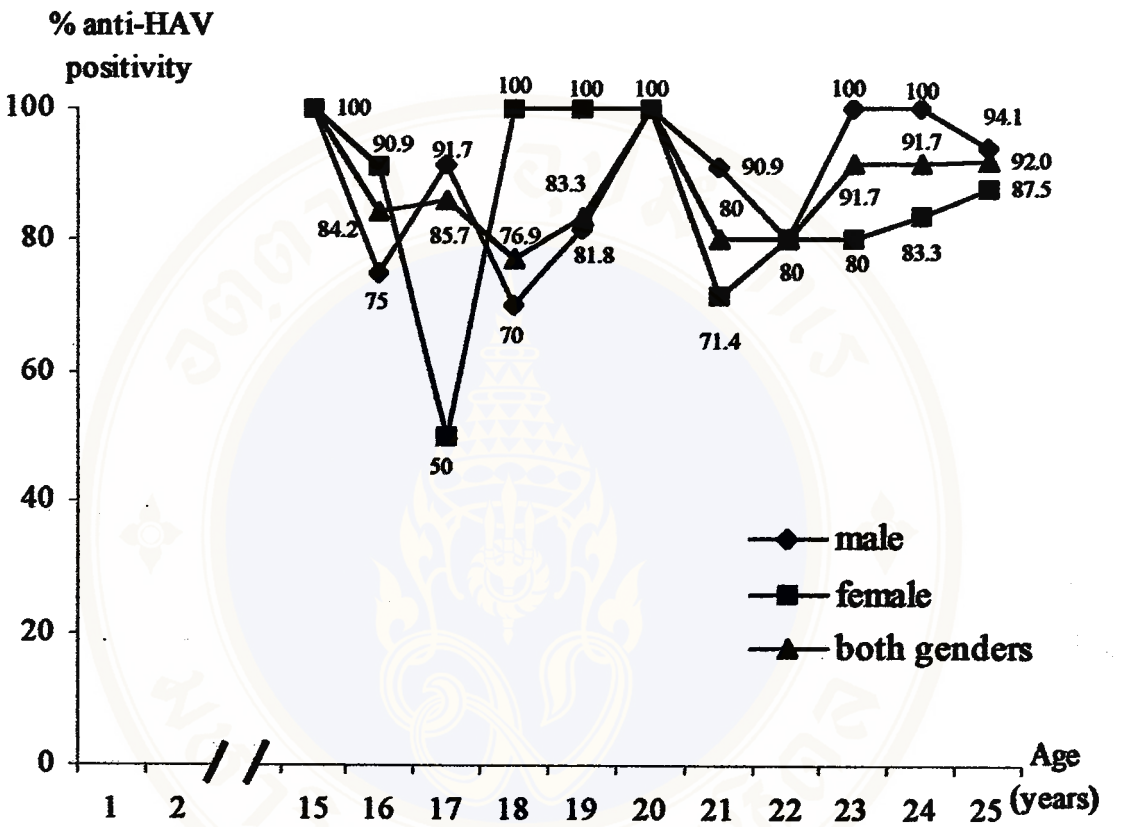


Figure 10 Age and gender specific prevalence of anti-HAV in the studied Akha-hill tribe youth, Amphoe Mae-Suai, Chiangrai Province, Thailand.

4.3 Socio-Economic Factors, Personal Hygiene Behaviors, Child Health Care Behaviors, and Environmental Sanitation Conditions associated With the Antibody to Hepatitis A Virus

From the laboratory results of 190 studied subjects, they were divided into 2 groups, the first group consisted of 167 studied subjects with anti-HAV positive and the second group consisted of 23 studied subjects without anti-HAV. The studied factors including socio-economic factors, personal hygiene behaviors, child health care behaviors and environmental sanitation conditions of two groups were compared and the results of some factors associated with anti-HAV positivity are shown in Table 11, 12, 13, and 14.

4.3.1 Socio-economic factors

Socio-economic factors, which included age, gender, education, occupation, monthly income and numbers of family members in the household, were analyzed. The study was found that monthly income and numbers of family member in the household were associated with anti-HAV positivity ($p = 0.040$ and 0.007, respectively). Those are shown in Table 11.

Table 11 Association between socio-economic factors and anti-HAV positivity

Socio-economic factors	Anti-HAV positive group (n=167)	Anti-HAV negative group (n=23)	p-value from χ^2 or Fisher's Exact test
Age (years)			
15-19	71 (42.52%)	10 (43.48%)	0.930
21-24	96 (57.48%)	13 (56.52%)	
Gender			
Male	105 (62.87%)	12 (52.17%)	0.323
Female	62 (37.13%)	11 (47.83%)	
Education			
No education	86 (51.50%)	8 (34.78%)	0.279
Primary school	59 (35.33%)	10 (43.48%)	
Secondary school or higher	22 (13.17%)	5 (21.74%)	
Occupation			
Labourer	37 (22.16%)	9 (39.13%)	0.116
Agriculturist	117 (70.06%)	13 (56.52%)	
Student	16 (9.58%)	1 (4.35%)	
Monthly income (Baht)			
≤ 2,000	74 (44.31%)	4 (17.39%)	0.040*
2,001 - 4,500	36 (21.56%)	6 (26.09%)	
> 4,500	40 (23.95%)	7 (30.43%)	
Did not know	17 (10.18%)	6 (26.09%)	
Numbers of household members			
≤ 6 persons	96 (57.49%)	13 (56.52%)	0.007*
7-11 persons	66 (39.52%)	6 (26.09%)	
> 11 persons	5 (2.99%)	4 (17.39%)	
Accessibility to HAV infection			
Yes	76 (45.51%)	14 (60.87%)	0.167
No	91 (54.49%)	9 (39.13%)	
Previous jaundice history			
Yes	8 (4.79%)	2 (8.70%)	0.347
No	159 (95.21%)	21 (91.30%)	
History of having jaundiced patients in family			
Yes	5 (2.99%)	1 (4.35%)	0.544
No	162 (97.01%)	22 (95.65%)	

* statistical significance at $\alpha = 0.05$

4.3.2 Personal hygiene behaviors

Personal hygiene behaviors, which included method of improving households' drinking water, drinking water container, method of cleaning drinking water container, frequency of cleaning drinking water container, utensil used for drawing drinking water from drinking water container, use of drinking cup, hand-washing behaviors, and food hygiene were analyzed. The study was found that, only one personal hygiene behavior, (hand-washing with soap after using toilet) was associated with anti-HAV positivity ($p = 0.013$). The details are shown in Table 12.

Table 12 Association between personal hygiene behaviors and anti-HAV positivity

Personal hygiene factors	**Anti-HAV positive group** (n=167)	**Anti-HAV negative group** (n=23)	**p-value from χ^2 or Fisher's Exact test**
Method of improving households' drinking water			
Boiled or filter	19 (11.38%)	2 (8.70%)	0.517
Occasionally boiled or not done	148 (88.62%)	21 (91.30%)	
Drinking water container			
Earthen pot or plastic icebox	93 (55.69%)	12 (52.17%)	0.904
Bottle	30 (17.96%)	5 (21.74%)	
Gallon or others	44 (26.35%)	6 (26.09%)	
Method of cleaning drinking water container			
Detergent or dishwashing liquid	53 (31.74%)	7 (30.43%)	0.456
Brushing and pouring with water only	75 (44.91%)	13 (56.52%)	
Only pouring with water or cleaning with sand or not done	39 (23.35%)	3 (13.04%)	
Frequency of cleaning drinking water container			
Everyday, 2-3 days or every week	164 (98.20%)	23 (100.00%)	ND
Not done	3 (1.80%)	0	

Table 12 Association between personal hygiene behaviors and anti-HAV positivity (continued)

Personal hygiene factors	Anti-HAV positive group (n=167)	Anti-HAV negative group (n=23)	p-value from χ^2 or Fisher's Exact test
Utensil used for taking drinking water from drinking water container			
Pouring from the container	26 (15.57%)	5 (21.74%)	0.628
Dipper or drinking cup or directly drink from the container	141 (84.43%)	18 (78.26%)	
Use of sharing drinking cup			
Separate	6 (3.59%)	0	ND
Sharing or directly drink from the container	161 (96.41%)	23 (100.00%)	
Hand-washing after using toilet			
With soap	71 (42.51%)	16 (69.57%)	0.013*
With only water including no hand-washing	96 (57.49%)	7 (30.43%)	
Hand-washing before preparing food			
With soap	59 (35.33%)	9 (39.13%)	0.721
With only water including no hand-washing	108 (64.67%)	14 (60.87%)	
Hand-washing before eating meal			
With soap	62 (37.13%)	11 (47.83%)	0.323
With only water including no hand-washing	105 (62.87%)	12 (52.17%)	
Food characteristic			
Freshly prepared	95 (56.89%)	13 (56.52%)	0.878
Cooked food and reheated before eating	23 (13.77%)	4 (17.39%)	
Cooked food and occasionally reheated before eating	49 (29.34%)	6 (26.09%)	
Cooked food storage			
Food cabinet or shield covered	142 (85.03%)	20 (86.96%)	0.550
Didn't shield	25 (14.97%)	3 (13.04%)	

Table 12 Association between personal hygiene behaviors and anti-HAV positivity (continued)

Personal hygiene factors	Anti-HAV positive group (n=167)	Anti-HAV negative group (n=23)	p-value from χ^2 or Fisher's Exact test
Eating method			
Always used spoon	61 (36.53%)	6 (26.09%)	0.351
Hand and spoon	20 (11.98%)	5 (21.74%)	
Chopsticks and spoon	86 (51.50%)	12 (52.17%)	
Usage of shared spoon			
Yes	45 (26.95%)	6 (26.09%)	0.931
No	122 (73.05%)	17 (73.91%)	
Place used for having meal			
On the table or on Kun-toak	129 (77.25%)	20 (86.96%)	0.219
On the ground or not fixed	38 (22.75%)	3 (13.04%)	
Substance used for cleaning dishes			
Dishwashing or detergent and water	134 (80.24%)	20 (86.96%)	0.327
Water only	33 (19.76%)	3 (13.04%)	
Raw clams eating behavior			
No	153 (91.62%)	23 (100.00%)	ND
Yes	14 (8.38%)	0	
Raw food eating behavior			
Never eat raw food	73 (43.71%)	8 (34.78%)	0.611
Occasionally eat raw food	76 (45.51%)	13 (56.52%)	
Always eat raw food	18 (10.78%)	2 (8.70%)	
Unclean strawberry eating behavior			
No	163 (97.60%)	22 (95.65%)	0.479
Yes	4 (2.40%)	1 (4.35%)	

* statistical significance at $\alpha = 0.05$

ND = Not determined

4.3.3 Child health care behaviors

Child health care behaviors, which included children's drinking water, disposal management of child's feces, washing management of child's contaminated feces clothes, and disposal management of child's contaminated feces washing water, were analyzed. The study was not found the association between child health care behavior and anti-HAV positivity, $p > 0.05$. The details are shown in Table 13.

Table 13 Association between child health care behaviors and anti-HAV positivity

Child health care behavior factors	Anti-HAV positive group (n=35)	Anti-HAV negative group (n=5)	p-value from χ^2 or Fisher's Exact test
Children's drinking water			
Boiled water	10 (28.57%)	1 (20.00%)	0.578
Mountain pipe water	25 (71.43%)	4 (80.00%)	
Disposal management of child's feces			
Disposal in the latrine	32 (91.42%)	3 (60.00%)	0.109
Leave on the ground or throw outside the yard	5 (14.28%)	2 (40.00%)	
Washing management of child's contaminated feces clothes			
Separated washing	24 (68.57%)	4 (80.00%)	0.523
Pool washing with others household member's clothes	11 (31.43%)	1 (20.00%)	
Disposal management of child's contaminated feces washing water			
Disposal in the latrine	1 (2.86%)	0	ND
Pored down through the yard	34 (97.14%)	5(100.00%)	

ND = Not determined

4.3.4 Studied households' environmental sanitation conditions

The studied households' environmental sanitation conditions factors of 190 subjects which included use of latrine, latrine emptying method, waste water management, households' garbage management, and households' insect and rodent eradication were analyzed. The study was found that use of latrine, latrine emptying method, household's refuse management and insect and rodent eradication were associated with anti-HAV positivity, ($p = 0.008$, 0.003, <0.001 and <0.001, respectively). The details are shown in Table 14.

Table 14 Association between households' environmental sanitation conditions and anti-HAV positivity

Environmental sanitation conditions	Anti-HAV positive group (n=167)	Anti-HAV negative group (n=23)	p-value from χ^2 or Fisher's Exact test
Use of latrine			
Owner or sharing with their extended family	106(63.47%)	21 (91.30%)	0.008*
Sharing with neighbor or defecation in the forest	61 (36.53%)	2 (8.70%)	
Latrine emptying method			
Municipal or independence service defecation removal	55 (32.95%)	9 (39.13%)	0.003*
Breaking the tank	19 (11.38%)	8 (34.78%)	
Others	93 (55.65%)	6 (26.09%)	
Waste water management			
Drained to waste water canal or pond	19 (14.38%)	1 (4.35%)	0.268
Drained through the yard	148 (88.62%)	22 (95.65%)	
Household refuse management			
Leave in a pit	30 (17.96%)	13 (56.52%)	<0.001*
Leave on the ground and occasionally burned	96 (57.49%)	8 (34.78%)	
Leave on the ground or outside the village	41 (24.55%)	2 (8.70%)	
Household insect and rodent			
Not have	13 (7.78%)	1 (4.35%)	0.407
Having insect and rodent	154(92.22%)	22 (95.65%)	
Insect and rodent control [a] (n=176)			
Chemical or trap	74 (48.05%)	21 (95.45%)	<0.001*
No control	80 (47.91%)	1 (4.55%)	

* statistical significance at $\alpha = 0.05$

[a] omitted households without insect and rodent

CHAPTER V

DISCUSSION

The results from this cross-sectional study demonstrated a high prevalence of anti-HAV (87.89%) among Akha-hill tribe youth of Ban Doi-Chang, Amphoe Mae-Suai, Chiangrai Province, Thailand. The high prevalence of anti-HAV of these studied subjects could be explained that people usually lived in unsatisfactory sanitary conditions resulting in contamination by HAV occurring early in life (48). Confirming Burkes' findings in the previous two decades, that 97% of Thai adults with age 16 years or older were anti-HAV positive (6) and Thai medical students whose age 20-21 years showed 73% of HAV seroprevalence in the same year (110). For the last ten years, anti-HAV prevalence in the rural eastern part of Thailand was 67.9% at Pong-Nam-Ron, Chanthaburi Province and 59.3% at Bo-Thong Districts, Chonburi Province (111). This study of anti-HAV prevalence in the youth of Akha-hill tribe demonstrated a high prevalence which contrast to data from the earlier study of anti-HAV in Bangkok students (10-19 years old) with 12.7% in 1996 (112). The seroprevalence of hepatitis A virus infection in urban part of Thailand has changed from hyperendemic into intermediate endemic status by the reason of improvement in socio-economic situation and sanitation (8,112).

There were no differences in the age-related seroprevalence that might be due to the infection occurring in the early age-group among these populatiion. Gender and education were not associated with anti-HAV positivity in this study. These results

supported the findings observed in Swedish population (113) and mother's education might be better marker of socio-economic status than the subject's education in population that HAV infection occurred early in childhood (114, 115).

Socio-economic factors were closely associated with the percentage of antibody-positive persons (57). In this present study, there was an association between family income and anti-HAV positivity ($p = 0.040$). This finding was similar to the study observed in the high prevalence of primary school children of lower income population in rural area of Ban-Lad-Na-Peang, Khon-Kaen Province, 1994 (116), and in other studies of high HAV infection prevalence related to the prevailing socio-economic conditions (50, 58, 62, 117-119).

This study was found an association between numbers of family members and anti-HAV positivity ($p = 0.007$). This result was consistent with higher prevalence of anti-HAV demonstrated in rural areas compared to urban areas of Greece, Switzerland, and Australia (48), large size family in young adults in North-East Italy which was exposed to HAV (120). The study of an urban Canadian area found more frequent HAV infection in larger families with numerous children among students (121).

Households with children under 5 years were not found to be significantly associated with anti-HAV positivity in this study ($p = 0.227$). It contrasted with several studies which the history of contacting with children infected hepatitis A virus was a significant risk factor (57, 85, 118, 122). Most HAV infected children under 6 years of age were asymptomatic or unrecognized infection (123). Infected children and infants could shed HAV for longer periods than adults, up to several months after onset of

clinical illness (10) so that they might play an important role in HAV transmission and serve as a source for interfamilial spread (71, 86, 124).

History of contacting with HAV infected persons is the most common risk factor for transmission (125, 126) and household contact was the most frequently reported source of infection (127). The usual source of infection is human feces (16). There was no relationship between previous jaundice history nor history of having jaundiced patients in family and anti-HAV positivity in this study that was similar to the study of pre-existing immunity against HAV in family member with index cases of hepatitis (128).

HAV can survive for prolong periods in the environment (37, 129, 130) therefore, food, water and shellfish are major vehicles of exposure implicated in transmission of HAV infection resulting in common sources of several outbreaks (13, 14, 88, 131). However, in this study was not found association between anti-HAV positivity and drinking water improvement, kind of drinking water container, method and frequency of cleaning drinking water container, sharing drinking cup or utensil used for drawing drinking water from drinking water container ($p > 0.05$). These findings revealed that food sanitation, food habit or eating hygiene were not associated with HAV positivity. Although mountain pipe water supply was available for all studied subjects, there was absence of water supplying treatment in this community. In addition, this community has used the same source of water, mountain pipe water, for daily use and consumption. These studied subjects might be infected with HAV in the childhood supported by the high anti-HAV positive in this study (87.89%). This study did not provide the laboratory for investigation of HAV contaminated in drinking

water or food, we could not rule out the possibility that consumption of HAV contaminated in drinking water and food may contribute to seropositivity for hepatitis A virus.

This study was found that, hand-washing after using toilet was associated with anti-HAV positivity ($p = 0.013$). This finding supported other studies that hand-washing was an important role of preventing HAV transmission (132-135). In this view point, proper hand-washing was considered to reduce the risk of HAV spread directly by hands and also to minimize the contamination of surfaces and objects contacted by hands.

Young children and infants may carry the HAV to susceptible hosts, usually caretakers. Secondary infections are recognized when adult contacts become ill (86, 136). The spread of HAV infection occurs when caretakers change and handle the dirty diapers (10, 118). There was no association between child health care behaviors and anti-HAV positivity in this study. The lack of association in these results might be due to a small numbers of caretakers who could give the child health care information. There were only 40 of 75 caretakers in all 190 participants who gave, which child health care data available for analysis.

There was an association between the use of latrine and anti-HAV positivity ($p = 0.008$). This study supported the findings from the unfortunate endemic investigation of HAV in school children at Nakhon-Si-Thammarat, Southern Thailand, in 1992, those symptomatic hepatitis patients showed evidence associated with no latrine in the family (137). Furthermore, defecation in the forest and proper hand-washing followed defecation were considered to be source of infection.

Full latrine management was associated with anti-HAV positivity ($p = 0.003$). It was possible that insanitary management with human excreta and breaking the tank spread the large amounts of HAV in feces, when the latrine was full with human excreta. Data from interviewing showed that some studied subjects broke the latrine tank while it rained. The rain rapidly removed unpleasant odor and disgusting scenery.

This study was not found an association between wastewater disposal and anti-HAV positivity. There was an absence of wastewater disposal in this community study. Several households disposed their wastewater from daily life through the yard and some disposed through canal or pond that they constructed. In laboratory studies, more than 90% of HAV survived more than 12 weeks in ground water, wastewater, and soil suspensions at 5°C (37). Hence, untreated wastewater before disposal, especially washing and bathing water from HAV infected person might leave the virus persisting in the environment for a long time. Although this recent study was not found the relation between sewage disposal and anti-HAV positivity, but it reflected that this community should be aware about HAV outbreak.

It was found that, household refuse management of the studied subjects was associated with anti-HAV positivity ($p < 0.001$). Garbage may be sources of several infections like cholera, parasitic helminthes, typhoid, etc., and breeding places of vectors such as flies, cockroach, rats, etc. Appropriate refuse disposal can reduce the transmission of food-borne and water-borne diseases. The results showed that household refuse disposed in a pit was better than leaving it on the ground and occasionally burned. Insect and rodent control were also found associated with anti-HAV ($p < 0.001$) in this study. Food may be contaminated by feces, domestic flies and

other carriers of diseases such as cockroaches, rats, etc. In this study, it was found that most of households in this community were infested with insects and vehicles of diseases. It is likely that they may play a part of the HAV contamination. The appropriate environmental management should be emphasized in this studied community for reducing the HAV infection and transmission.

CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

Conclusion

The general characteristics of studied youth participants revealed that mean of age was 21 years and higher in male proportion than female. There was no statistically significant difference between late adolescent and young adult ($p > 0.05$). Most of them had finished primary school or lower. The household income reported here seems to be low.

Observed environmental sanitation conditions data demonstrated a little better rural life style of hill people. They had available water throughout the year and facilitation in electricity. Most of their houses were temporary with poor in utilizing house space, cleanliness, and also to light and airflow. Furthermore, there were lack of available latrine, poor wastewater drainage, insanitary household refuse disposal, poor food sanitation, and poor in residential environmental surrounding. Unimproved mountain pipe water was the only source of drinking and using water of the studied community. The prevalence of bacterial contamination in drinking water screened by SI_2 media was 73.53%.

The high prevalence of anti-HAV positivity (87.89%) among these Akha-hill tribe youth prevailing in this study concluded that this rural community was high rates of HAV infection and the infection occurred early in the childhood. The prevalence of anti-HAV among studied participants was not different between gender. It did not demonstrate that the prevalence increased with age (15-24 years). It was found that

income related with anti-HAV positivity ($p = 0.040$). Anti-HAV positivity found more frequent in lower income. Anti-HAV positivity was associated with environmental sanitation conditions factors including, numbers of family members, human excreta disposal, household refuse disposal, and control of vectors and rodent ($p < 0.05$).

Hand-washing after using toilet, one of personal hygiene factors, was related with anti-HAV positivity ($p = 0.013$). Child health care behaviors including child's drinking water, child's excreta disposal, management with contaminated feces clothes and management with washing water contaminated feces, were not significantly associated with anti-HAV positivity. The other factors such as wastewater disposal, housing, food sanitation, method of improving households' drinking water, type of water container, cleanliness of drinking container, sharing drinking cup, dipping drinking cup in drinking water container, hand-washing before preparation food, hand-washing before eating, eating behavior, and raw food habit behaviors, were not significantly associated with anti-HAV positivity ($p > 0.05$).

Recommendations

Hepatitis A can affect anyone. Transmission is usually by drinking water or eating food that has been contaminated with fecal matter containing the virus, and direct contacting with an infected person. General measures for hepatitis A prevention include hygienic and sanitary measures to prevent transmission of any enteric illness. In household settings, adequate hand-washing, and proper food preparation are methods for reducing spread of the infection. At the community level, adequate chlorinating of drinking water, provision of safe drinking and proper disposal of sanitary waste will reduce the incidence of hepatitis A. Travelers, who visit and stay

for prolong period in rural areas of endemic diseases, should receive the hepatitis A vaccines. Although the study was carried out in the minority group of Thailand, these recommendations may applicable to the Nation Health Development Plan.

For the prevention and control the diseases

1. Health education

1.1 The health education about hygienic behaviors covering the following topics:

1.1.1 Proper hand-washing with soap before eating, preparation food, after defecation, and handling of food and water

1.1.2 Hygienic handling of food and water.

1.1.3 Improving eating and drinking hygiene habits such as:

Use of sharing spoon for eating, avoidance directly dipping drinking cup through drinking water container or avoidance directly drink from drinking container. Ready cooked food should be warm before eating.

1.1.4 Use of sanitary latrines and appropriate defecation.

1.2 The health education about sanitary storage of already cooked food.

1.3 Information and education about viral hepatitis should be provided for individual person and group by using posters, leaflet or community radio broadcasting. Community health workers should be trained for generalized prevention and control the disease to community level.

2. Improving environmental sanitation covering the following topics:

2.1 Provision and motivation latrine coverage and utilizing in every households.

2.2 Encouraging community to improve sewerage system by manual constructing wastewater drain canal from each household and drain through constructed village's wastewater pond.

2.3 Provision of safe and clean domestic water supply for drinking and using, and safe drinking water storage.

2.4 Promotion of sanitary housing including house cleanliness, good ventilation, and kitchen and latrine cleanliness.

2.5 Sanitary human excreta disposal while removal, emptying and transporting to disposal site.

3. Non-immune travellers or travellers from industrialized countries are likely to be susceptible to infection with hepatitis A virus, and should receive prophylactic immunoglobulin or the hepatitis A vaccine before travelling to this community.

For further study

1. Research study should be done for analysis and investigation risk factors, hygienic behaviors, environmental sanitation, and health behaviors and practices of mother or child caretakers, which effect or contribute to the endemic and epidemic areas of hepatitis A infection because it will give more reliable results for the influencing factors.

2. Investigation of HAV particles contaminated in the food, water and environment samples should be done in this community for community management and control the infection.

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APPENDICES

Appendix A

Interview Form

Subject's number .....

Participant's name..... Interviewer's name.....

Address .....Interview's date .....

<u>Part 1</u> Personal data

1. Age ..... year .....month
2. Gender ( ) 1. Male ( ) 2. Female
3. Family status
   ( ) 1. Head of family ( ) 2. Housewife
   ( ) 3. Son or daughter ( ) 4. Others (specified).....
4. Marital status
   ( ) 1. Single ( ) 2. Married
   ( ) 3. Widowed ( ) 4. Divorced
5. Education
   ( ) 1. No education ( ) 2. Primary school
   ( ) 3. Secondary school ( ) 4. High school
   ( ) 5. Vocational ( ) 6. University
   ( ) 7. Others (specified).....
6. Occupation
   ( ) 1. Government employee ( ) 2. Employee
   ( ) 3. Farmer ( ) 4. Agriculturist
   ( ) 5. Housewife ( ) 6. Others (specified).....
7. Family income ..... Baht / month
8. Economic status
   ( ) 1. Not enough and must be loan for expenditure
   ( ) 2. Not enough
   ( ) 3. Enough for expenditure
   ( ) 4. Enough with saving
9. Numbers of household members ..... person (s)
10. Below 5 years member in household ..... person (s)
    Immunization history .....
11. Illness treatment of household members
    ( ) 1. Go to the nearest hospital ( ) 2. Go to the nearest community health center
    ( ) 3. Go to the nearest clinic ( ) 4. Self medicine treatment
    ( ) 5. Not done ( ) 6. Others (specified).....
12. Accessibility to HAV infection
    ( ) 1. No ( ) 2. Yes, by .....
13. Previous jaundice history
    ( ) 1. Yes ( ) 2. No
14. History of having jaundiced patients in family
    ( ) 1. Yes ( ) 2. No

Part 2 Environmental sanitation data

1. Drinking water source
   ( ) 1. Pipe water ( ) 2. Rain water
   ( ) 3. Well water ( ) 4. River water
   ( ) 5. Others (specified).....
2. Household drinking water
   ( ) 1. Pipe water ( ) 2. Rain water
   ( ) 3. Well water ( ) 4. River water
   ( ) 5. Others (specified).....
   SI-2 media screening result.....
3. Using water
   ( ) 1. Pipe water ( ) 2. Rain water
   ( ) 3. Well water ( ) 4. River water
   ( ) 5. Others (specified).....
4. Use of latrine
   ( ) 1. Owner
   ( ) 2. Sharing with neighbor
   sharing between.....household (s),
   number of person who shared latrine.....person (s)
   ( ) 3. Defecation through the river
   ( ) 4. Defecation in the forest
   ( ) 5. Others (specified) .....
5. Latrine emptying method
   ( ) 1. Municipal disposal service
   ( ) 2. Independent disposal service
   ( ) 3. Breaking the tank
   ( ) 4. Others (specified) .....
6. Do you ever use your excreta as fertilizer?
   ( ) 1. Yes ( ) 2. No
7. Do you manage with your household wastewater?
   ( ) 1. Yes ( ) 2. No
   If yes, how do you manage with your household wastewater?
   ( ) 1. Waste water drain canal
   ( ) 2. Drained through waste water well
   ( ) 3. Drained through water source
   ( ) 4. Drained through the yard
   ( ) 5. Others (specified) .....
8. Do you manage with your household refuse?
   ( ) 1. Yes ( ) 2. No
   If yes, how do you manage with your household garbage?
   ( ) 1. Leave in a pit
   ( ) 2. Leave on the ground and occasionally burned
   ( ) 3. Leave on the ground
   ( ) 4. Others (specified).....
9. Has insect and rodent in your house?
   ( ) 1. No ( ) 2. Yes ☐ Control with (specified) .....
   ☐ No control

Part 3 Hygiene behaviors data

1. Method of improving drinking water
   ( ) 1. Boiled ( ) 2. Filtered
   ( ) 3. Not done ( ) 4. Others (specified).....
2. Drinking water container
   ( ) 1. Earthen pot ( ) 2. Bin
   ( ) 3. Bottle ( ) 4. Others (specified).....
3. Method of cleaning drinking water container
   ( ) 1. Detergent or dishwashing liquid
   ( ) 2. Brushing and pouring with water only
   ( ) 3. Only pouring with water
   ( ) 4. Others (specified).....
4. Frequency of cleaning drinking water container
   ( ) 1. Everyday ( ) 2. Every week
   ( ) 3. Not done ( ) 4. Others (specified).....
5. Utensil used for taking drinking water from drinking water container
   ( ) 1. Pouring from the container ( ) 2. Dipper
   ( ) 3. Drinking cup ( ) 4. Others (specified).....
6. Use of the drinking cup
   ( ) 1. Separate
   ( ) 2. Sharing between .....person (s)
   ( ) 3. Others (specified).....
7. Hand-washing after using toilet
   ( ) 1. Always with ☐ Only water ☐ Soap and water
   ( ) 2. Occasionally with ☐ Only water ☐ Soap and water
   ( ) 3. No hand-washing
8. Hand-washing before preparing food (including ready cooked food which buying)
   ( ) 1. Always with ☐ Only water ☐ Soap and water
   ( ) 2. Occasionally with ☐ Only water ☐ Soap and water
   ( ) 3. No hand-washing
9. Hand-washing before eating meal
   ( ) 1. Always with ☐ Only water ☐ Soap and water
   ( ) 2. Occasionally with ☐ Only water ☐ Soap and water
   ( ) 3. No hand-washing
10. Food characteristic of family member
    ( ) 1. Freshly prepared
    ( ) 2. Cooked food and reheated before eating
    ( ) 3. Cooked food and occasionally reheated before eating
    ( ) 4. Others (specified).....
11. Cooked food storage
    ( ) 1. Food storage cabinet ( ) 2. Sanitary shield covered
    ( ) 3. Did not shield ( ) 4. Others (specified).....

12. Eating method
( ) 1. Always use spoon ( ) 2. Hand and spoon
( ) 3. Hand only ( ) 4. Others (specified).....
13. Usage of shared spoon
( ) 1. Yes ( ) 2. No, because .....
14. Place used for having meal
( ) 1. On the table ( ) 2. On the ground floor of the house
( ) 3. Not fixed ( ) 4. Others (specified).....
15. Raw food eating behavior
( ) 1. Never ( ) 2. Occasionally ( ) 3. Always
16. Raw clams eating behavior
( ) 1. Never ( ) 2. Occasionally ( ) 3. Always
17. Unclean strawberry eating behavior
( ) 1. Never ( ) 2. Occasionally ( ) 3. Always
18. Substance used for cleaning the dishes
( ) 1. Dishwashing liquid or detergent and water
( ) 2. Water only
( ) 3. Others (specified).....

Below 5 years child health care behavior data

1. Children's drinking water
( ) 1. Boiled water
( ) 2. Pipe water
( ) 3. Others (specified).....
2. Disposal management of child's feces
( ) 1. Disposal in the latrine
( ) 2. Defecation in the spittoon then disposal in the latrine
( ) 3. Defecation on the latrine floor then flushing through waste water canal
( ) 4. Through the water source
( ) 5. Defecation on the ground then.....
☐ wiped and disposed in the latrine
☐ wiped and disposed in elsewhere (specified).....
( ) 6. Others (specified) .....
3. Washing management of child's contaminated feces
( ) 1. Separated washing
( ) 2. Pooled washing with others household member's clothes
( ) 3. Others (specified) .....
4. Disposal management of child's contaminated feces washing water
( ) 1. Disposal in the latrine
( ) 2. Poured down the latrine floor through waste water canal
( ) 3. Poured down through the yard
( ) 4. Others (specified) .....

Appendix B

Subject's number.....

Observation Record

Name.....Gender ( ) M ( ) F Age (Date of birth) .....

Address.....

Residential environmental surrounding

1. Manure from raising domestic animal surrounded the residence
   ( ) Not any manure
   ( ) Some and created a little bit odor nuisance
   ( ) Plenty and created too much foul odor nuisance
2. Cleanliness of surrounded residence
   ( ) Clean
   ( ) Fair
   ( ) Dirty
3. Place to keep domestic animal
   ( ) Kept in premises
   ( ) Kept in the house but do not created odor nuisance
   ( ) Kept in the house and created odor nuisance
4. Is there any refuse around the residence?
   ( ) Neither around nor on the street
   ( ) Not around but over there on the street
   ( ) Lots of refuse

Available of sanitary latrine

( ) Owner of sanitary latrine
( ) Owner of insanitary latrine
( ) Sharing with neighbor
( ) Defecation in the forest

Wastewater around residence

( ) No wastewater remained
( ) Some wastewater remained
( ) Too much wastewater remained and offensive smell

Refuse disposal

1. Sanitary refuse storage container in the residence
   ( ) Used sanitary refuse storage container
   ( ) No refuse storage container
   ( ) Insanitary refuse storage container
2. Habit of refuse disposal
   ( ) Always
   ( ) Occasionally
   ( ) No refuse disposal

Housing

1. House stability
   ( ) Good
   ( ) Fair
   ( ) Poor
2. Divided space
   ( ) Good
   ( ) Fair
   ( ) Poor
3. House cleanliness
   ( ) Good
   ( ) Fair
   ( ) Poor
4. Indoors lighting and ventilation
   ( ) Good lighting that can read or write and fresh air flow
   ( ) Fair lighting that can read or write but no air flow
   ( ) Poor lighting that can not read or write and no air flow with musty smell

Food sanitation

1. Food storage or protection
   ( ) Sanitary food storage cabinet or shield
   ( ) Insanitary food storage cabinet or shield
   ( ) No food storage cabinet or shield
2. Kitchen cleanliness
   ( ) Clean and tidy
   ( ) Clean but untidy
   ( ) Unclean and untidy
3. Dining place
   ( ) Clean
   ( ) Fair
   ( ) Dirty
4. Utensil cleanliness and good keeping
   ( ) Clean and good keeping
   ( ) Clean but not good keeping
   ( ) Dirty utensil

Insect and rodent control

( ) Control
( ) No control

Appendix C

Chemical reagents for SI-2 media preparation:

Peptone	10.00 gm
Lactose	10.00 gm
Sodium Desoxycholate	1.00 gm
Bromcresol Purple	0.02 gm
Distilled water	1,000.00 ml

BIOGRAPHY

NAME	Miss Supranee Tongpradit
DATE OF BIRTH	16 February 1965
PLACE OF BIRTH	Ubolrachathanee, Thailand
INSTITUTIONS ATTENDED	Khon Kaen University, 1982-1985: Bachelor of Science (Nursing)
	Mahidol University, 1998-2000: Master of Science (Public Health), Major Infectious Diseases
RESEARCH GRANT	International Medical Consultants (IMC Inc.) and Faculty of Public Health, Mahidol University, 1999
POSITION & OFFICE	1986 – 1995, Obstetric and Gynecological Ward, Srinakarin Hospital, Khon Kaen University, Khon Kaen, Thailand. Position: Registered Nurse
	1995 – Present, Research in Microbiology and Paediatric Infectious Diseases Division, Research Center, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. Position: Researcher