

HAV and HEV: What's New?



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The 14th Asian Pan-Pacific Society of Pediatric Gastroenterology, Hepatology and Nutrition
APPSPGHAN
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BANGKOK

**Toward Good Health and
Well-being of Children**



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Publication : more than 500 publications

Citation : > 14000 Cites

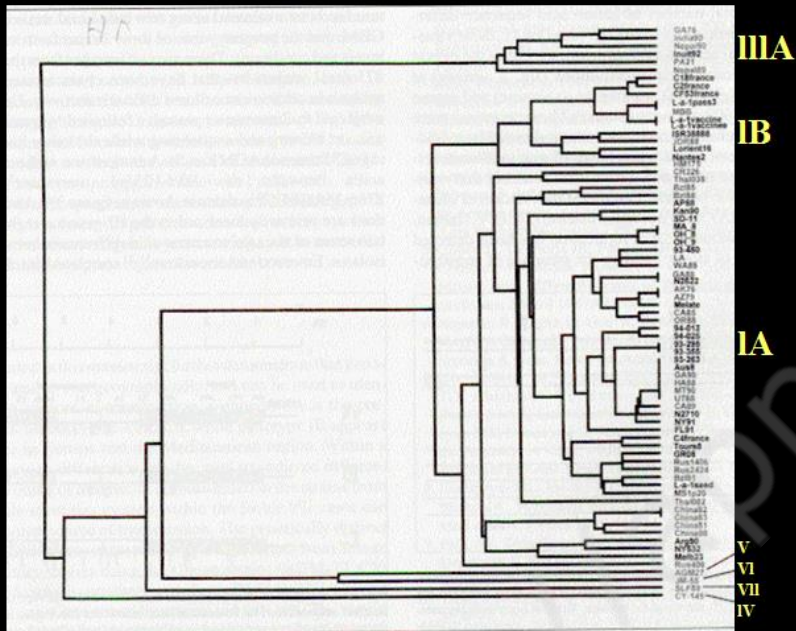
**Center Of Excellence In Clinical Virology, Faculty of
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Hepatitis A virus

- **Picornavirus (RNA)**
- **Humans are only natural host**
- **Stable at low pH**
- **Inactivated by high temperature formalin, chlorine**
- **Entry into mouth**
- **Viral replication in the liver**
- **Virus present in blood and feces after about 2 weeks**
- **Illness usually self-limited**

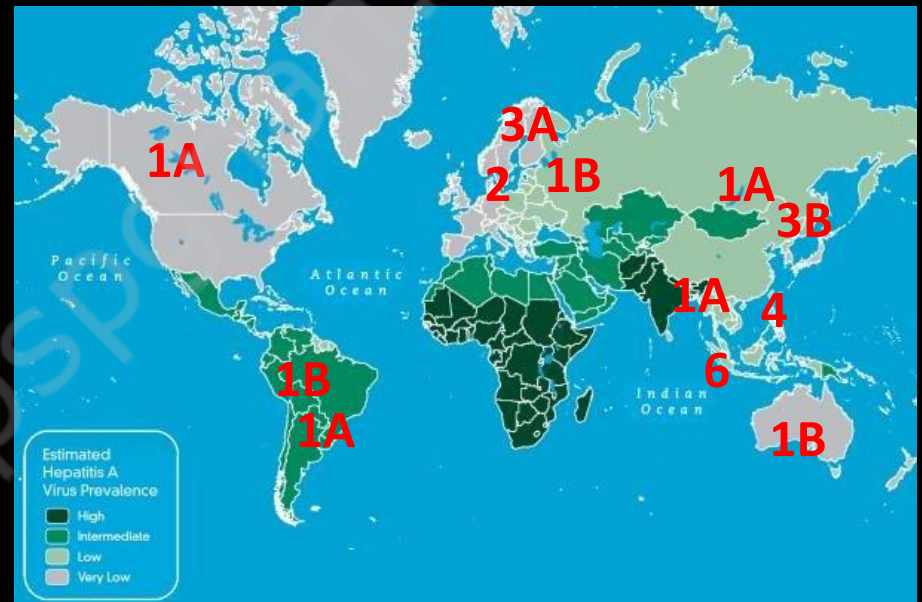
Hepatitis A Virus Genotype

HAV genotype



Dendrogram representing the relatedness of VP1 amino terminal nucleic acid sequences

Robertson BH 1997.



https://www.researchgate.net/publication/262383253_Enterick_hepatitis_viruses/figures?lo=1

HAV Extent of the problem

- **The incidence of enteric infections have fallen**
- **The incidence of hepatitis A has increased**

Global hepatitis A epidemiology

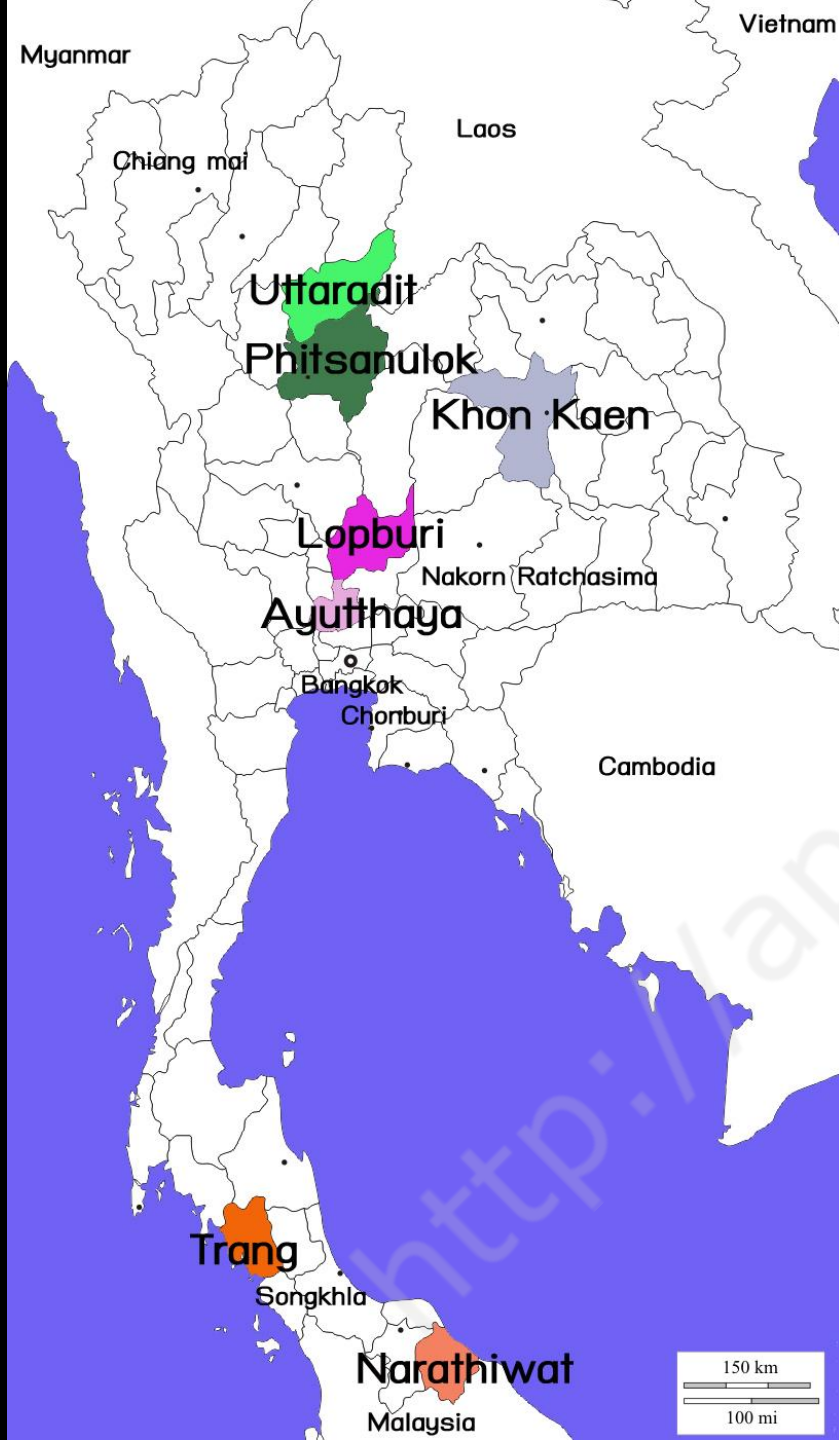
- **Developed countries**
- **Developing countries Improved living standard**
- **Developing countries, Poor sanitation and hygiene**

Geographic distribution of HAV infection





Epidemiology of hepatitis A



Northern part

Uttaradit : 904 (389 males, 515 females)

Phitsanulok : 518 (193 males, 325 females)

North-eastern part

Khon Kaen: 1,638 (854 males, 784 females)

Central part

Lopburi: 781 (389 males, 392 females)

Ayutthaya: 757 (313 males, 444 females)

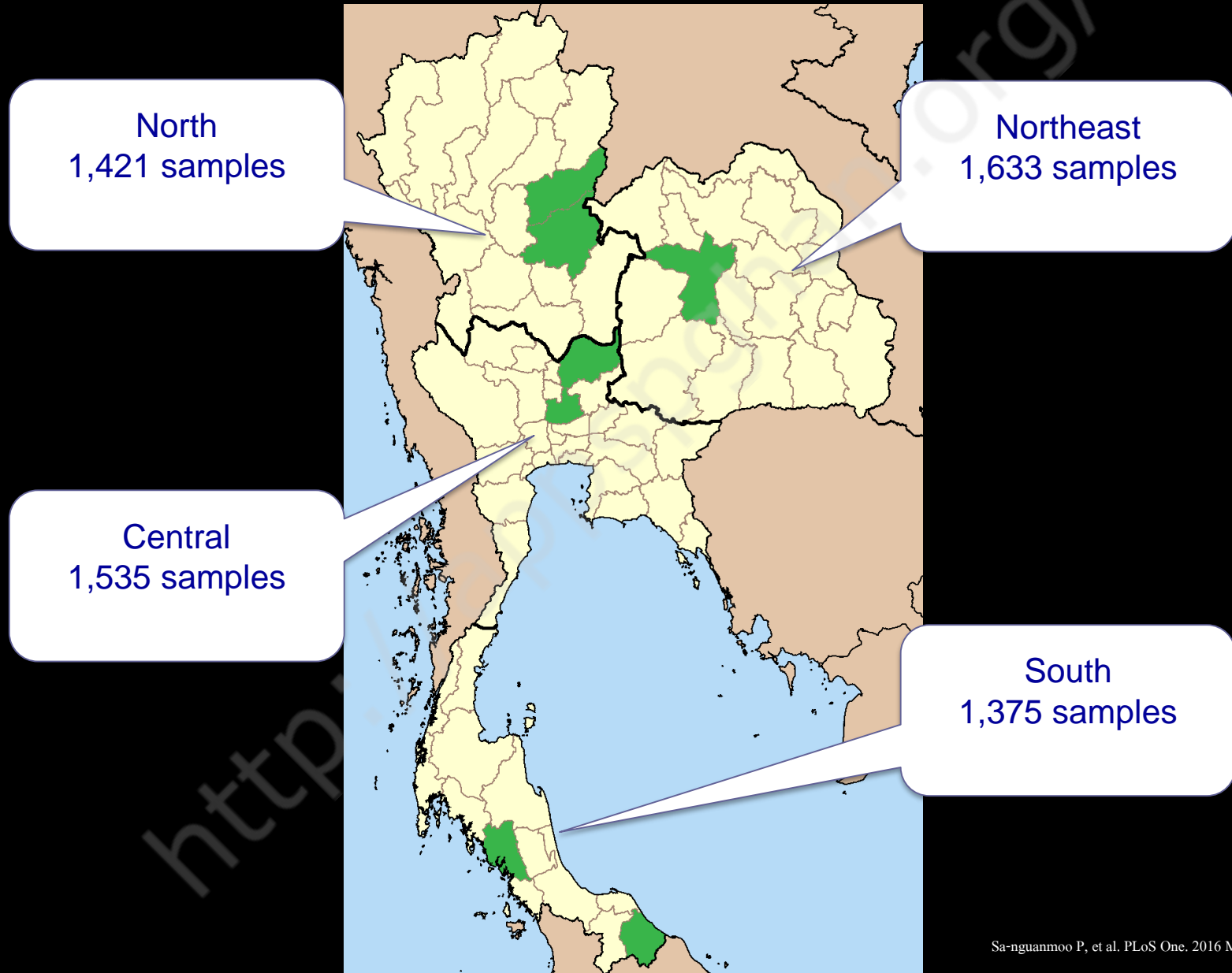
Southern part

Trang: 733 (297 males, 436 females)

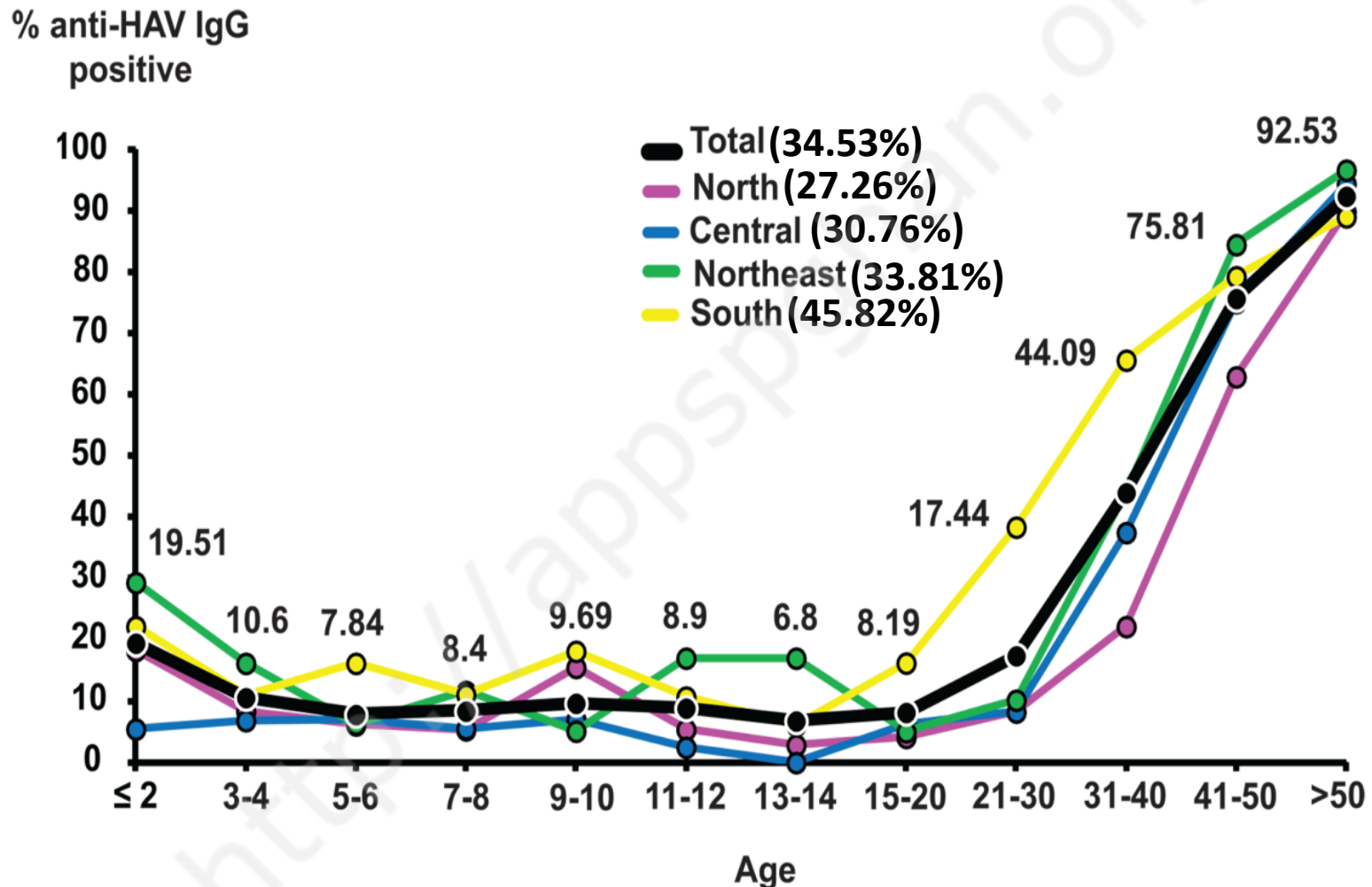
Narathiwat: 648 (173 males, 475 females)

Total: 5,979

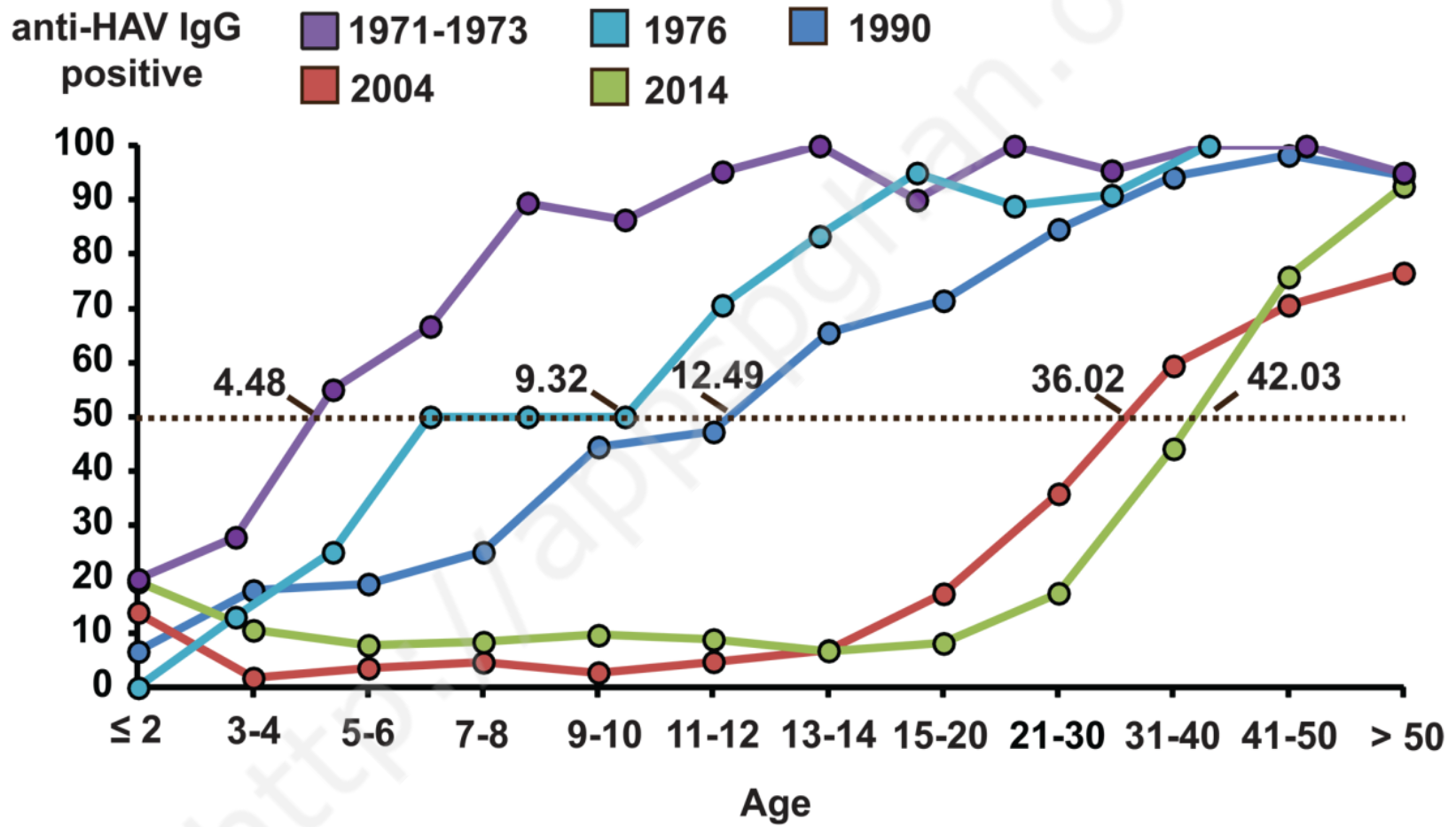
Sample Collection



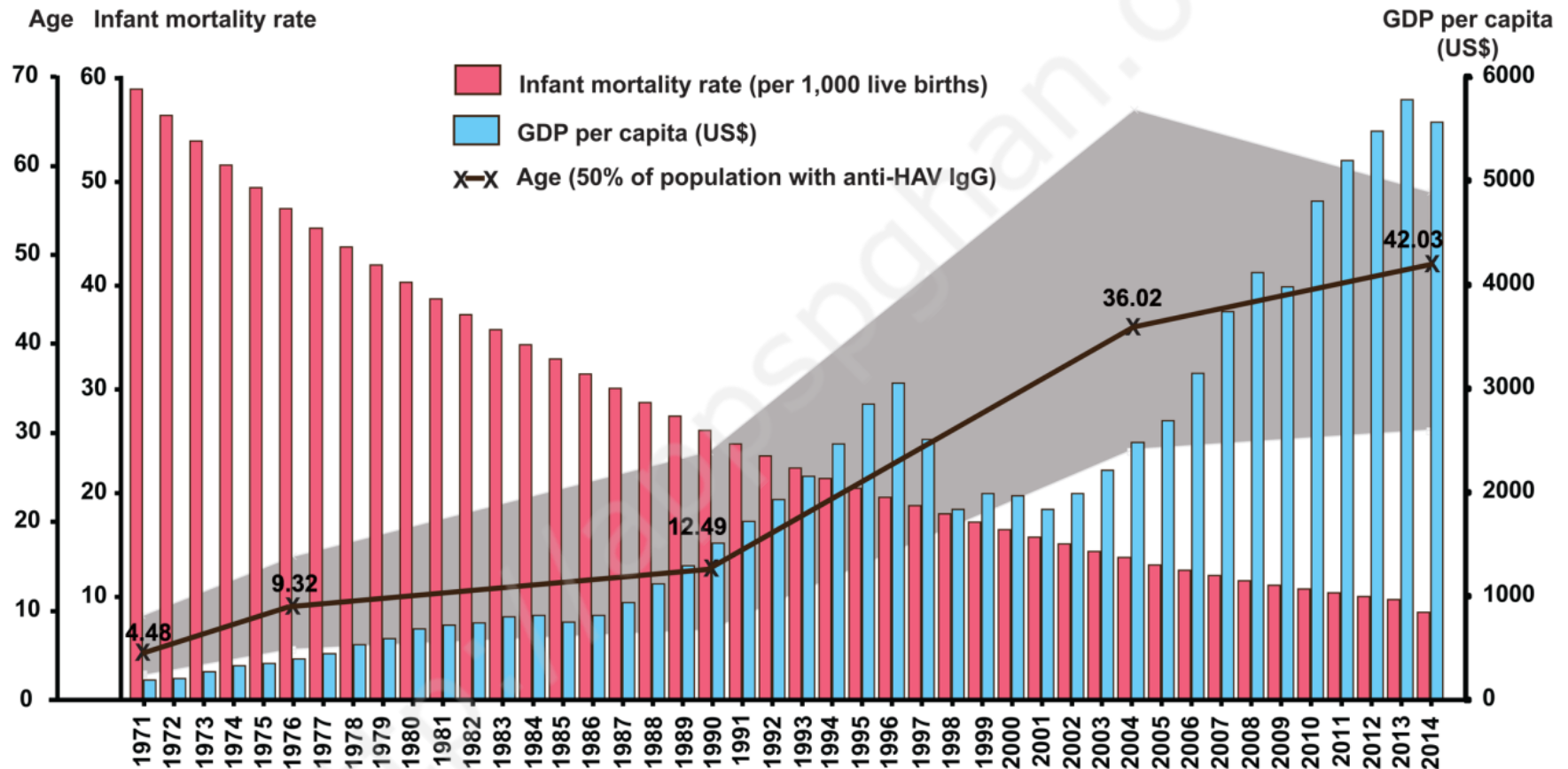
Comparison of anti-HAV IgG positive in each age group among region.



Comparison of anti-HAV IgG positive from 1971 to 2014.

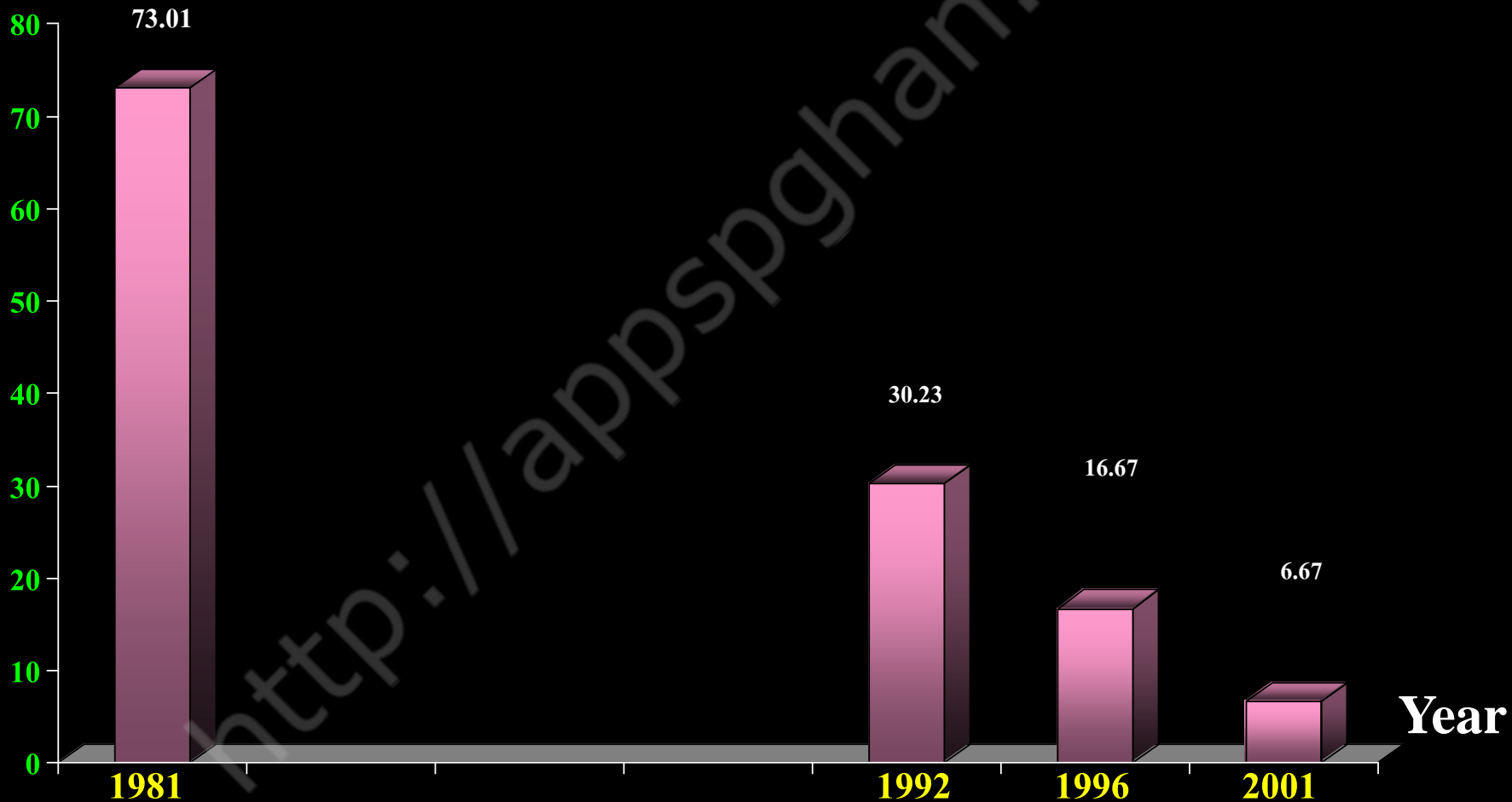


Correlation between infant mortality rate (per 1,000 live births), Gross Domestic Product (GDP) and age of 50% anti-HAV IgG seropositive in population



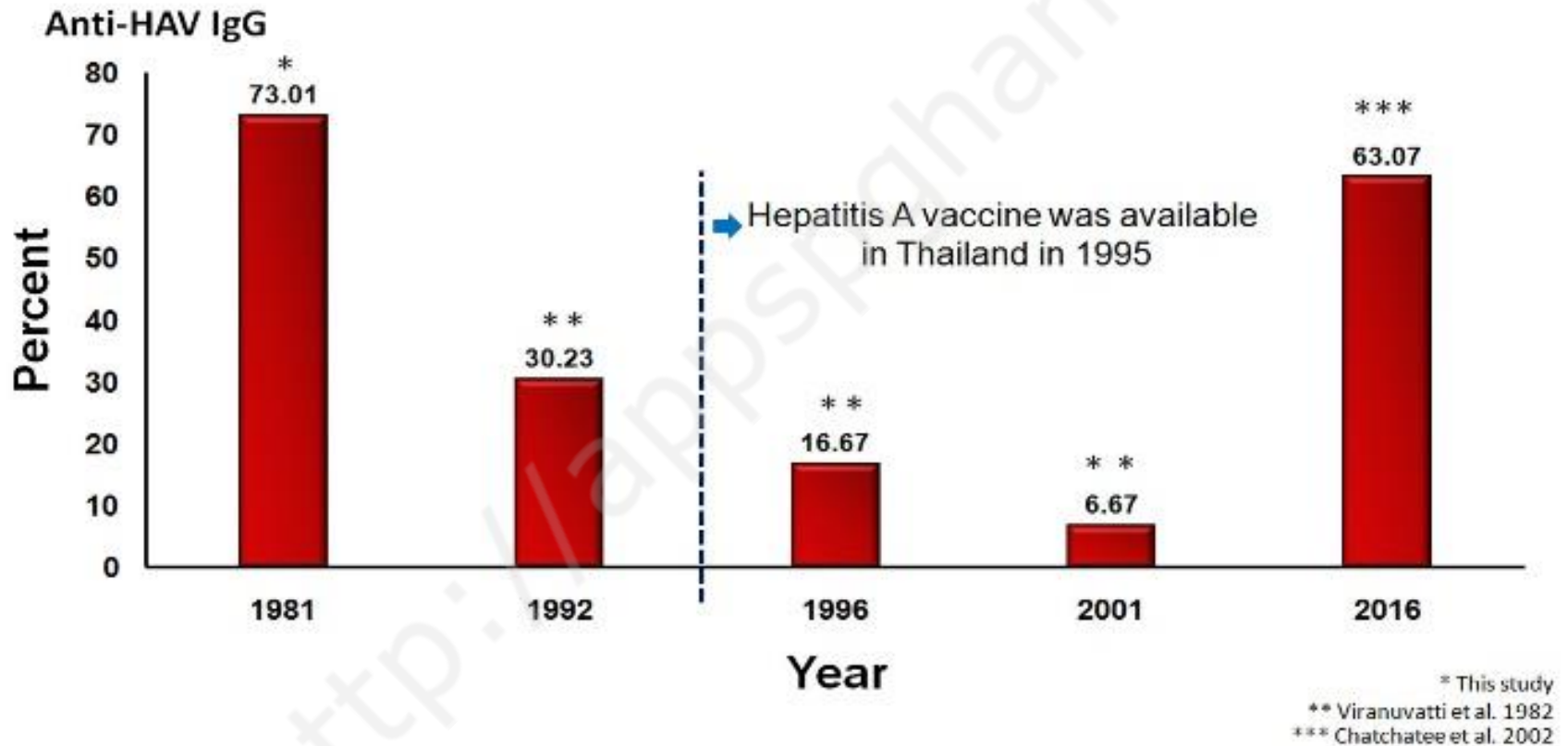
Seroprevalence of anti HAV among medical student in Bangkok

Anti-HAV (%)



Chatchatree et al 2002

Changes in hepatitis A virus (HAV) seroprevalence in medical students in Bangkok, Thailand, from 1981 to 2016



Sintusek P, Sa-Nguanmoo P, Posuwan N, Jaroonvanichkul V, Vorayingyong A, Poovorawan Y. BMC Res Notes. 2018 Sep 3;11(1):640.



Outbreak of hepatitis A

Hepatitis A outbreak

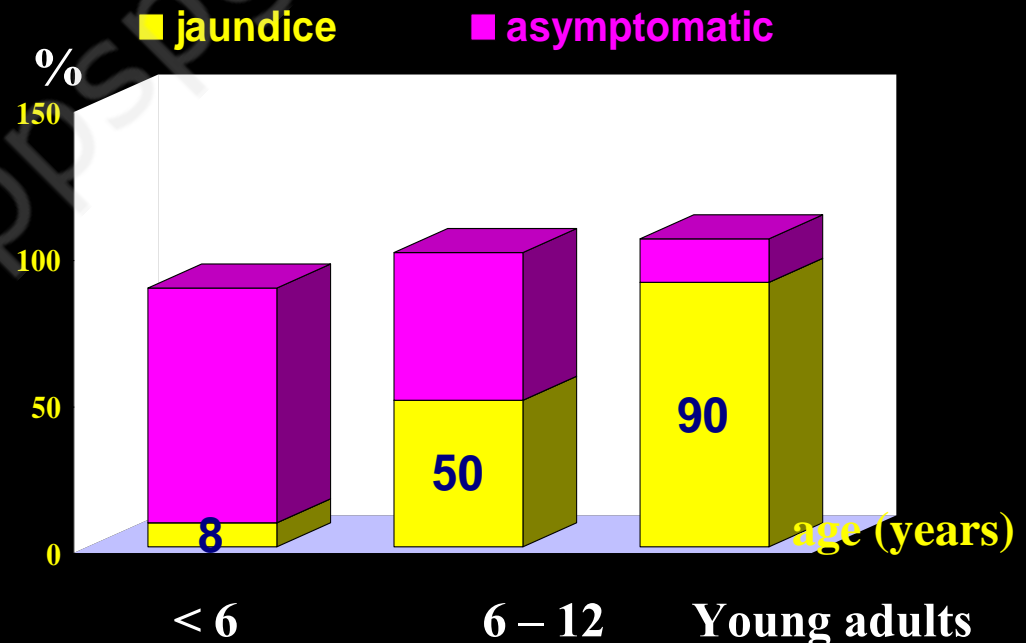
Child care institution, Thailand

- Number of children 112 cases
- antiHAV IgM positive 65 cases
- Children with clinical hepatitis 5 cases
- Symptomatic : asymptomatic 1:13
- No virus was detected in saliva
- HAV-RNA can be detected in stool for at least 3 wks.

Hepatitis A - The disease

- Severity of the hepatitis A is highly correlated with :

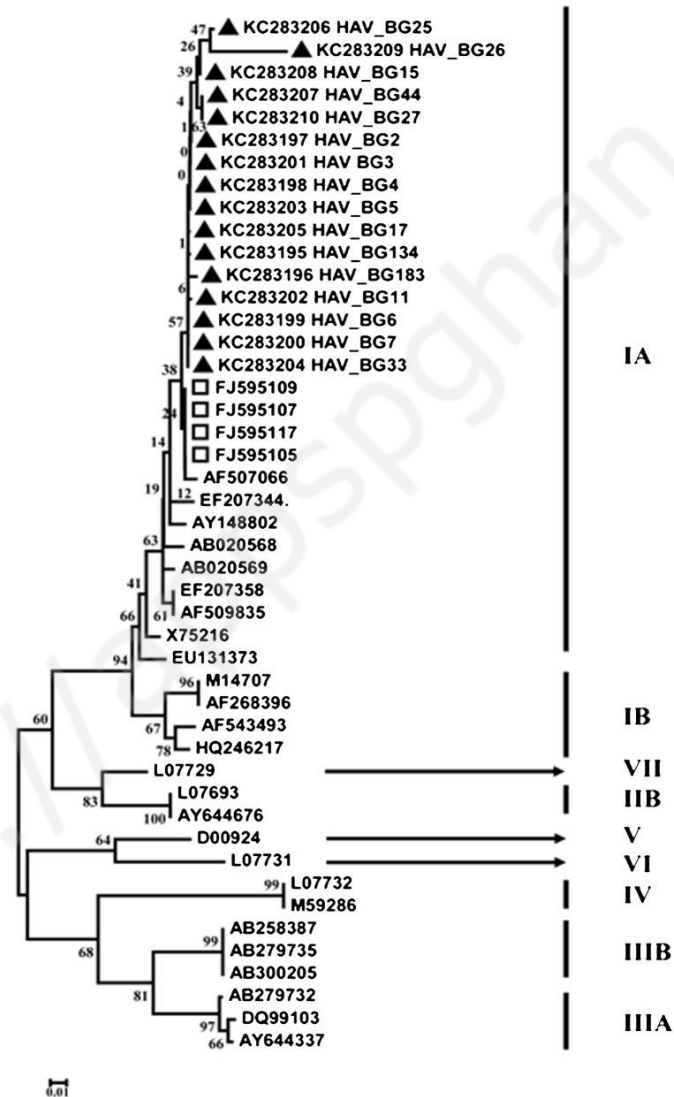
- the age of the subject
- previous liver disease



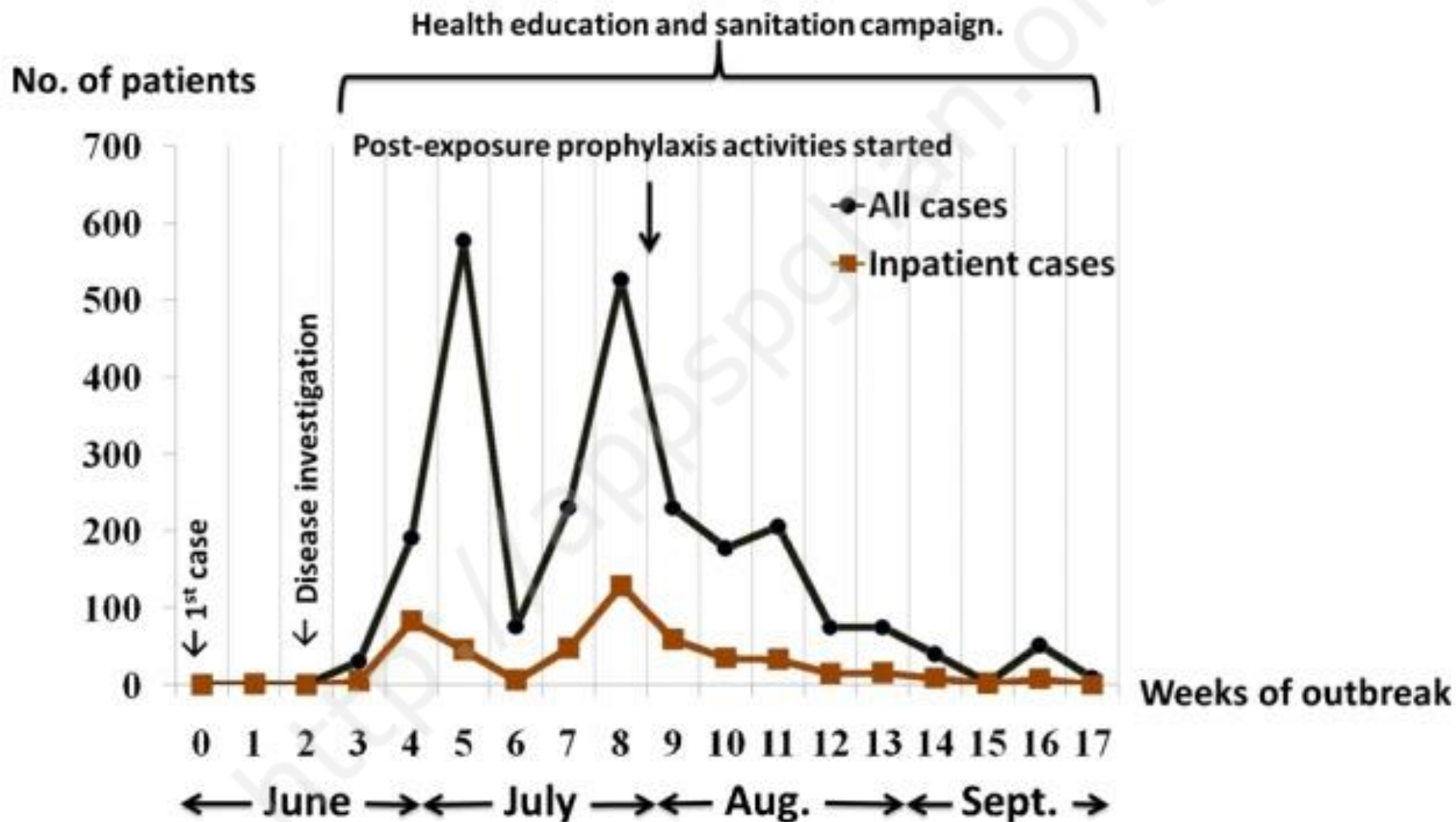
**Buengkan province where the large-scale hepatitis A virus (HAV) outbreak
occurred in 2012**



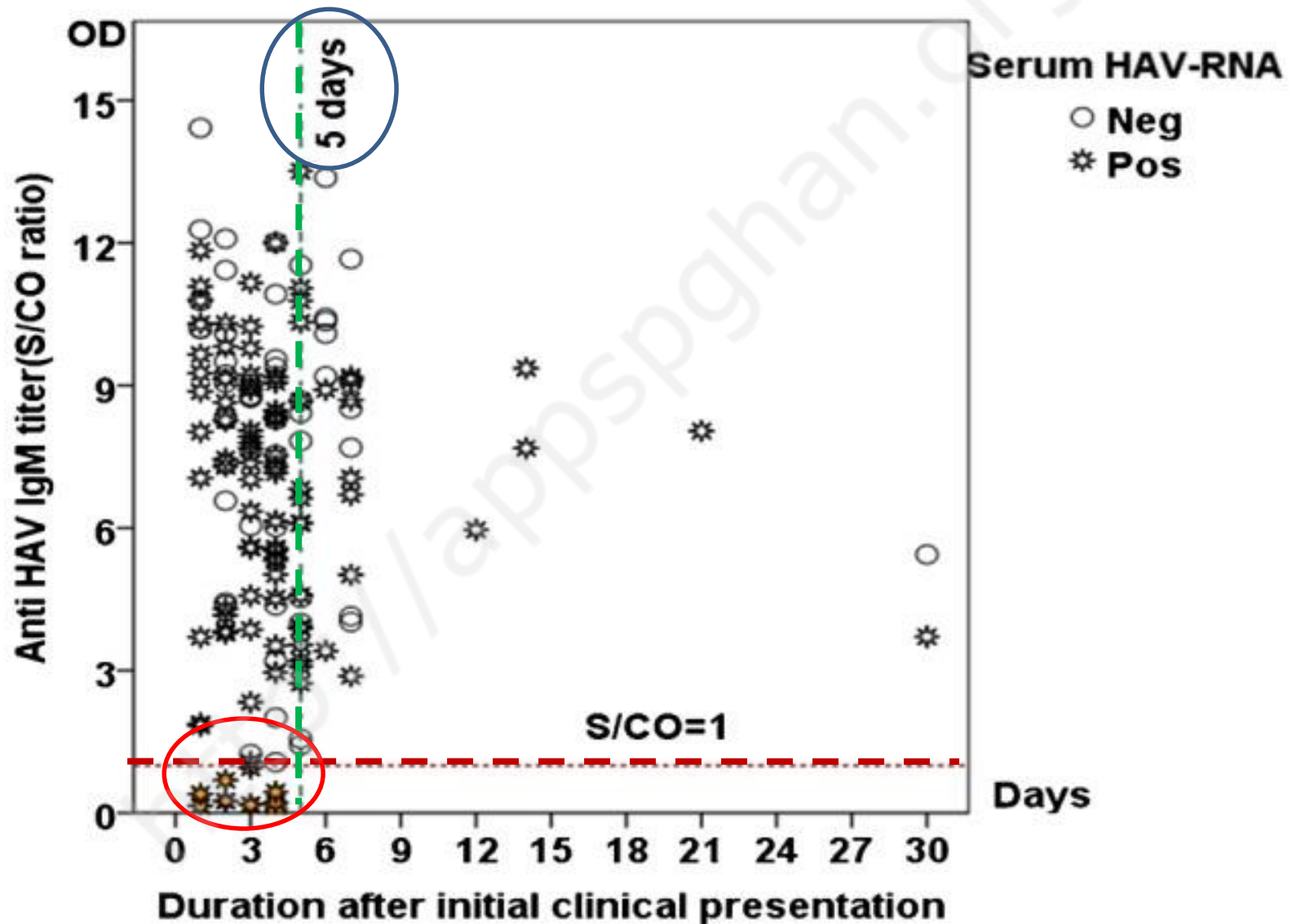
Phylogenetic tree of hepatitis A virus (HAV) strains based on the VP1-2A region



Three waves of acute hepatitis A patients during the epidemic and intervention phase.



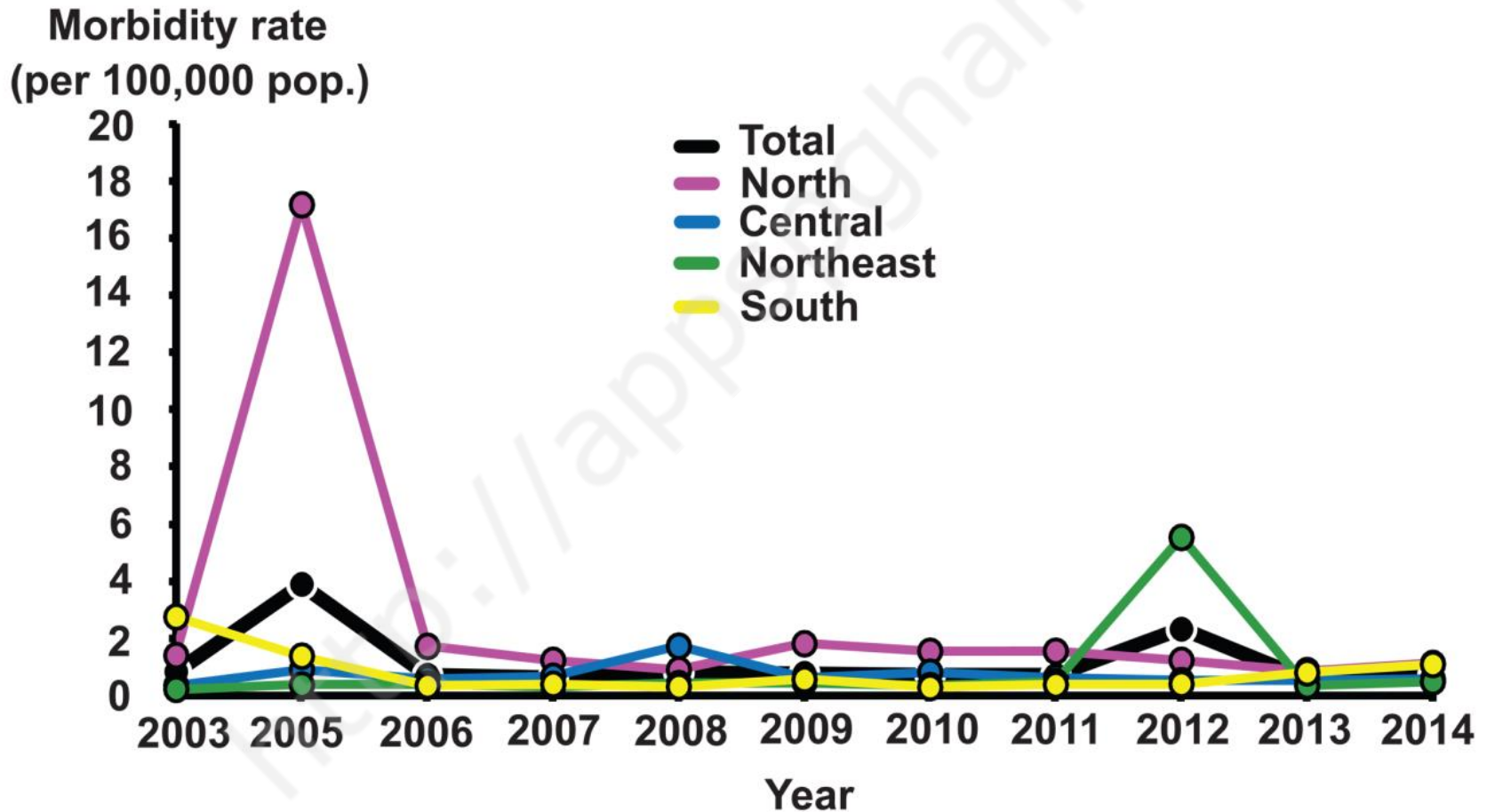
Anti-HAV IgM and HAV-RNA assays when analyzed individually



Hepatitis A virus infection

- **Symptomatic and severity increased with age**
- **Mortality in children <0.1%**
In patient with age >40 yr.. = 1%
- **High mortality in CLD**

Total hepatitis A infected cases and morbidity rate in Thailand



Hepatitis A high-risk groups

- **Preschool children**
- **Homosexual men**
- **Intravenous drug users**
- **Travellers to areas of high endemicity**
- **Hospital workers**
- **Nursery / child day-care centre employees**
- **Food handlers**

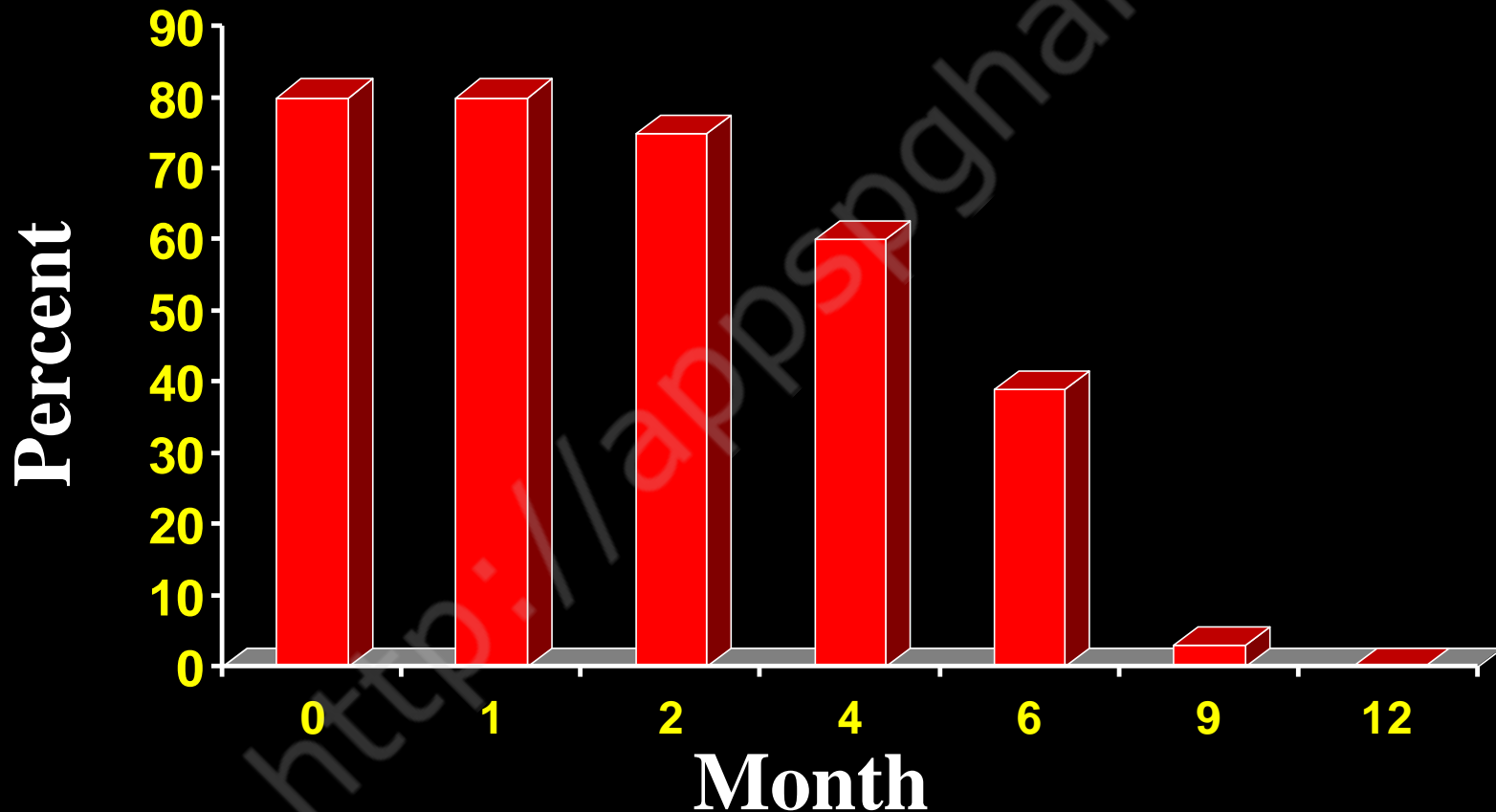
Control of hepatitis A

- **implementation of standard and public health**
- **Pre & post exposure prophylaxis**
 - **Active immunization**
 - **Passive immunization**

Passive antiHAV from mothers to infants



Passive anti HAV in infants



Control of hepatitis A

- Implementation of standard and public health
- Pre & post exposure prophylaxis
 - Active immunization
 - Passive immunization

Hepatitis Vaccine

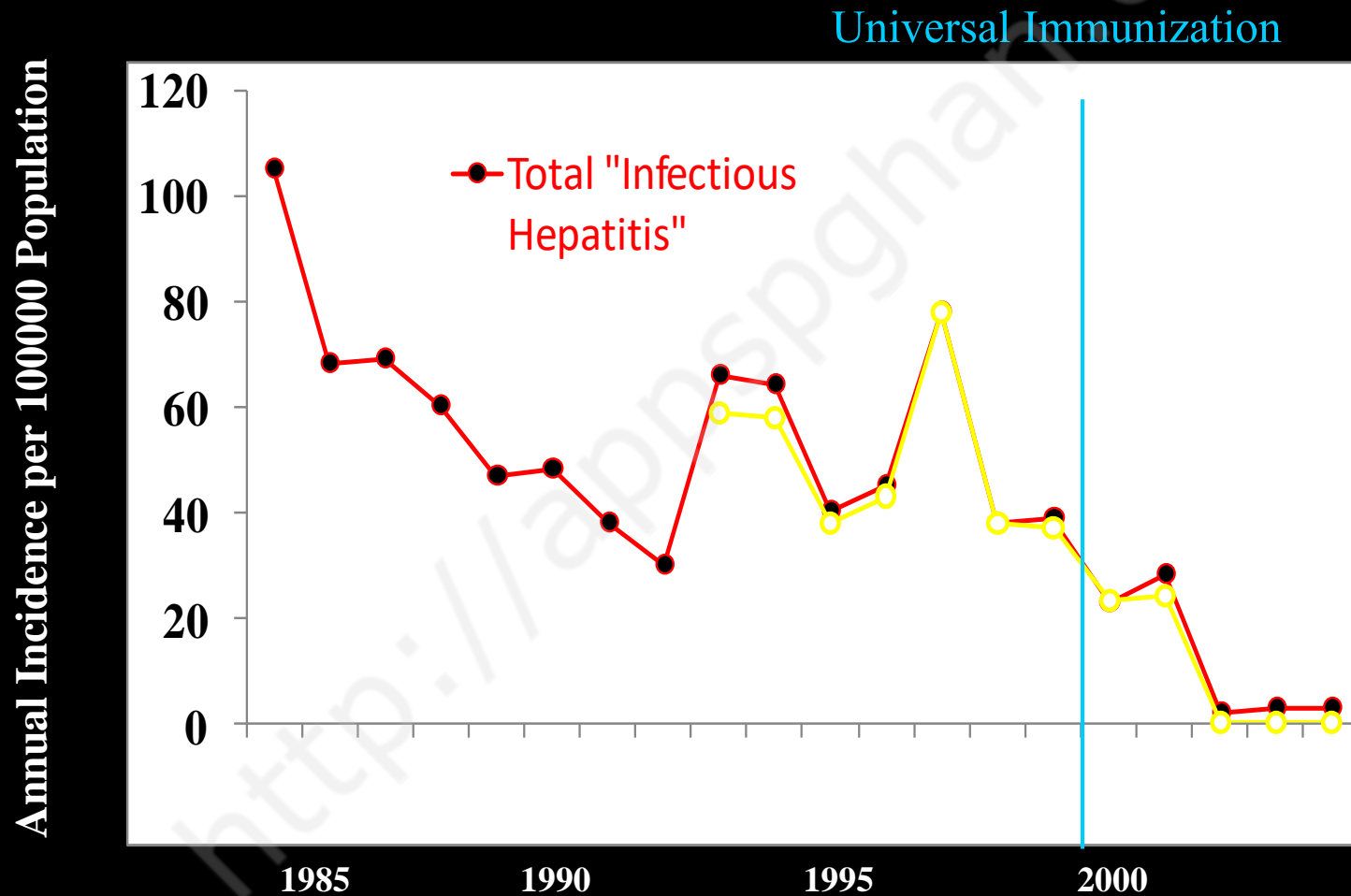
Hepatitis A vaccine

Live attenuated

Inactivated

<http://apppsgnhan.com/>

Incidence of Hepatitis A in Israel Following Universal Immunization of Toddlers



Hepatitis E



Hepatitis E virus

Background

1794: First outbreak in Lüdenscheid, Germany

1991: Named as hepatitis E virus (HEV)

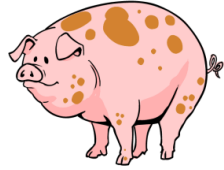
1997, 1999: Reported in domestic pigs and wild boars

2010 - : Rat, rabbit, ferret, mink, fox, bat and moose

Host

Genotype 1 and 2: humans

Genotype 3 and 4: humans, pig, wild boar, deer, moongoose, monkey, rat



Poor sanitation at the Yusuf Batil refugee camp in South Sudan caused an outbreak of hepatitis E earlier 2012.

Disease burden

Every year there are an estimated 20 million hepatitis E infections, over 3 million acute cases of hepatitis E, and 56 600 hepatitis E-related deaths. (WHO, Fact sheet 2014)



The discovery of infectious hepatitis E virus in retail pork products may help explain the purported association between liver failure and pork consumption.

History of HEV outbreaks

HISTORY

30,000 cases in New
Delhi, India after
flooding

1955-56

1977

1978

52,000 cases
in Kashmir,
India

1986-88

11,000 cases in
Somalia
4,000 cases in
Mexico

1988-89

1995

2,600 cases in
Sudan.

2004

2008

20,000 cases in
Mandalay, Myanmar,
and found that 18%
mortality in
pregnant women

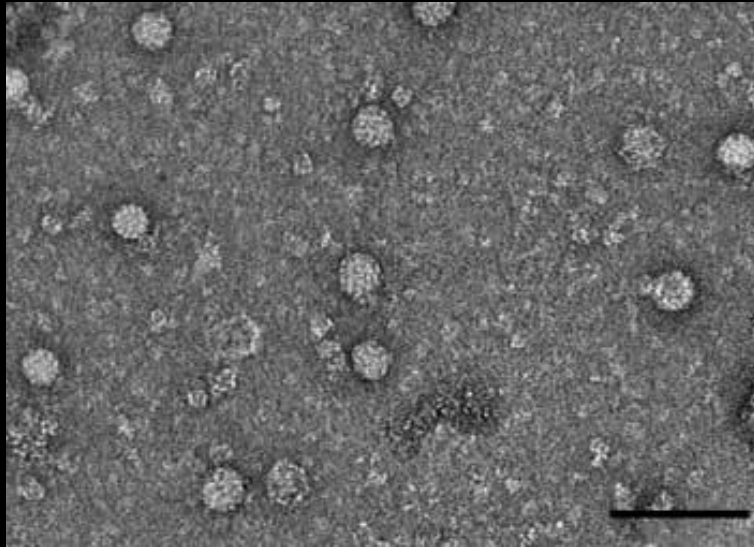
100,000 cases
in China

Patients
in Italy
and
Spain

10,000
cases in
Uganda

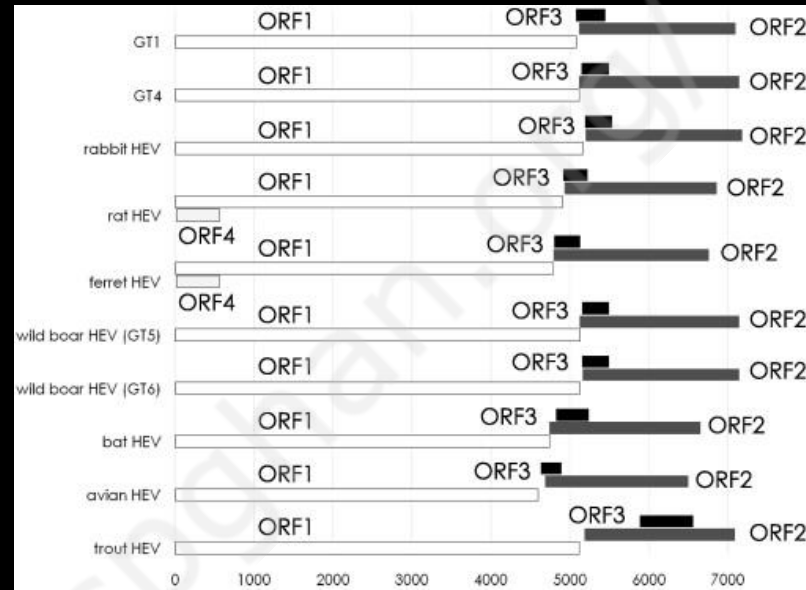


Virus structure



Hepeviridae, 25-35 nm

Genome organization

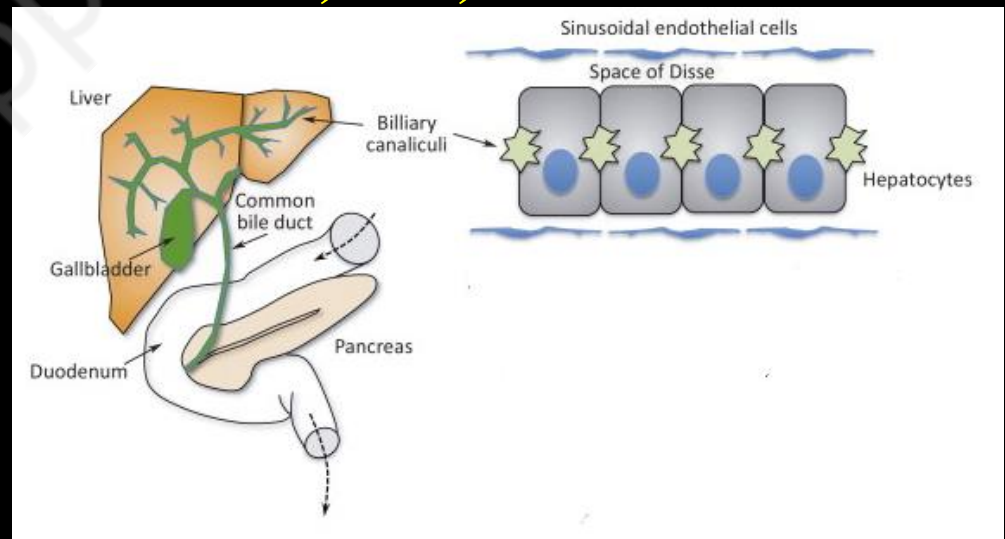


ssRNA, 7kb, ORF1-4

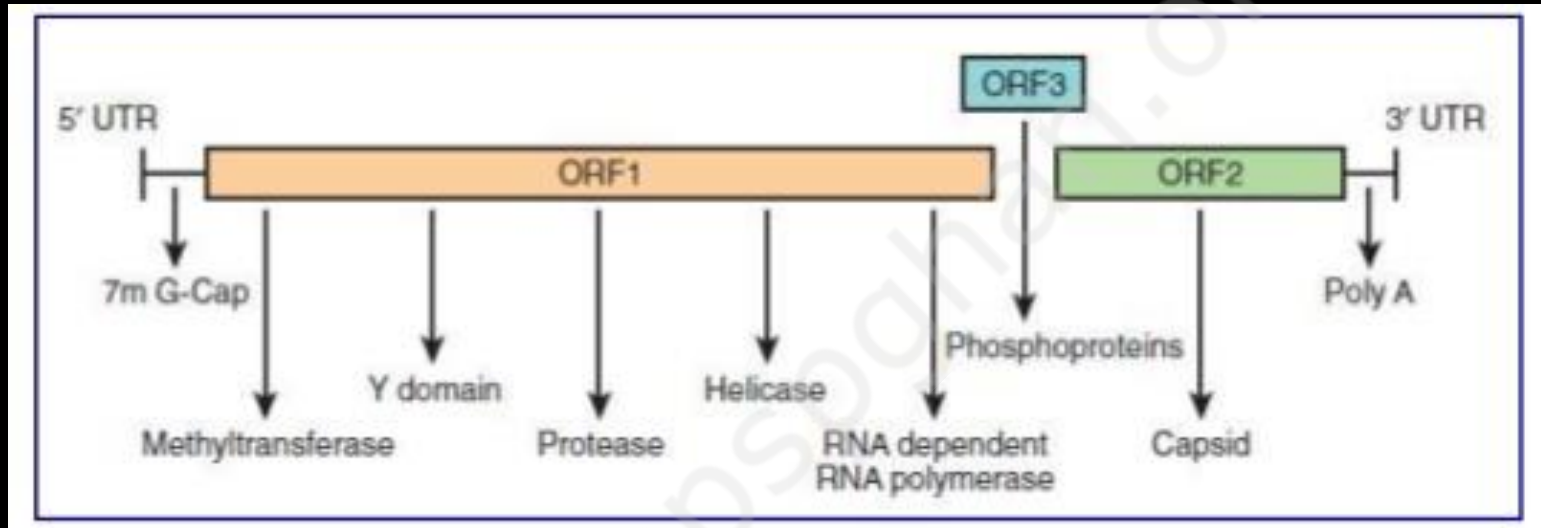
Replication of HEV

- Within cords of polarized hepatocytes

- Released across canicular membrane into the biliary canaliculus and to a lesser extent across the basolateral membrane into the space of Disse and the hepatic sinusoids that are bathed by blood. It is likely that most virus shed in feces.



Genomic organization of HEV

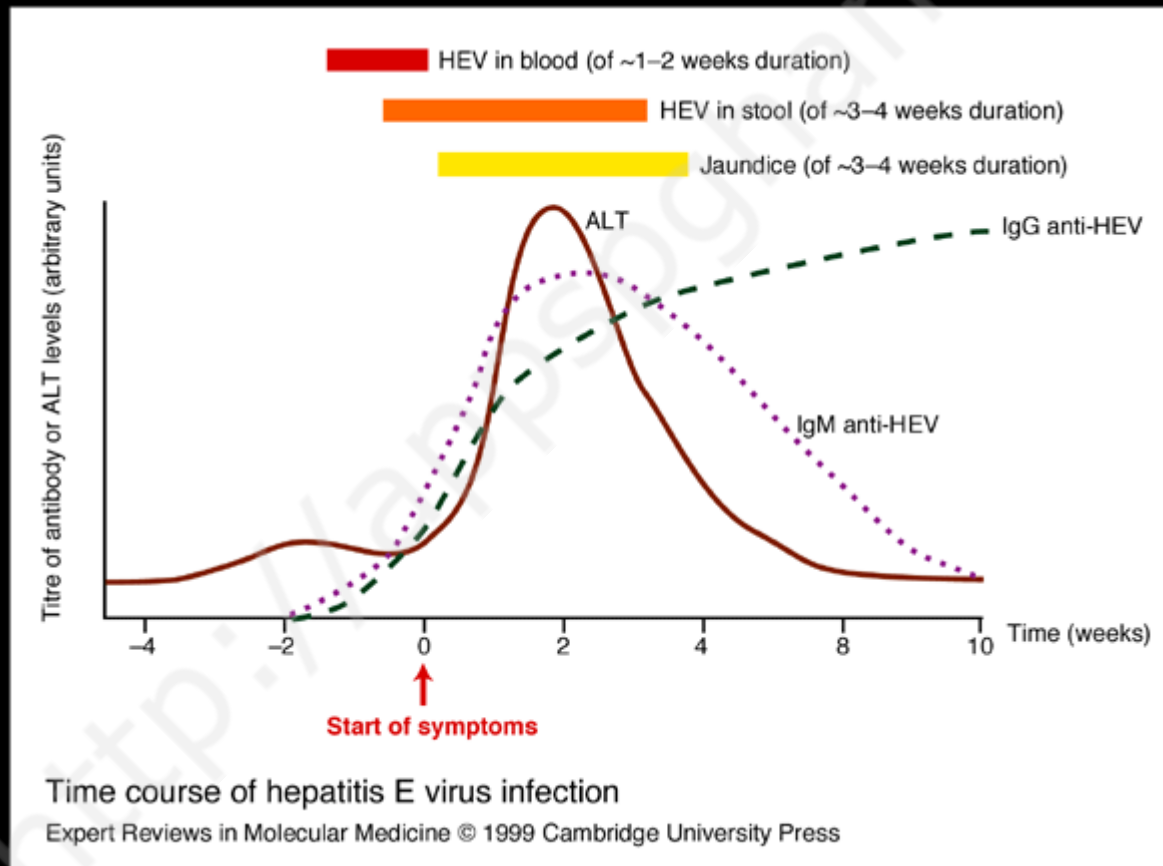


Three open reading frames (ORFs)

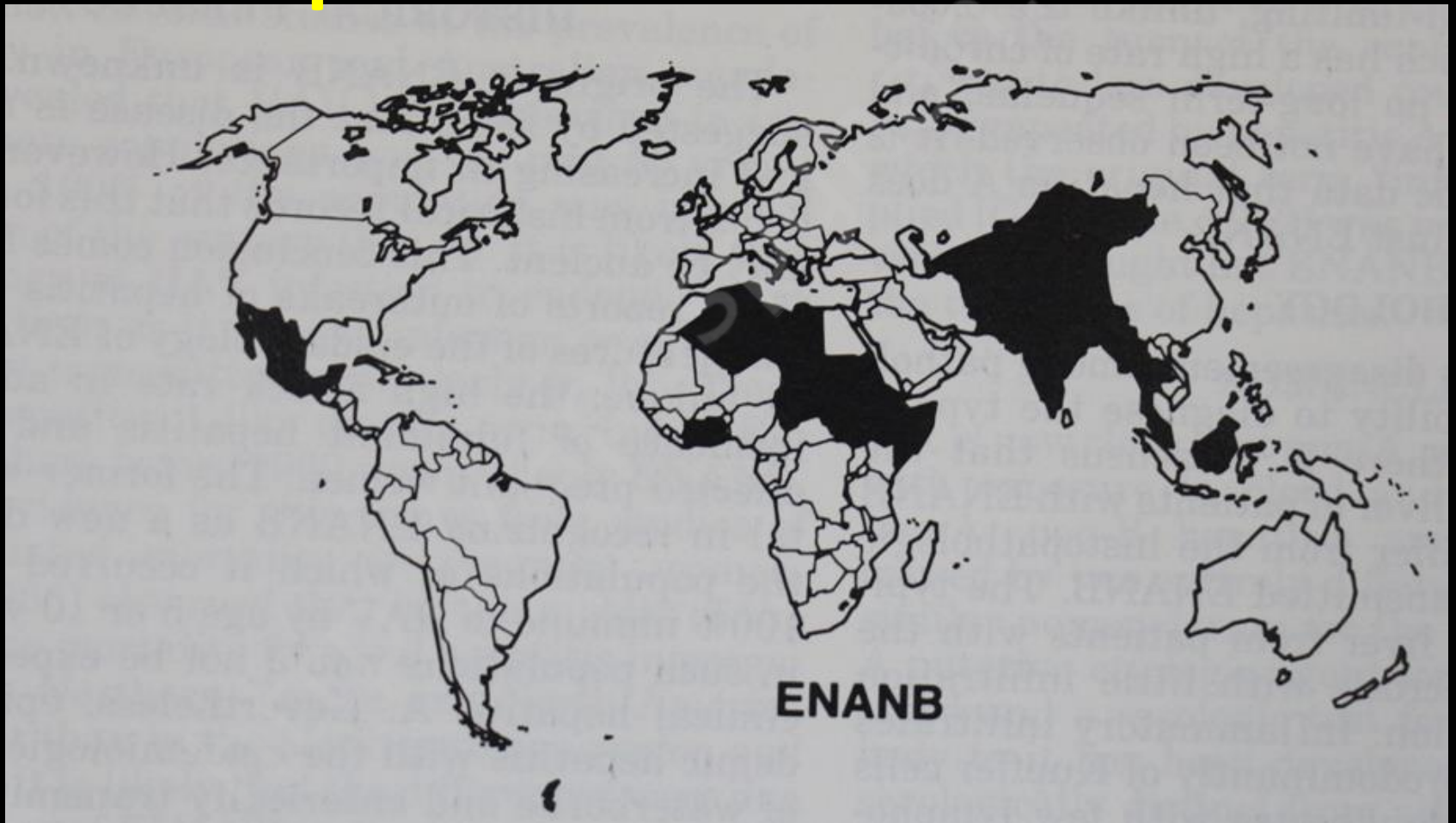
- **ORF 1** : encodes nonstructural protein (nsp)
- **ORF 2** : encodes viral capsid protein
- **ORF 3** : encodes small regulatory phosphoprotein

Clinical characteristic

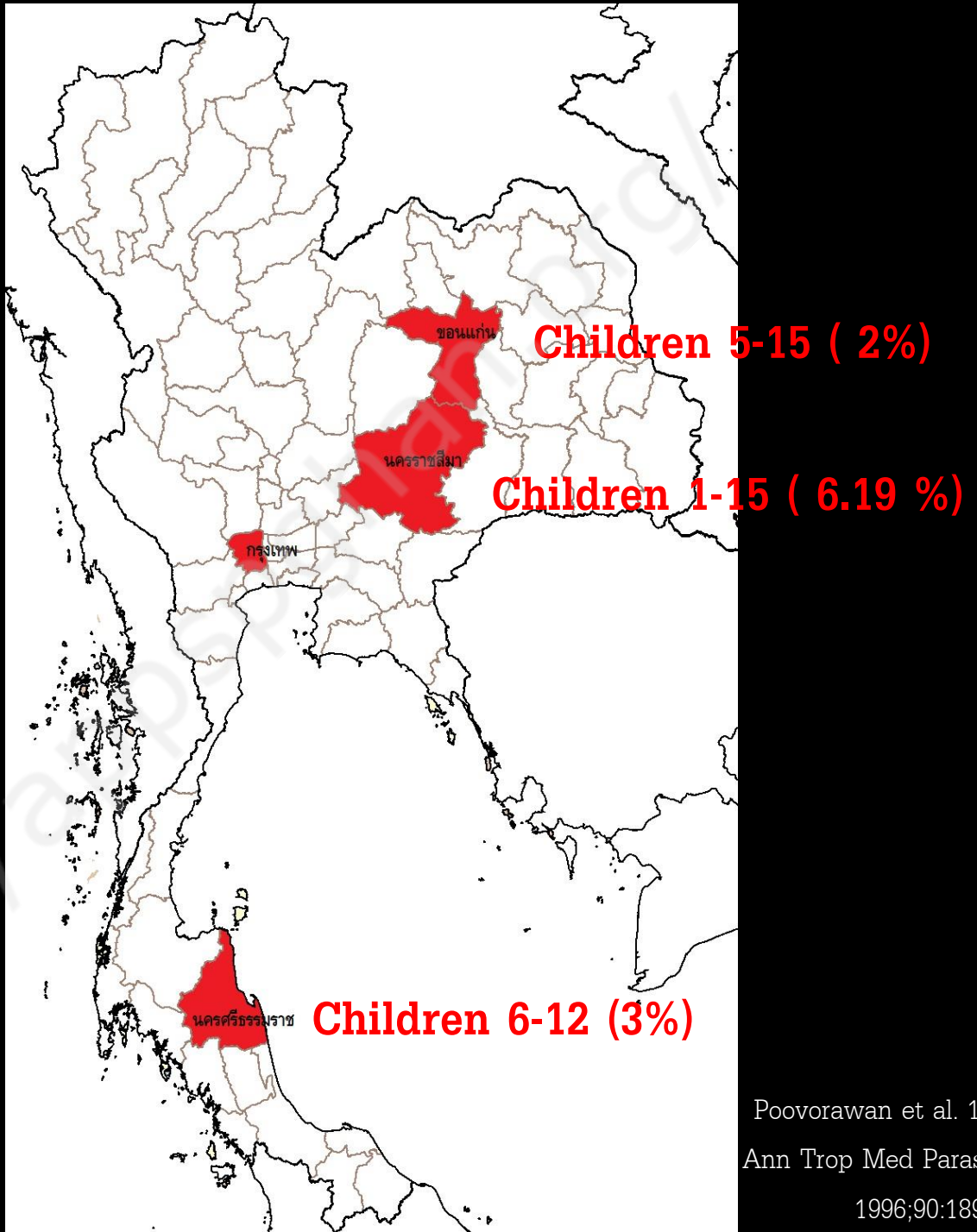
Incubation period: 3-8 wks **Symptoms: jaundice, anorexia, vomiting, fever**



Proceeding of the 1987 International Conference Symposium on Viral Hepatitis and Liver Disease



Prevalence of anti HEV in 1994, Thailand



Seroprevalence anti-HEV in Thailand 1994 Bangkok

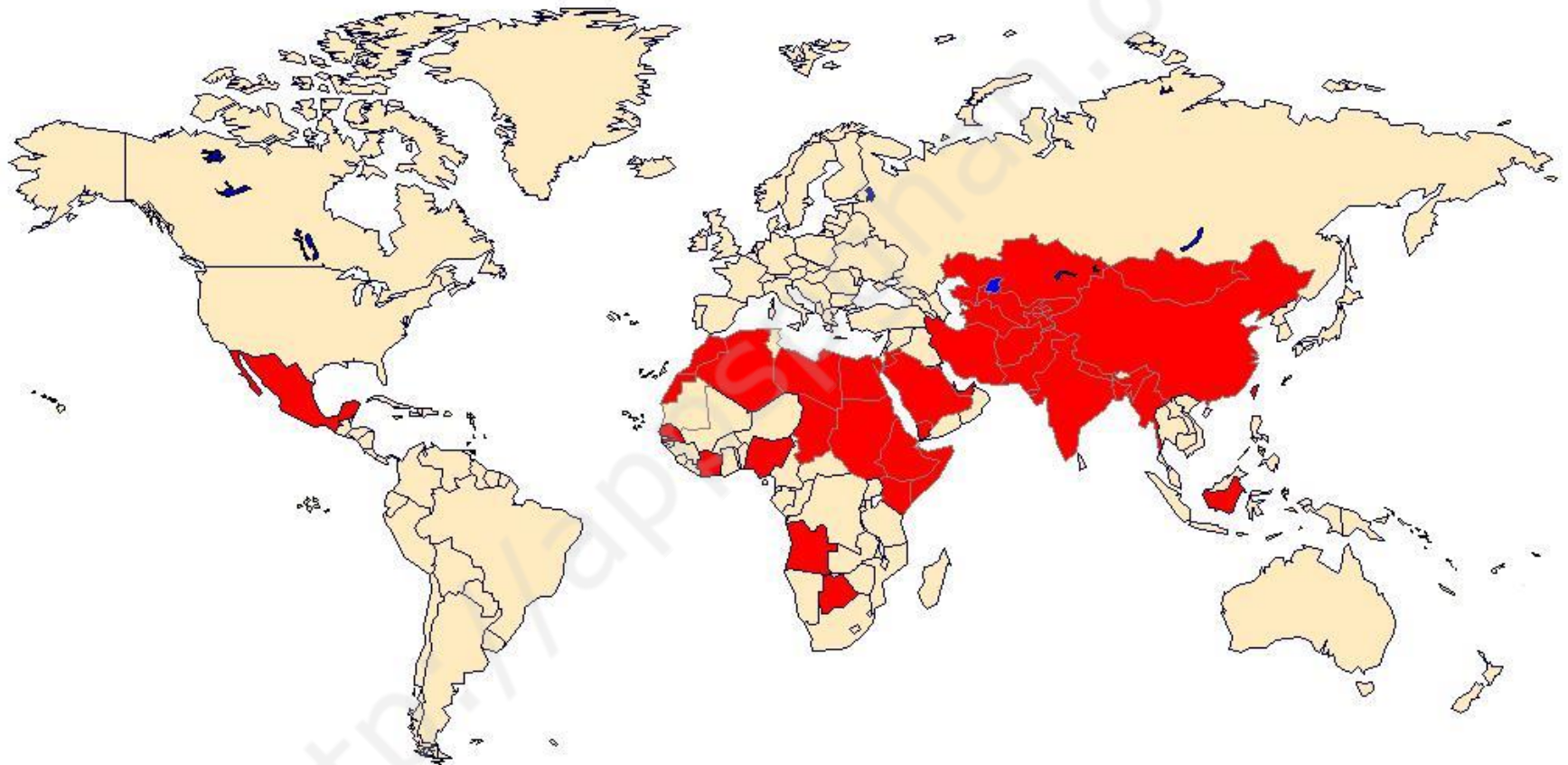
	No.	+ve	(%)
Blood donor 16-60 years	178	28	(15.7)
Pregnant women 16-45 years	178	16	(9.0)
Secondary school children 13-18 years	140	5	(3.6)

Poovorawan et al. 1996

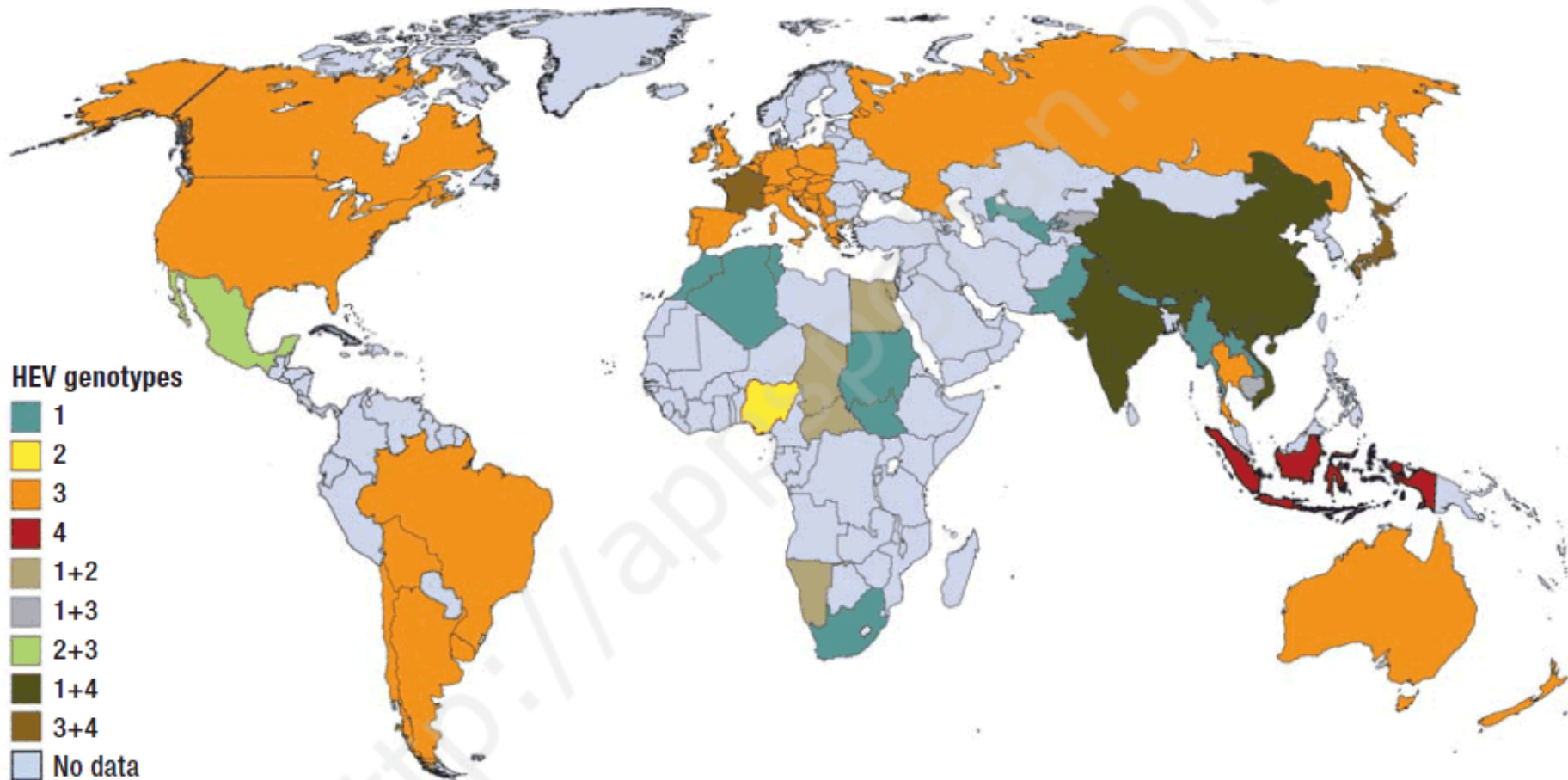
Ann Trop Med Parasitol 1996;90:189-96

Geographic Distribution of Hepatitis E

Outbreaks or Confirmed Infection in >25% of Sporadic Non-ABC Hepatitis



The global distribution of HEV genotype



Pischke, S et al. (2014). Hepatitis E in Germany—an Under-Reported Infectious Disease. *Deutsches Ärzteblatt International*, 111(35-36), 577.

Swine is the possible source of HEV infection in Thailand



Japan : HEV in wild boar



UK and France : HEV can be found in pork and pig products

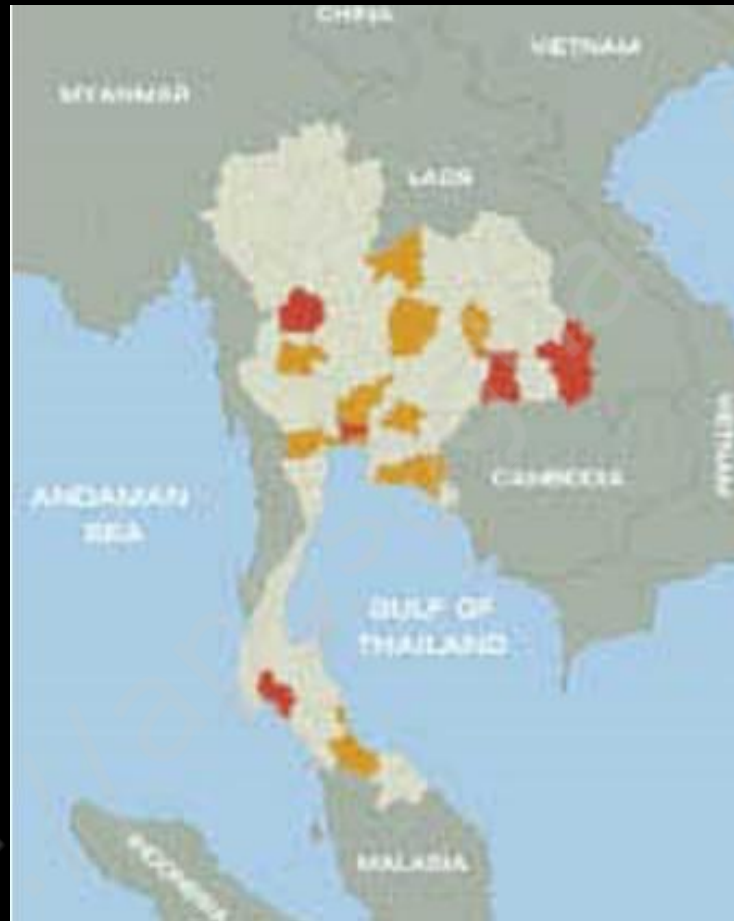


HEV infection at Chulalongkorn Hospital



www.med.cmu.ac.th

Risk factors and molecular characterization of acute sporadic symptomatic hepatitis E virus infection in Thailand



Regions of exposure (red) and residence (yellow) of acute HEV cases in Thailand.

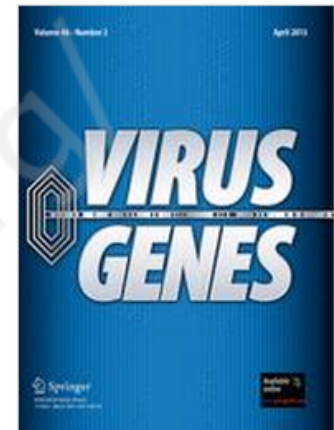
Regions of confirmed relevant exposure included Nonthaburi, Ubonratchathani, Kamphaengphet, Krabi, Surin and Bangkok; regions of residence included Ayutthaya, Ratchaburi, Rayong, Prachinburi, Uthaitхани, Chanthaburi, Loei, Samutprakarn, Samut Sakhon, Mahasarakham, Chaiyaphum, Saraburi and Songkhla.

Laboratory data of acute hepatitis E patients classified by patient risk. Plus–minus values are mean \pm SD for all comparisons. TB: total bilirubin, DB: direct bilirubin, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: alkaline phosphatase, INR: international normalized ratio. [†]Risk group defined as patients with one of the following clinical risks: very elderly (>80 years), liver cirrhosis or immune-compromised/post transplantation. *Statistically significant, [‡] mean (range), [§] Mann–Whitney U test

Laboratory data	All cases (n=40)	High risk group (n=16) [†]	Low risk group (n=24)	P value
TB (mg/dL)	10.4 \pm 9.9	9.4 \pm 9.5	11.1 \pm 10.3	0.62
DB (mg/dL)	8.5 \pm 8.6	7.1 \pm 7.7	9.6 \pm 9.2	0.40
AST (U/L) [‡]	226 (24-5440)	197 (117-5440)	324 (24-3266)	0.51 [§]
ALT (U/L) [‡]	463 (32-3986)	237 (54-3986)	495 (42-3750)	0.18 [§]
ALP (U/L)	192 \pm 96	221 \pm 119	156 \pm 57	0.01*
INR	1.25 \pm 0.34	1.35 \pm 0.30	1.17 \pm 0.30	0.20
Peak TB (mg/dL) [‡]	10.4 (0.7-56.5)	13.1 (4.6-56.5)	10 (0.7-41)	0.46 [§]
Peak DB (mg/dL) [‡]	9.2 (0.4-37.5)	10.3 (2.6-37.5)	7.7 (0.4-34)	0.69 [§]
Peak INR	1.4 \pm 0.4	1.5 \pm 0.6	1.2 \pm 0.2	0.07

Hepatitis E virus genotype 3f sequences from pigs in Thailand, 2011–2012

Juthatip Keawcharoen, Thanunrat Thongmee, Raphee Panyathong, Pichai Joiphaeng, Supansa Tuanthap, Kanisak Oraveerakul, Apiradee Theamboonlers, Yong Poovorawan

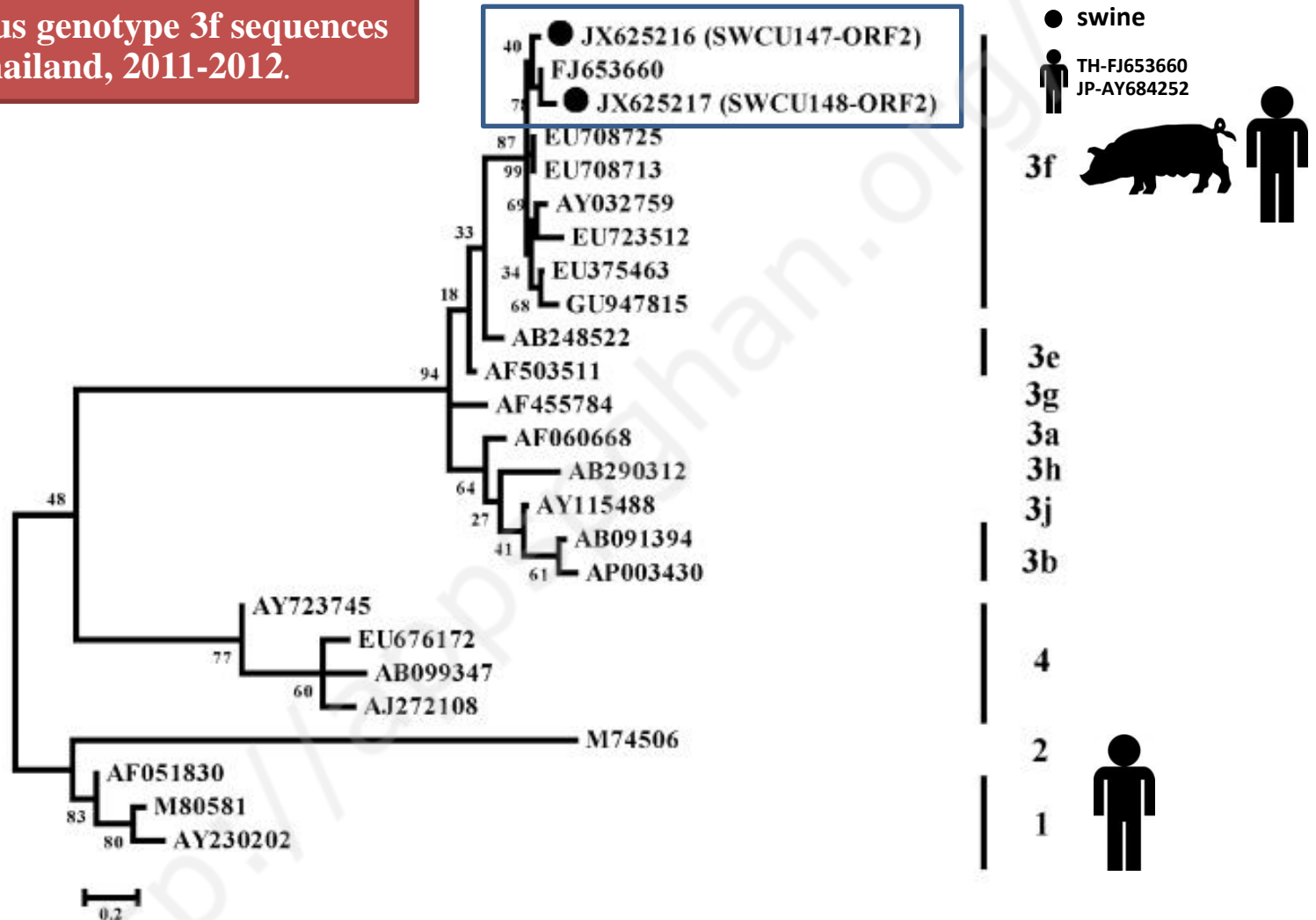


Three of 237 one to 22 week old pigs fecal specimen were positive for HEV-RNA

Phylogenetic analysis of partial ORF1 and ORF2 genes of Hepatitis E virus (HEV) strains from pigs in Thailand during 2011–2012 was performed. The result indicated that the current Thai strains belonged to the genotype 3 subgroup 3f, which were similar to the previous HEVs circulating in humans in Thailand

Hepatitis E virus genotype 3f sequences from pigs in Thailand, 2011-2012.

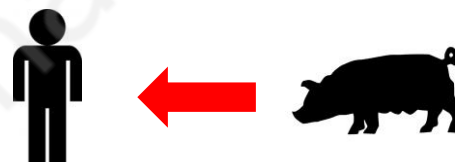
ORF2 of HEV



BRIEF REPORT

Swine as the possible source of hepatitis E virus transmission to humans in Thailand

Kamol Suwannakarn · Chitima Tongmee ·
Apiradee Theamboonlers · Piyawat Komolmit ·
Yong Poovorawan



We found that viruses recovered from Thai patients are closely related to genotype 3 and swine hepatitis E virus in Thailand. Based on analysis of a 302-base-pair ORF2 fragment the strains investigated belong to subgroup 3 e and are closely related to European strains. Based on the results obtained, swine are suspected to be a source of HEV transmission to human in Thailand

related to genotype 3 and swine hepatitis E virus in Thailand. Based on analysis of a 302-base-pair ORF2 fragment, the strains investigated belong to subgroup 3e and are closely related to European strains. Based on the results

Based on genetic variability, HEV has been divided into five genotypes. Genotypes 1–4 have been reported in mammals [1, 3, 7, 11], whereas genotype 5, avian HEV, has been detected in avian species and does not seem to

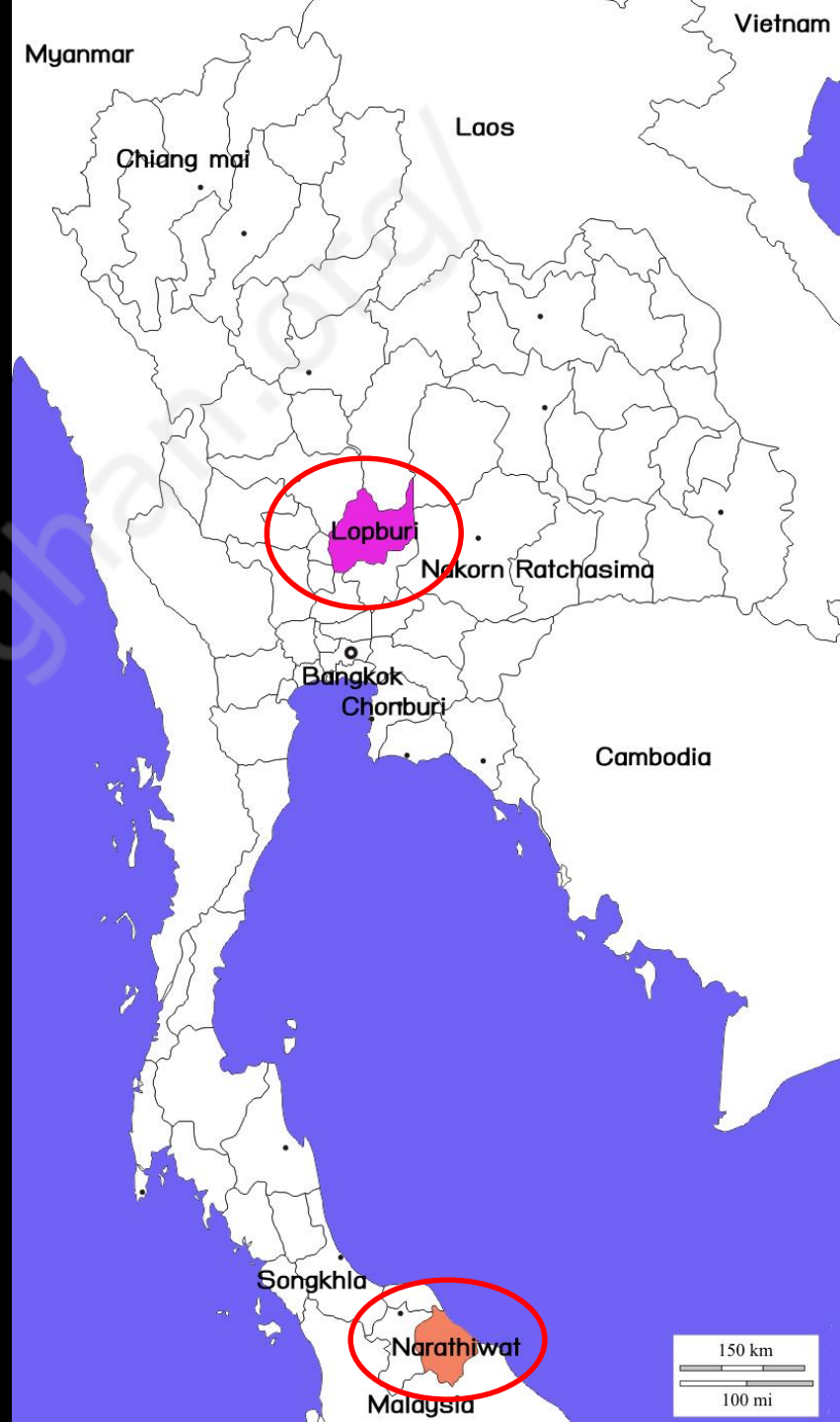
Narathiwat province



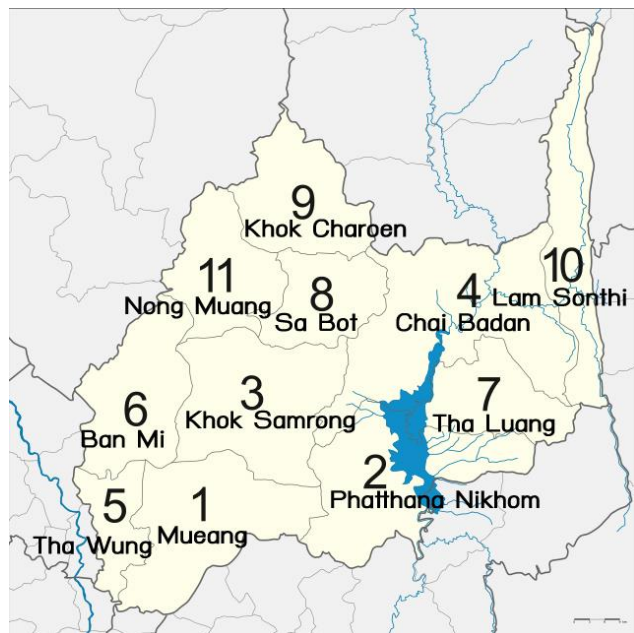
Target provinces

Lopburi: 778
(388 males, 390 females)

Narathiwat: 566
(134 males, 432 females)



Lopburi



Total population: 758,059^a

Pig population: 342,276^b

Islamic: 0.13%^c

Pig farm: 226^e

Population study: 778 (388 males, 390 females)

Total population: 757,397^a

Islamic: 82%^d

Pig farm: no data^e

Population study: 566 (134 males, 432 females)

^a Year 2012, The Bureau of Registration Administration, Department of Provincial Administration, Ministry of Interior (available on 203.146.15.175/ESSNEW/)

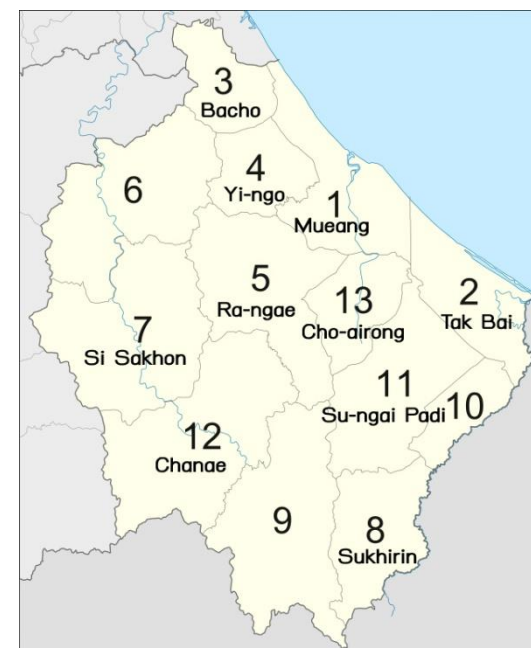
^b Year 2013, Information Technology Center, Department of Livestock Development (available on http://ict.dld.go.th/th2/images/stories/planning/2557/report_summary2013.pdf) Ministry of Agriculture and Cooperatives

^c Year 2012, Lopburi Province, (available on http://www.lopburi.go.th/plan_lopburi/plan_lop57-60.pdf)

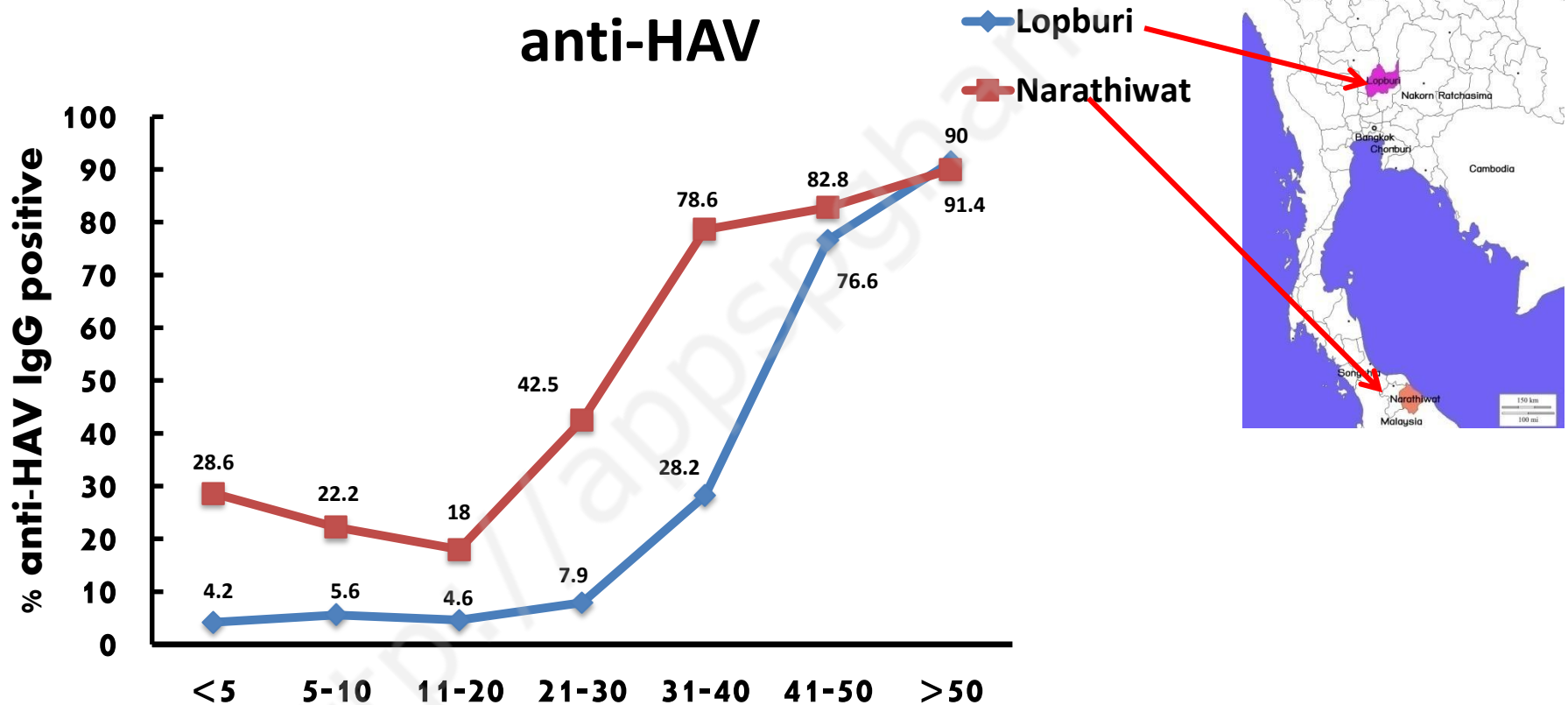
^d Year 2012, Narathiwat Province, (available on <http://103.28.101.10/briefprovince/filedoc/96000000.pdf>)

^e Department of Internal trade, Ministry of Commerce, (available on http://gis.dit.go.th/region/Report/rp_place_all.aspx?pid=40&poid=11&p=16)

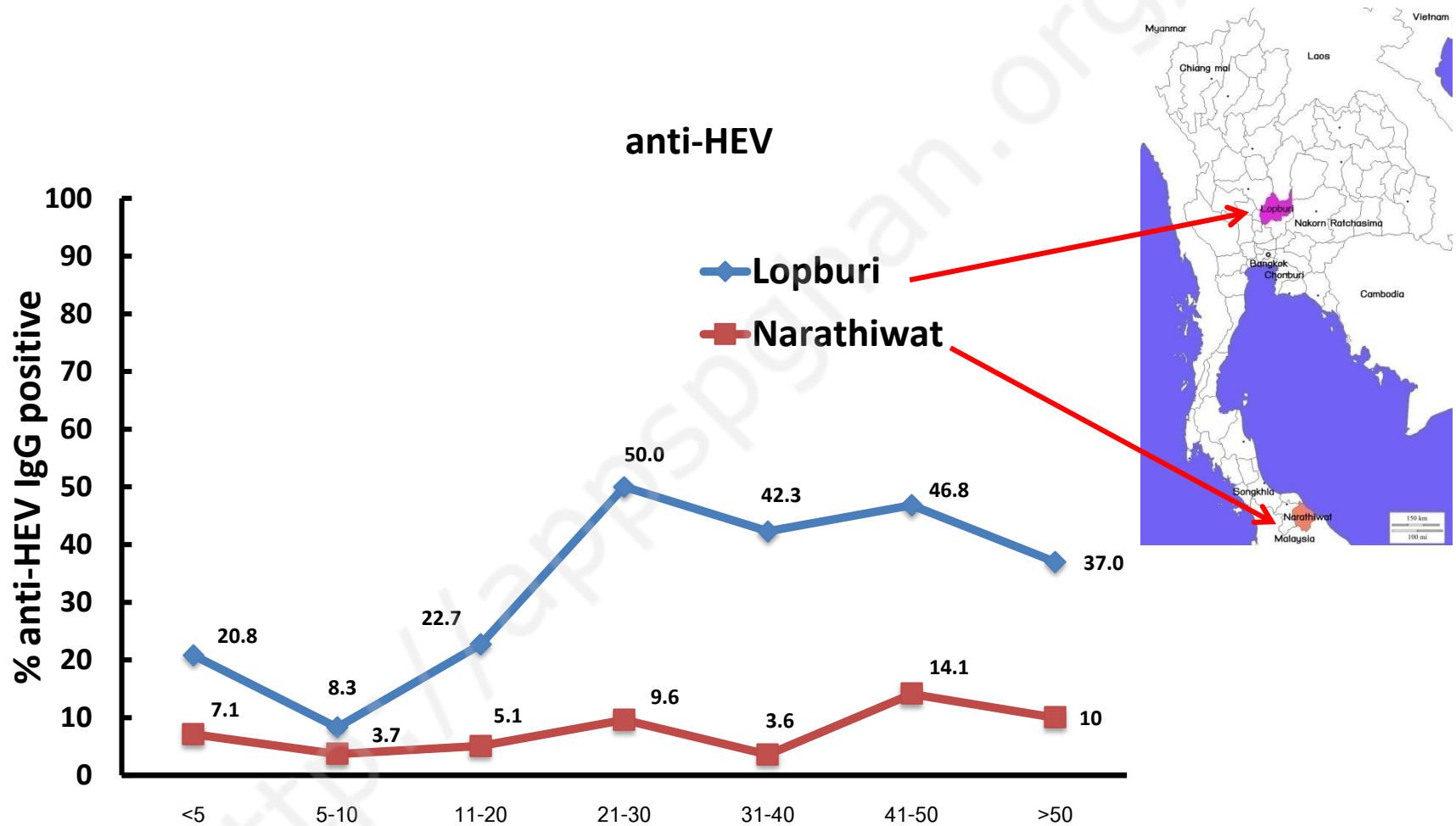
Narathiwat



Seroprevalence of anti HAV



Seroprevalence of anti HEV



RESEARCH ARTICLE

Swine Is a Possible Source of Hepatitis E Virus Infection by Comparative Study of Hepatitis A and E Seroprevalence in Thailand

Pattaratida Sa-nguanmoo¹, Nawarat Posuwan¹, Preeyaporn Vichaiwattana¹, Norra Wutthiratkowit², Somchai Owatanapanich³, Rujipat Wasitthanasem¹, Thanunrat Thongmee¹, Kittiyod Poovorawan⁴, Apiradee Theamboonlers¹, Sompong Vongpunsawad¹, Yong Poovorawan^{1*}

1 Center of Excellence in Clinical Virology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, **2** Narathiwat Ratchanakarin Hospital, Bang Nak, Narathiwat, Thailand, **3** King Narai Hospital, Khao Sam Yot, Lop Buri, Thailand, **4** Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

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OPEN ACCESS

Citation: Sa-nguanmoo P, Posuwan N, Vichaiwattana P, Wutthiratkowit N, Owatanapanich S, Wasitthanasem R, et al. (2015) Swine Is a Possible Source of Hepatitis E Virus Infection by Comparative Study of Hepatitis A and E Seroprevalence in Thailand. PLoS ONE 10(4): e0126184. doi:10.1371/journal.pone.0126184

Academic Editor: Srinand Sreevatsan, University of Minnesota, UNITED STATES

Received: February 13, 2015

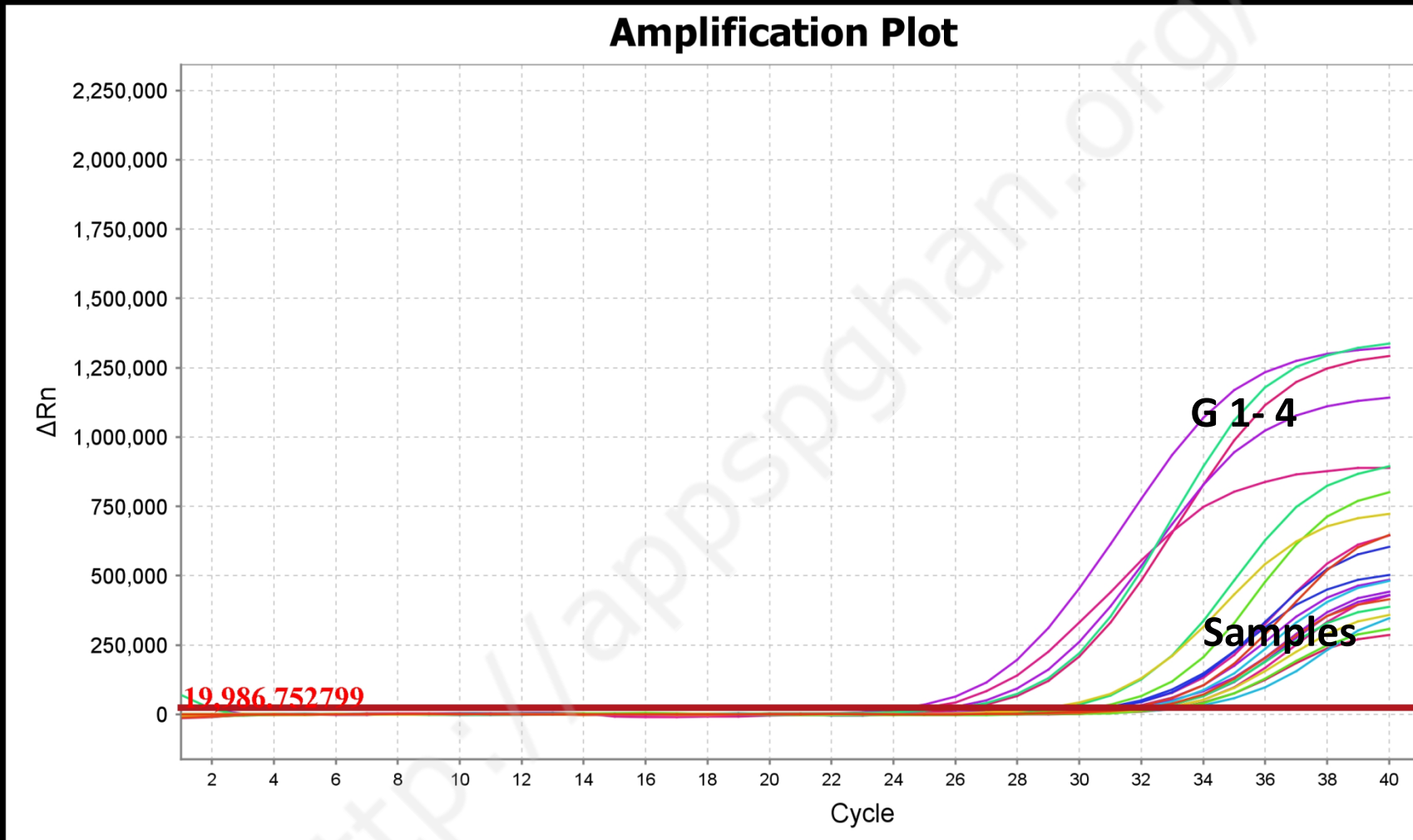
Abstract

Hepatitis A virus (HAV) and hepatitis E virus (HEV) infection in developing countries are associated with contaminated food or water. Although Thailand is non-endemic for HEV, sporadic infections may occur from zoonotic transmission. Individuals between 7 months to 69 years (mean age = 32.8) from predominantly Islamic Narathiwat ($n = 305$) and swine farm-dense Lop Buri ($n = 416$) provinces were screened for anti-HEV and anti-HAV antibodies by commercial enzyme-linked immunosorbent assay and automated chemiluminescent micro-particle immunoassay, respectively. Seroprevalence and relative antibody titers were analyzed according to age groups. HAV IgG antibody positive rates in Lop Buri and Narathiwat residents were 39.9% and 58%, respectively ($p < 0.001$). Greater than 90% of individuals

Diagnostic Development of Hepatitis E virus by Real-Time RT PCR



Real time PCR testing on samples



Legend

A B C D E F G H

Hepatitis E in Pork and Variety Meats in Fresh Markets



Sample collected from swine

Collection time :
November 2014 – February 2015



Liver : 1,144 samples
Pork : 560 samples
Intestine : 557 samples

Slaughter house

Klongtoey

Bile : 720 samples
Feces : 720 samples

Market

Klongtoey

Suanplu

Bangkruai

BangPo

S.K. floor 11

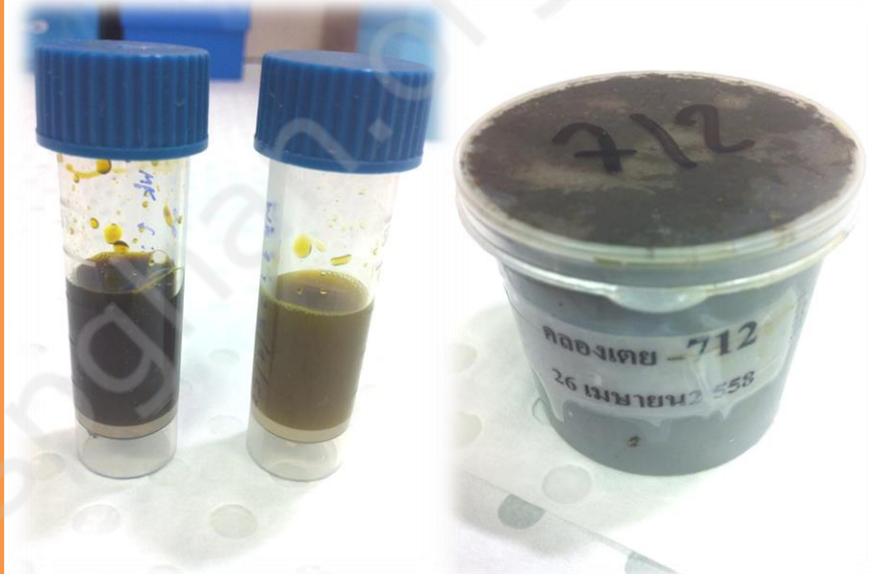
Bangson

Swine samples



Market

- Khlong Toei
- Suan Plu
- Bang Kruai
- Bang Po
- Bang Son
- Tao poon



Sluaghterhouse

- Khlong Toei

HEV in variety part of pig in fresh markets and slaughter house

Liver	3	in	1000
Bile	3.6	in	100
Feces	5	in	100
Pork	0.7	in	1000
Intestine	not found		

ORIGINAL PAPER

Hepatitis E Virus in Pork and Variety Meats Sold in Fresh Markets

Duangnapa Intharasongkroh¹ · Pattaratida Sa-nguanmoo¹ · Supansa Tuanthap¹ ·
Thanunrat Thongmee¹ · Ausanee Duang-in¹ · Sirapa Klinfueng¹ · Jira Chansaenroj¹ ·
Sompong Vongpunsawad¹ · Apiradee Theamboonlers¹ · Sunchai Payungporn² ·
Chintana Chirathaworn³ · Yong Poovorawan¹

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Abstract Swine is an economically important livestock, yet pork consumption and close contact with pigs are associated with the risk of hepatitis E virus (HEV) infection. Limited data on the prevalence of HEV in Southeast Asia have mainly examined farm animals. To investigate the potential zoonotic transmission of HEV from dietary consumption of pork and variety meats (i.e., offal or organ meats), we obtained 1090 liver, 559 pork meat, and 556

studies will be required to further assess potential dietary transmission of HEV elsewhere in the region.

Keywords Hepatitis E virus · Pork · Prevalence · RT-PCR · Thailand · Fresh market

Introduction



Nosocomial HEV infection



Blood transfusion and Hepatitis E virus infection

Prevalence of HEV among Thai blood donors



Source of samples in this study

N = 30,000

**Duration time : 3 months
October – December 2015**



**EDTA blood samples were collected
from The National Blood Center
(n = 30,000).**



**HAMILTON
Microlab
STAR/STARlet
IVD Pipettor**

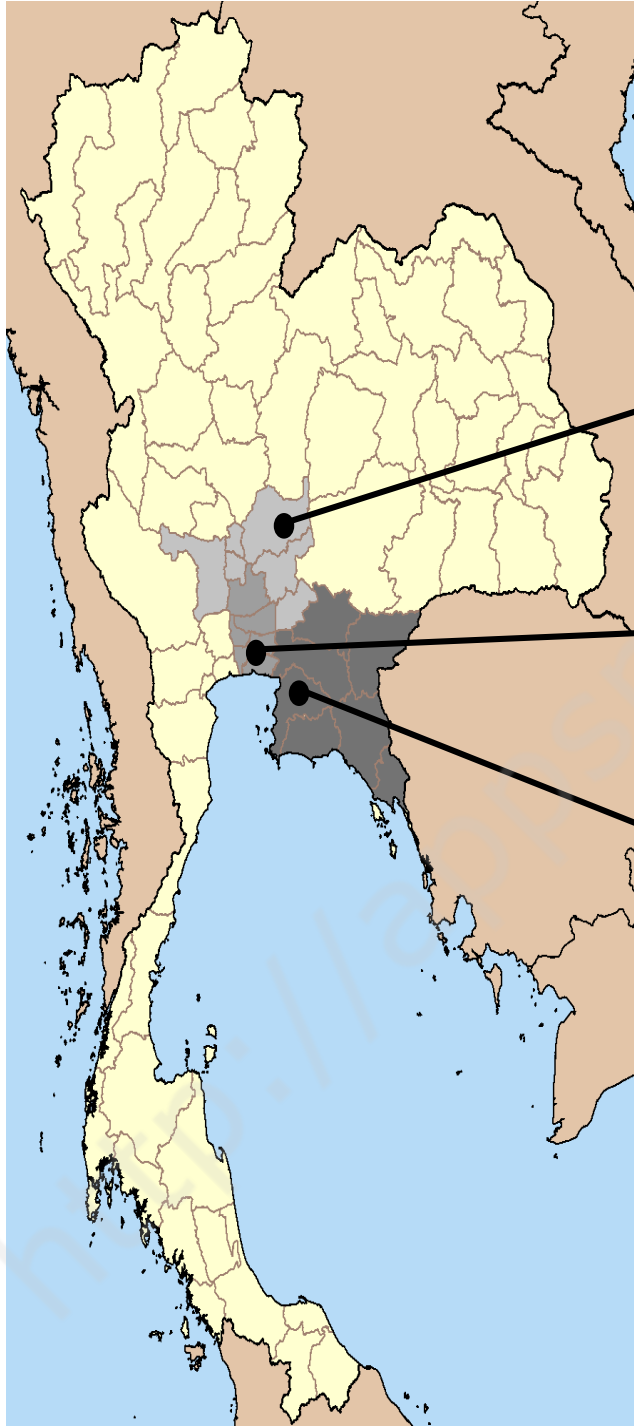
**All samples were performed in
minipool of 6 (5,000 pooled)
by using automated specimen
pooling**

**HEV RNA-positive rate of donor plasma samples
derived from three areas**

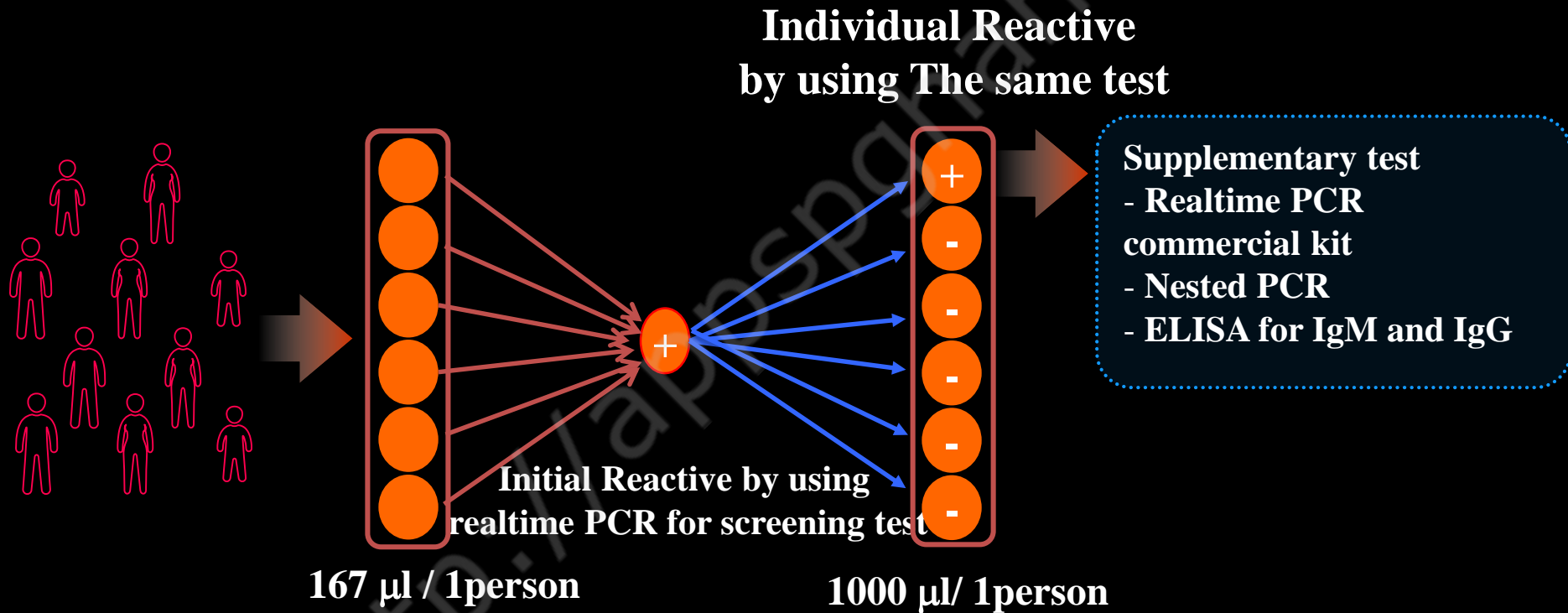
**Regional blood centre II, Lop Buri
(1/562)**

**National blood centre, Bangkok
(22/22,711)**

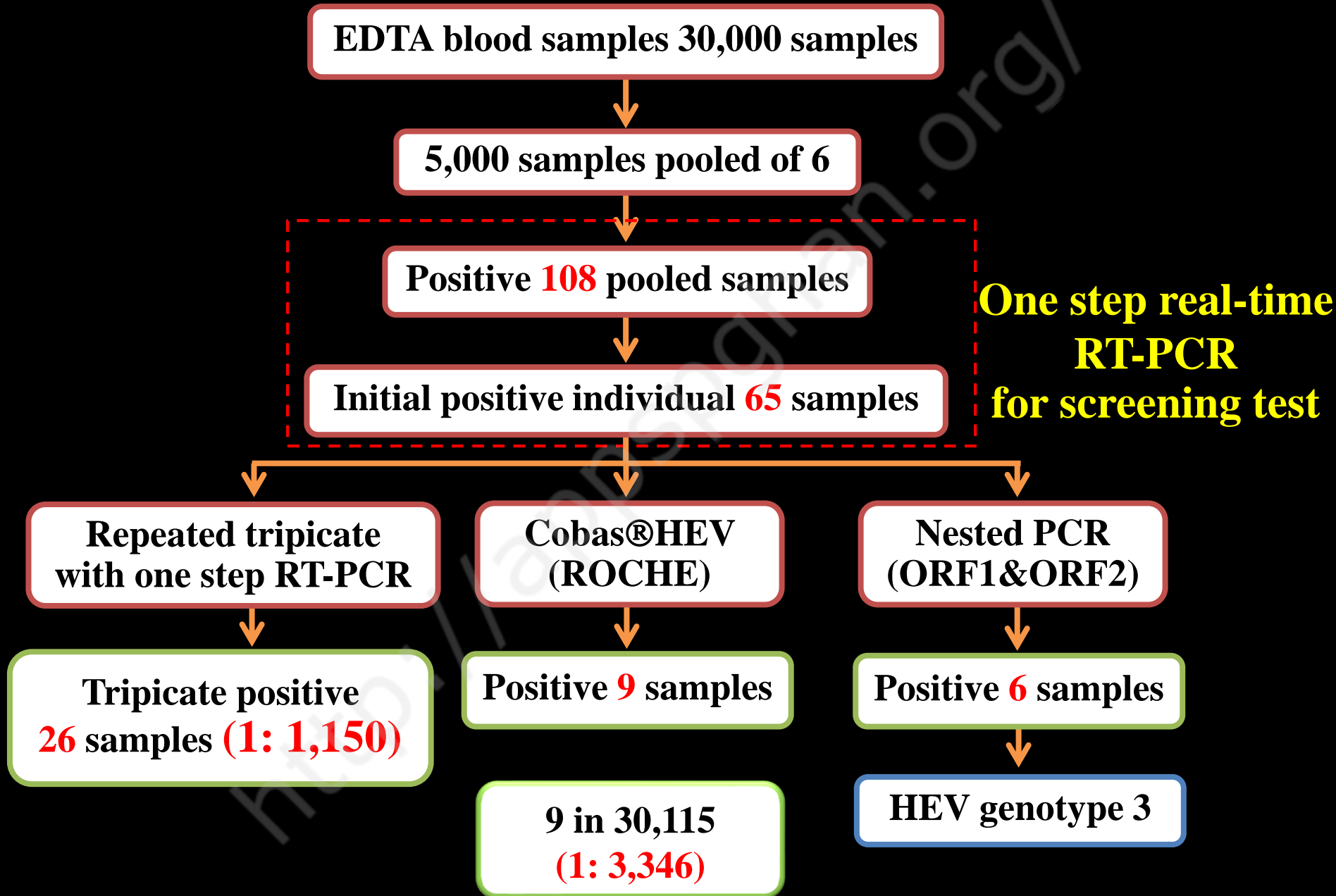
**Regional blood centre III, Chon Buri
(3/1,931)**



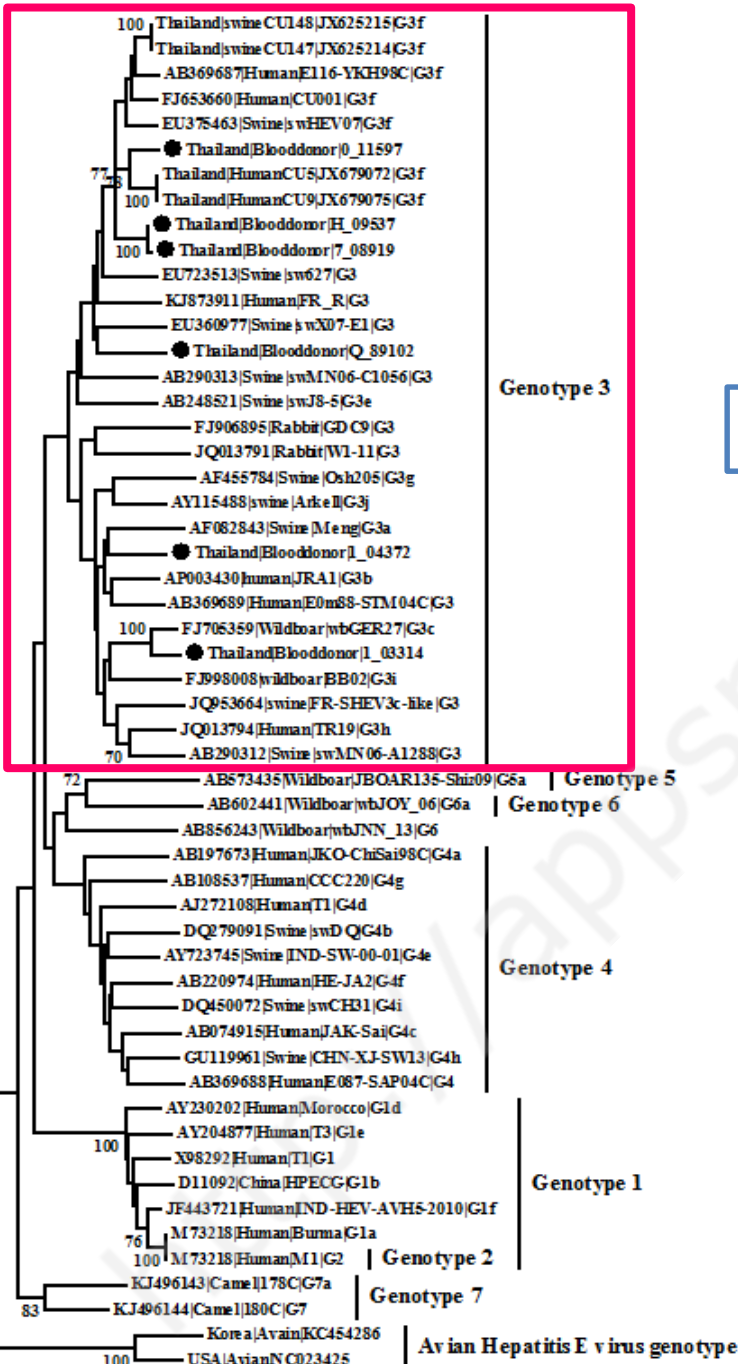
Pooled 6 samples collected from blood donor



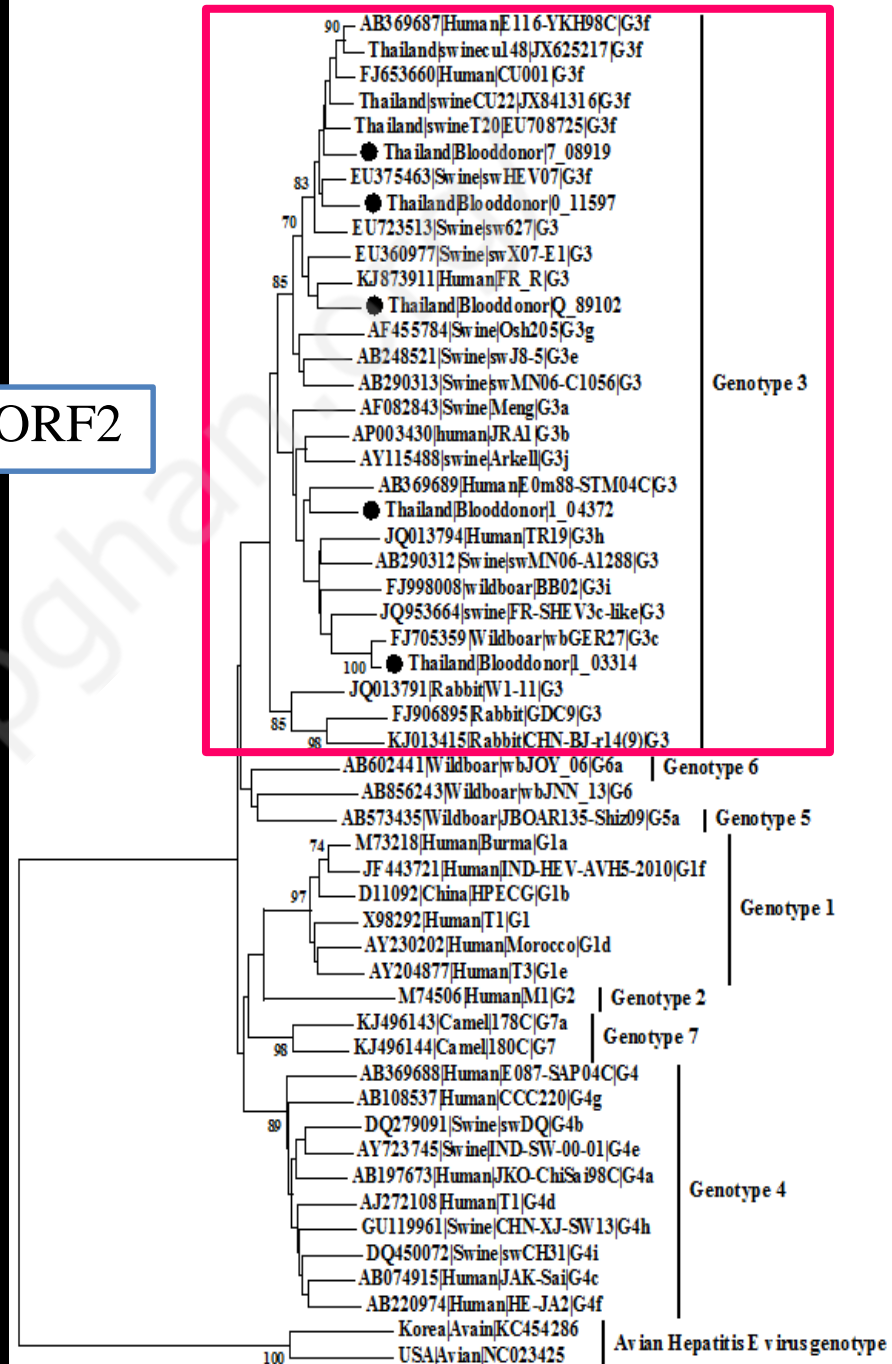
Hepatitis E in Thai Blood Donors



ORF1



ORF2



0.1

0.1

HEV transmission

HEV genotype 1 and 2

Developing countries

Epidemic

Susceptible host

Cynomologous macaques

Tamarins

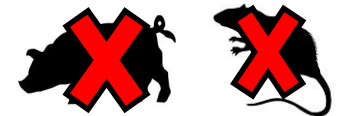
Rhesus

Chimpanzees

Owl monkeys

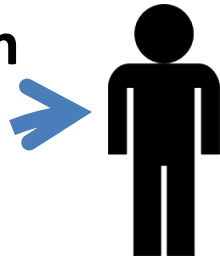


Drinking water



Trials to infect **pigs** and **rats** was not successful.

Person to Person
(less important)

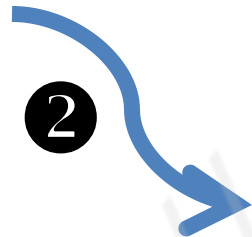


1

15-30 years old
Acute Self-limiting
hepatitis



2



Environmental sample contaminated
with human excretions
(Faecally contaminated water)

Mortality rate: 26.9%
Third trimester

HEV genotype 3 and 4

Developed countries
Sporadic

Porcine derived
products
(porcine heparin
products)



Asymptomatic



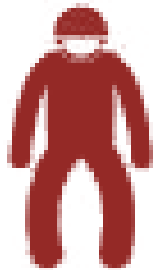
Middle-age

Men were found 4-fold
than women

Symptomatic



Elderly age



**Immunocompromised host
organ transplantation**

**Blood
Transfusion**



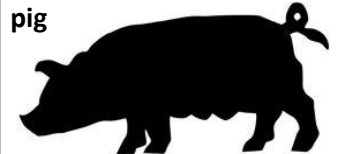
**Ingestion of HEV
containing meat,
liver, blood or
sausage**



deer



pig



wild boar



rabbit



Hepatitis E virus

2 disease entities

Genotype 1,2

- Epidemic, water borne disease
- Young adult
- High mortality pregnant women

Genotype 3,4

- Sporadic infection
- Developed countries
- Immunocompromised host
- Transmission :- zoonotic infection (swine)
:- blood transfusion
- Chronic hepatitis

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- Chulalongkorn University and Hospital
- NSTDA Research Chair Grant

