Hepatitis A: Mechanisms of Vaccine Induced Protection

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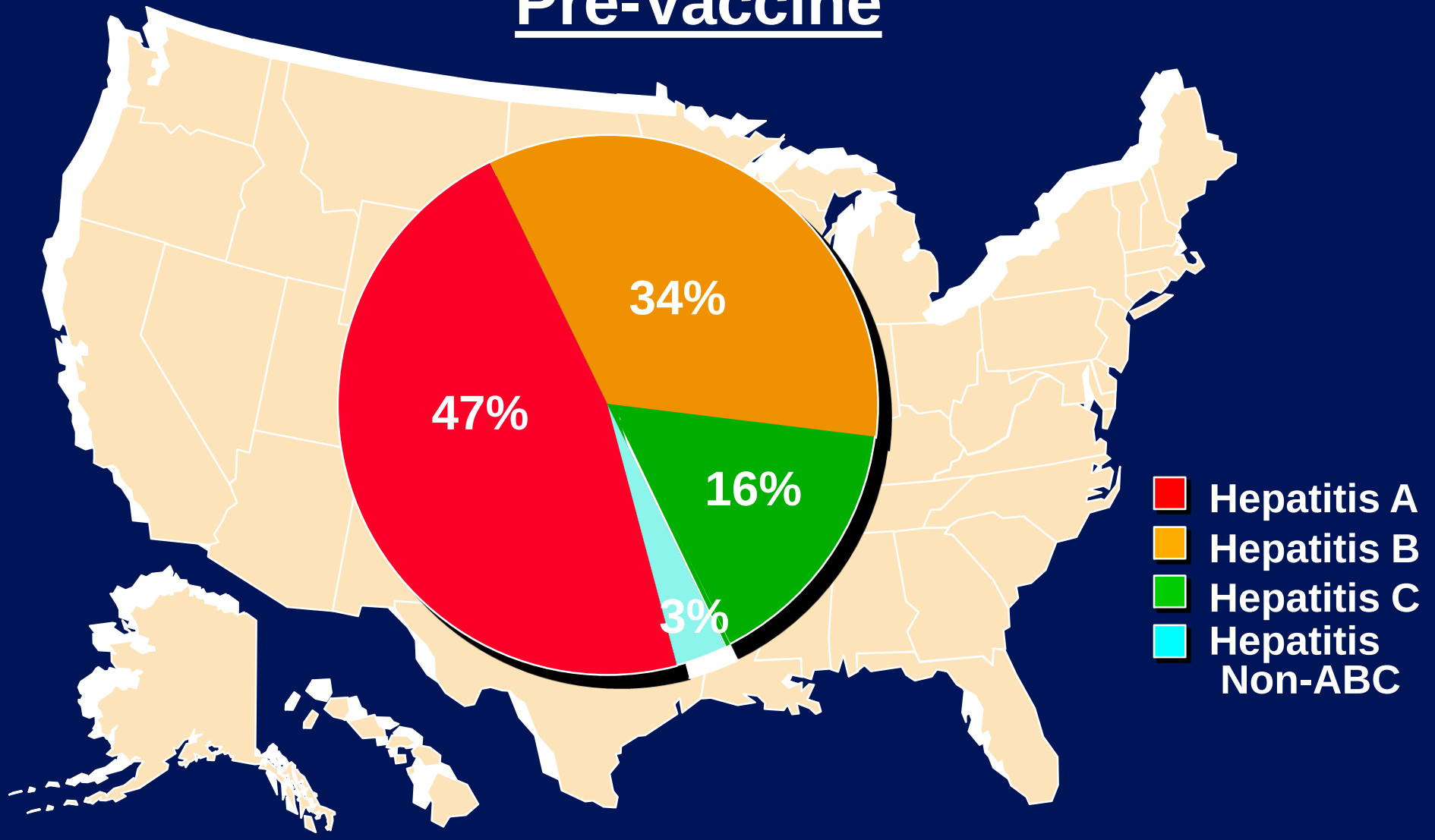
Types of Viral Hepatitis

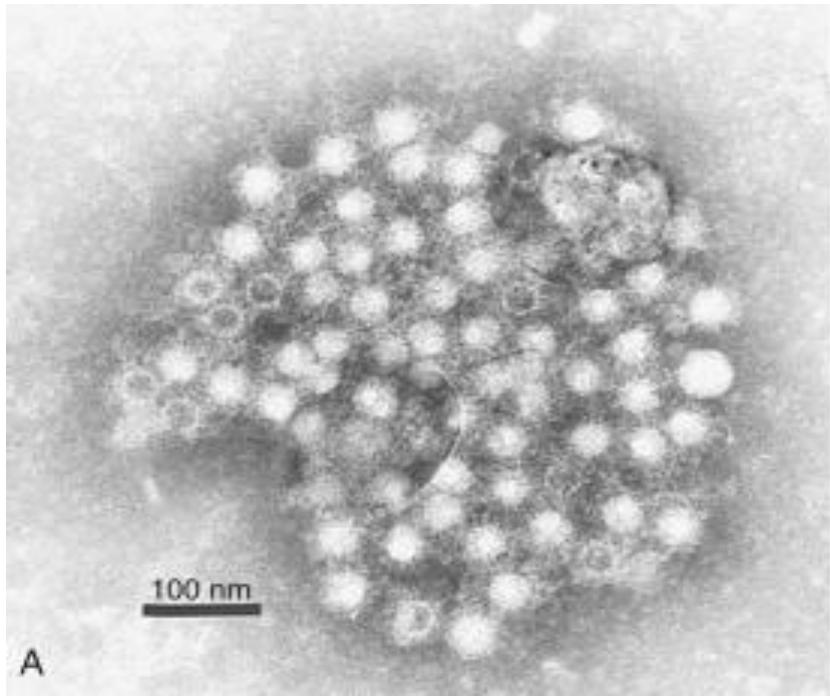
	A	B	C	D	E
Source of virus	**feces**	blood/ blood-derived body fluids	blood/ blood-derived body fluids	blood/ blood-derived body fluids	feces
Route of transmission	**fecal-oral**	percutaneous permucosal	percutaneous permucosal	percutaneous permucosal	fecal-oral
Chronic infection	**no**	yes	yes	yes	yes
Prevention	**pre/post-exposure immunization**	pre/post-exposure immunization	blood donor screening; risk behavior modification	pre/post-exposure immunization; risk behavior modification	ensure safe drinking water

Acute Viral hepatitis by Type: 1982-1993
Pre-Vaccine



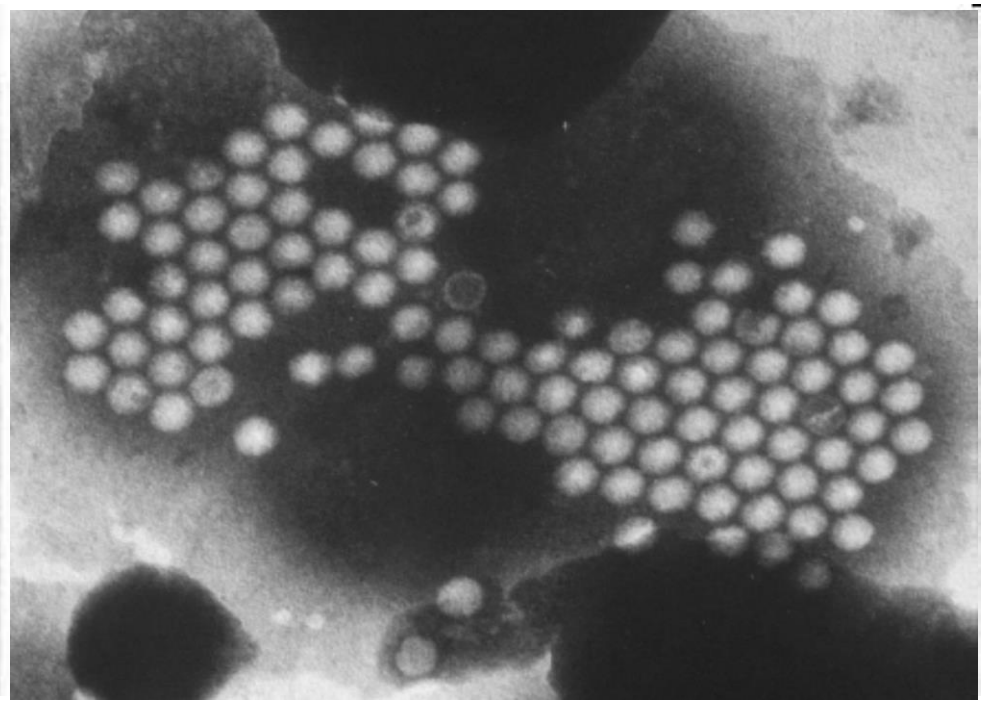
Source: CDC Sentinel Counties Study on Viral Hepatitis

Hepatitis A Virus by Electron Microscopy



HAV Immune-EM

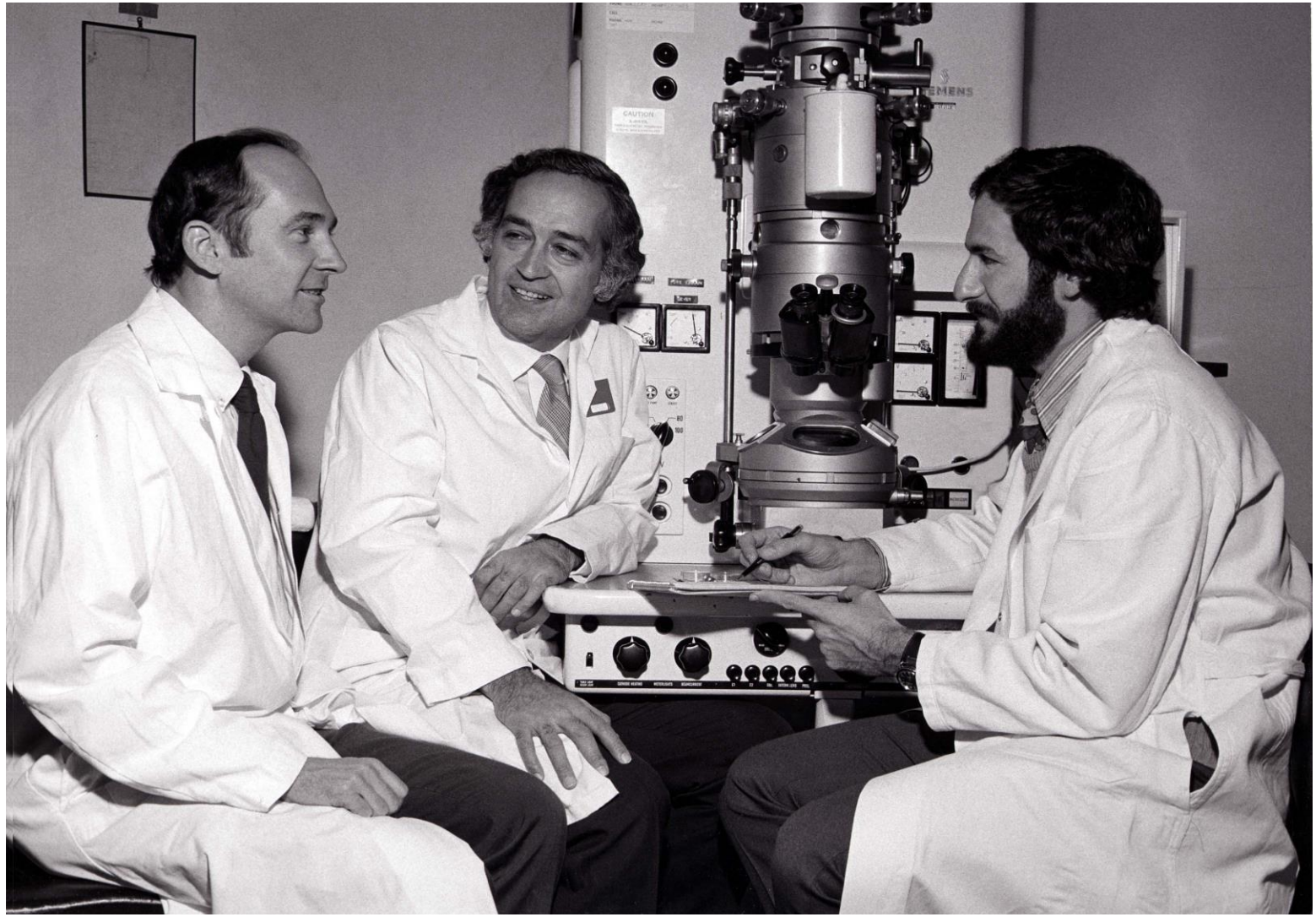
Feinstone, Kapikian, Purcell, 1973



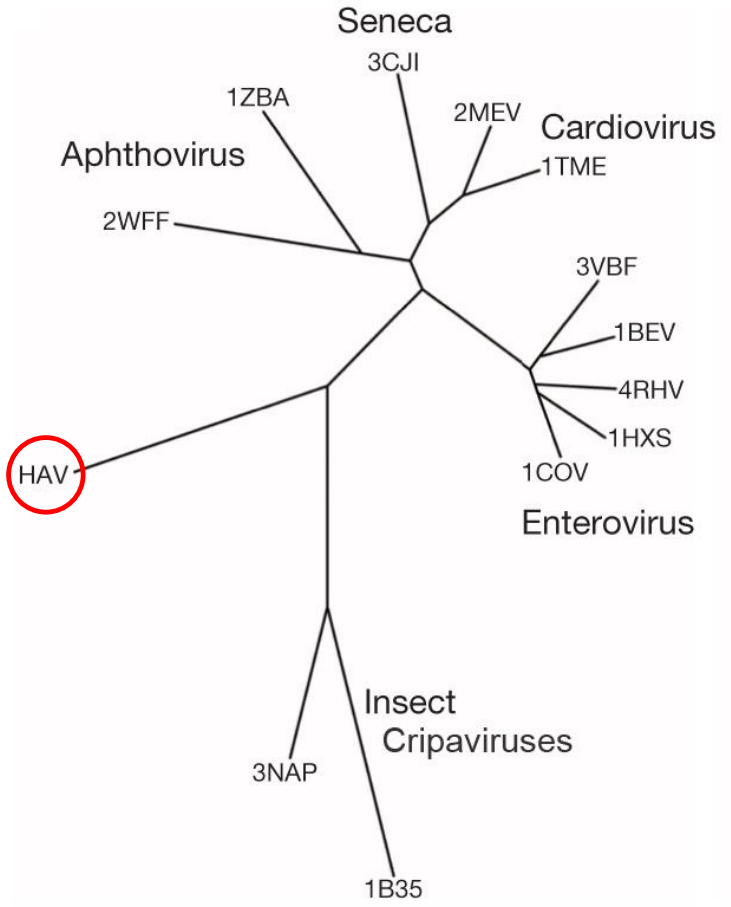
Purified HAV

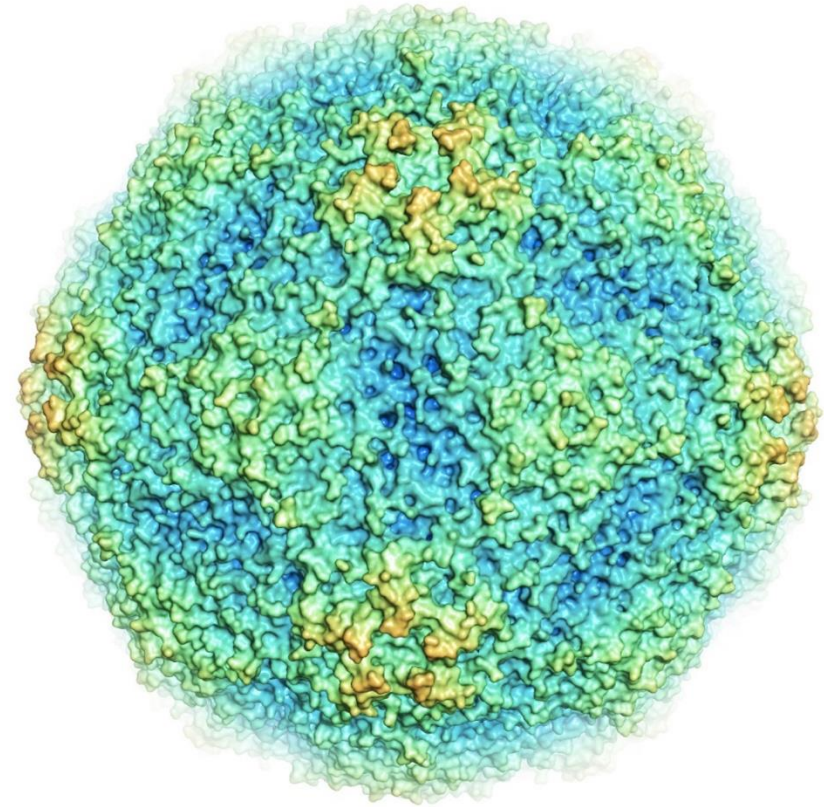
Moritsugu, 1976

The Team



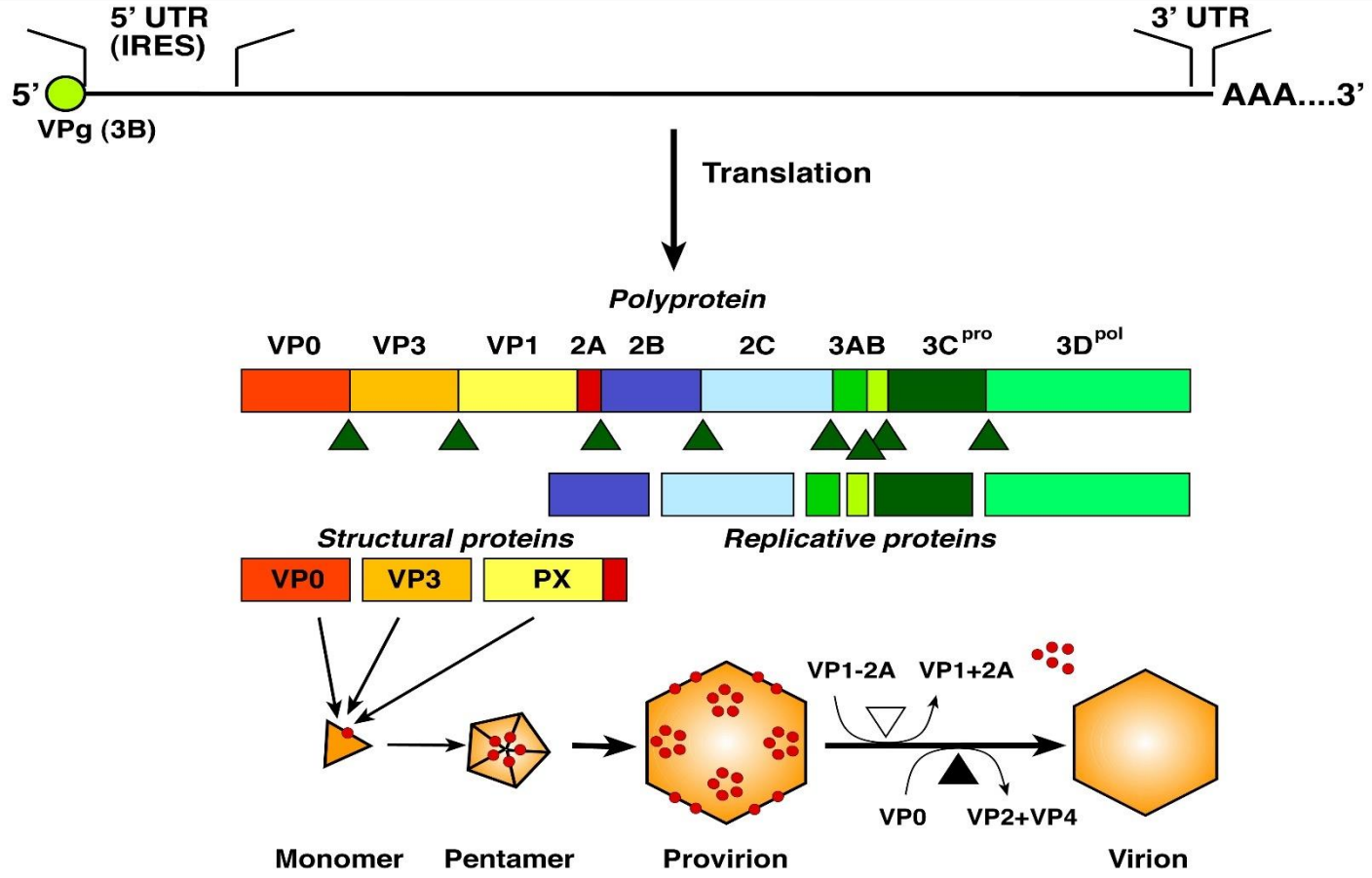
Structural Phylogenetic Analysis Reveals HAV to Be a 'Primitive' Picornavirus



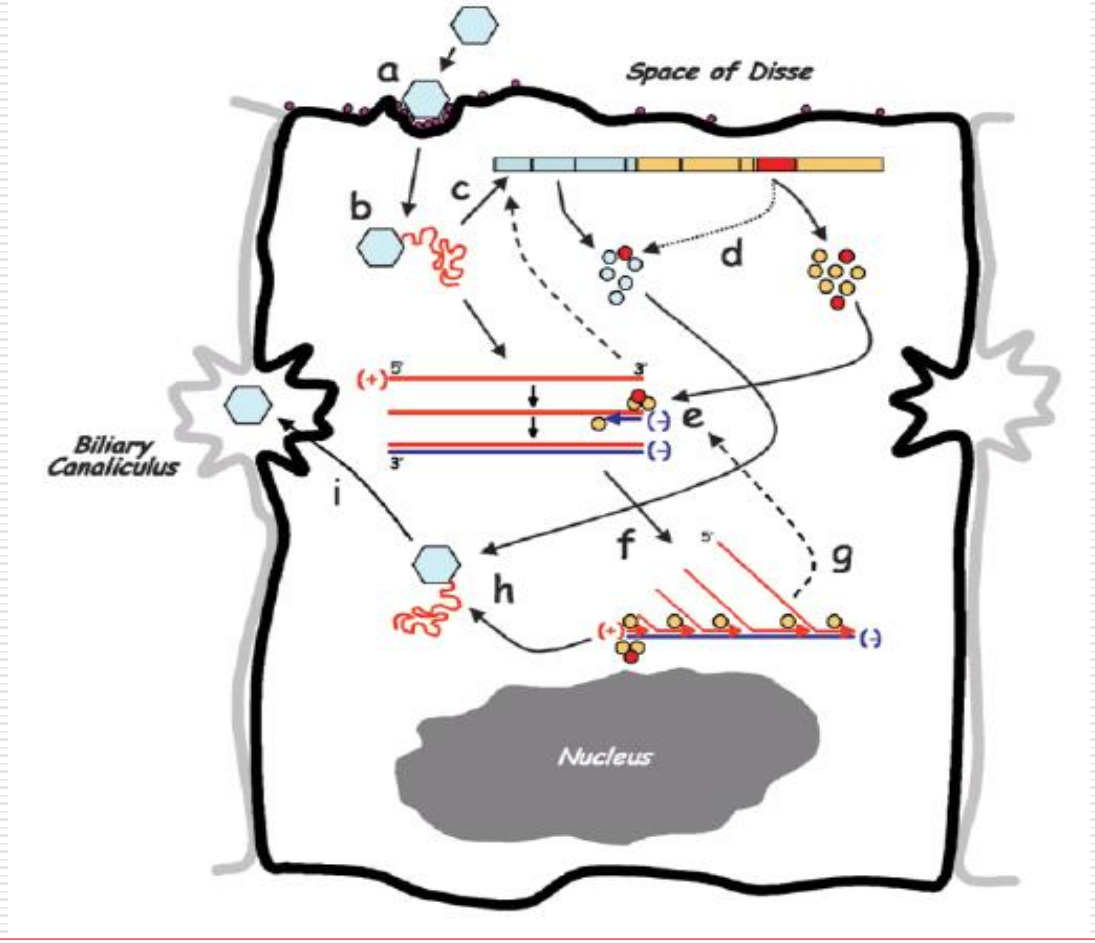


Wang et al., Nature 2014 doi:10.1038/nature13806

HAV
Genome – Gene products – Processing - Assembly



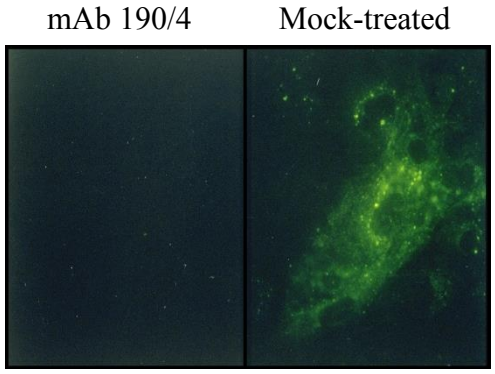
Replication Cycle of HAV



Martin and Lemon, 2006

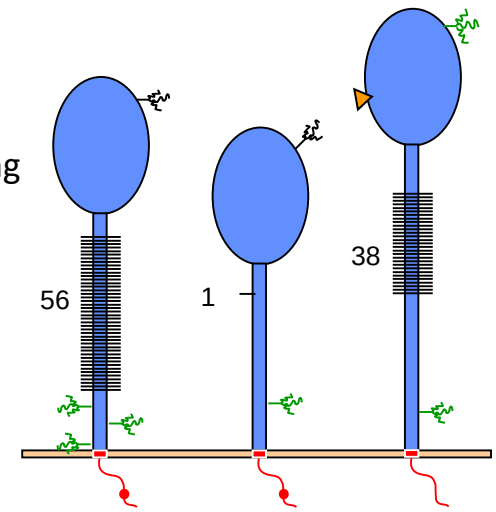
Identification of HAVCR1 as a cellular receptor for HAV

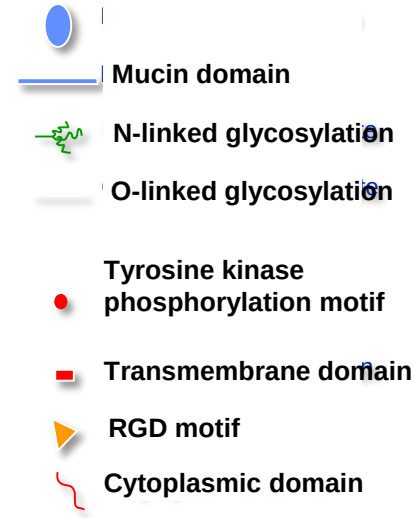
Protection against HAV infection



Expression cloning HAVCR1

Other members of the HAVCR family





HAVCR1 (TIM1) | **HAVCR2 (TIM3)** | **TIM4**

•HAV receptor

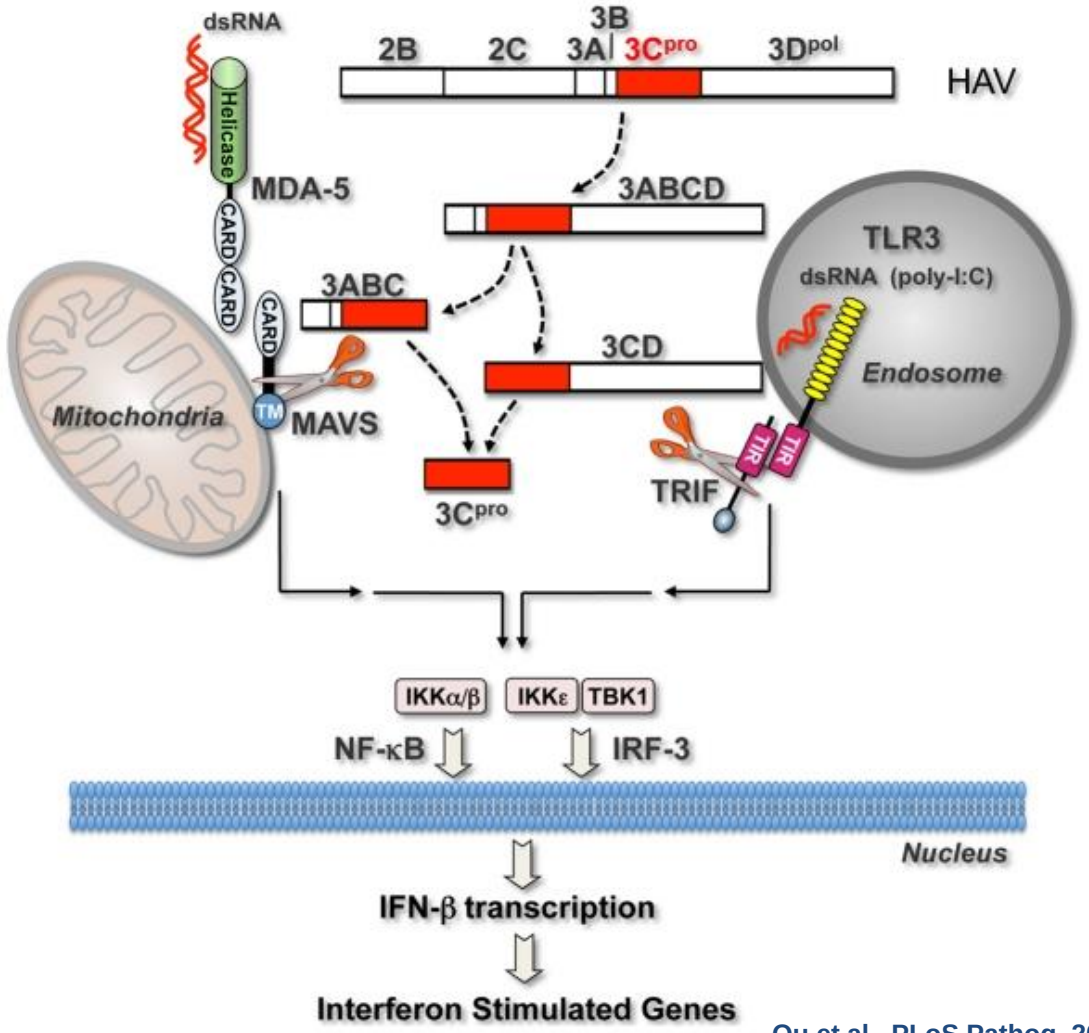
•Expressed in liver, kidney, lung, T helper 2, and NKT cells
•Significant allergy determinant gene in man and mouse
•Inverse association between HAV infection and development of asthma?
•Polymorphism affect T cell function associated with chronicity in HCV and HIV infection

•Expressed in T Helper 1 and macrophages
•Induces a negative signal
•Regulates autoimmune responses

HAVCR (TIM) family: phosphatydilserine receptors that regulate the immune response

•Expressed in antigen presenting cells
•Mediates phagocytosis of apoptotic cells
•Involved in allergic responses (peanut allergy)

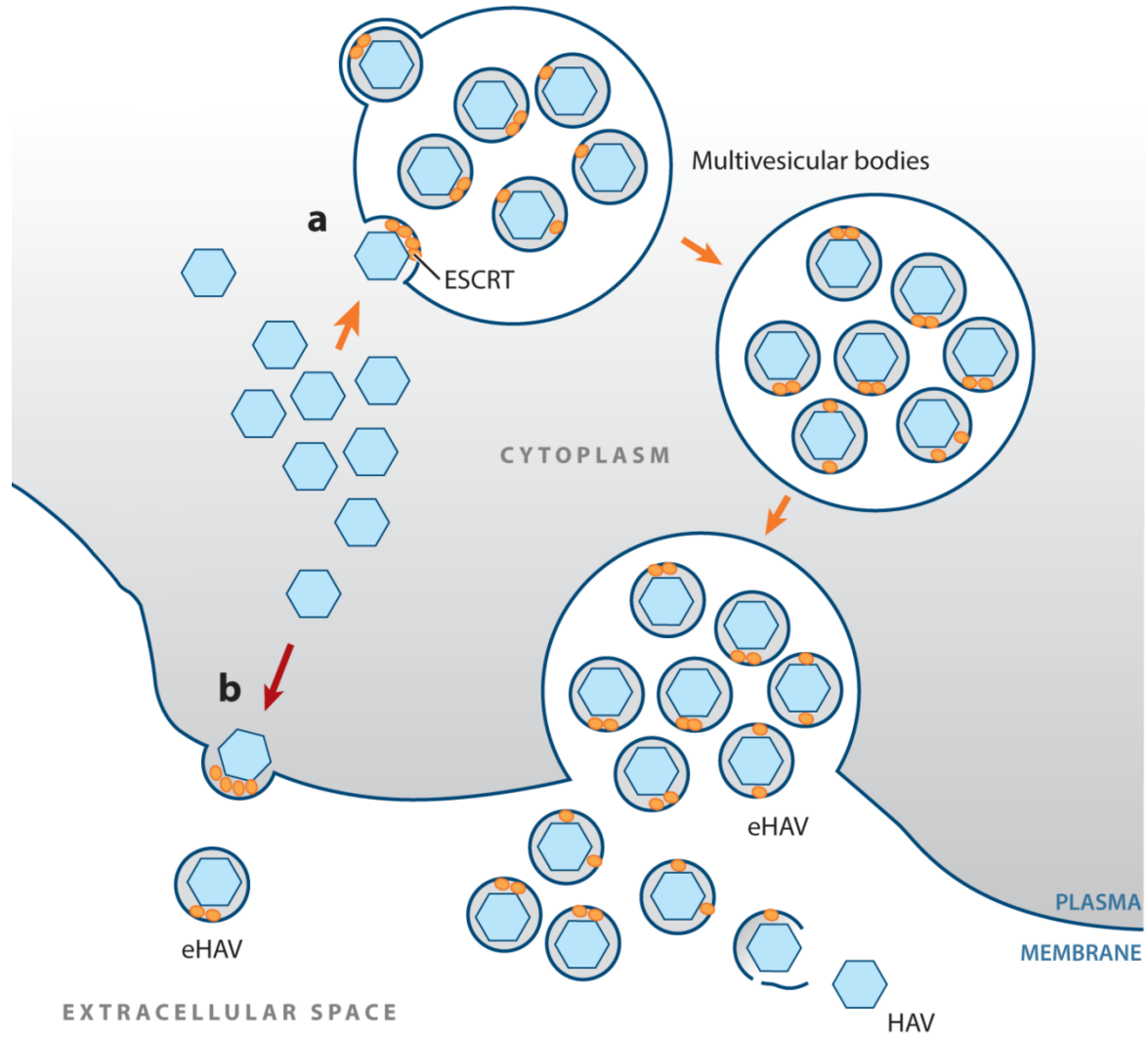
IFN-activating pathways disrupted by HAV 3C precursor-mediated proteolysis both MAVS and TRIF



Long incubation period
Persistent infection in cell culture

Qu et al., PLoS Pathog, 2011

Membrane Hijacking by Hepatitis A Virus



Z Feng and SM Lemon, *Ann Rev Virology* 1:26.1, 2014

HEPATITIS A - CLINICAL FEATURES

- Rare complications: Fulminant hepatitis
  Cholestatic hepatitis
  Relapsing hepatitis

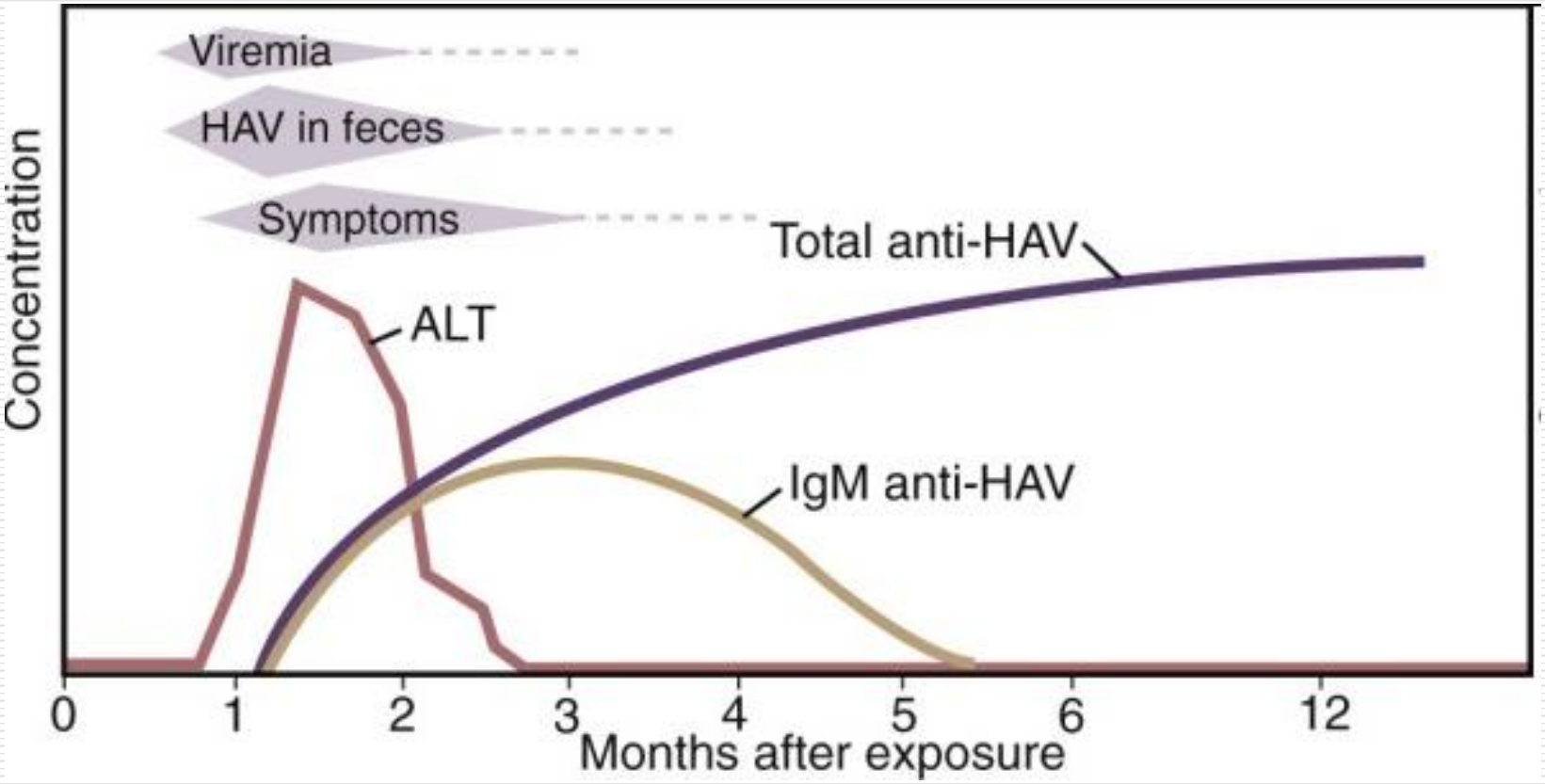
- Jaundice by age group:

<6 yrs	<10%
6-14 yrs	40%-50%
>14 yrs	70%-80%

- Incubation period: Average 30 days
  Range 15-50 days

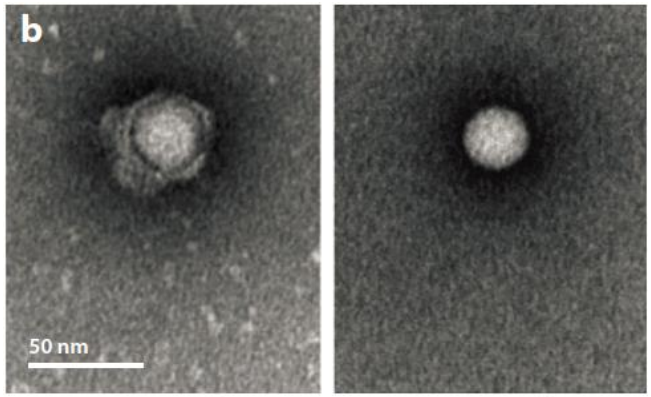
- Chronic sequelae: None

CLINICAL, VIROLOGIC AND SEROLOGIC EVENTS in HAV INFECTION

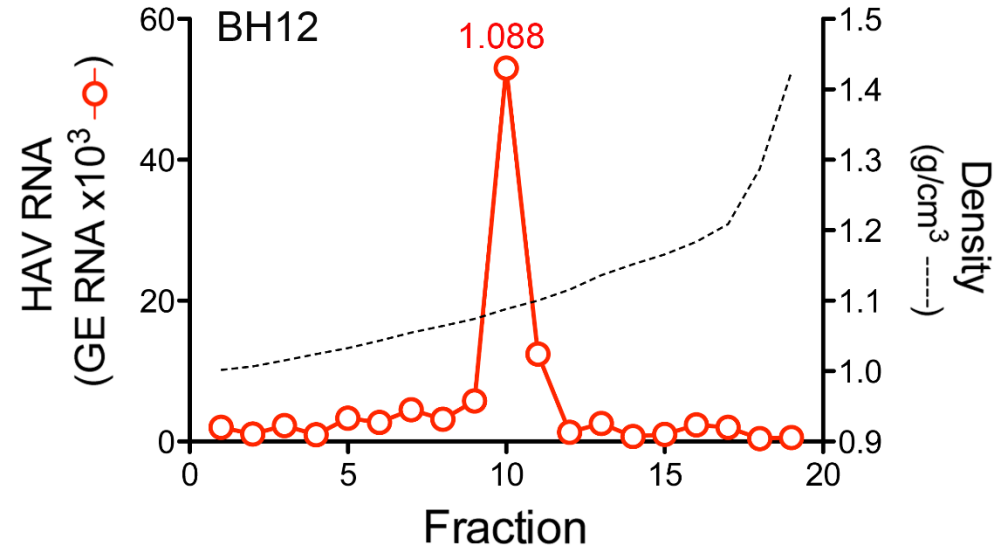


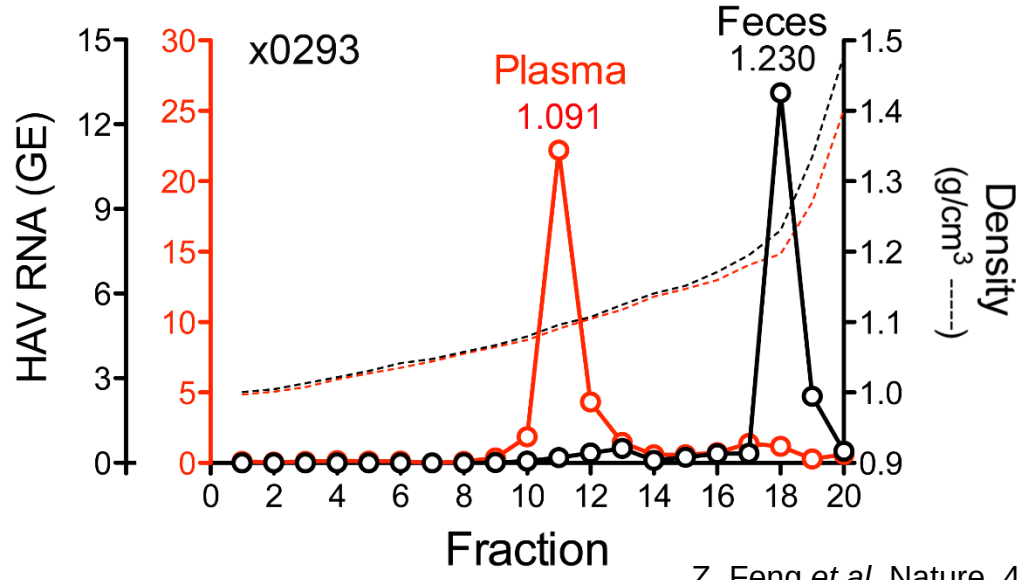
eHAV Circulates in Infected Humans and Chimpanzees while Virus Shed in Feces is Not Associated with Membranes

Human Serum



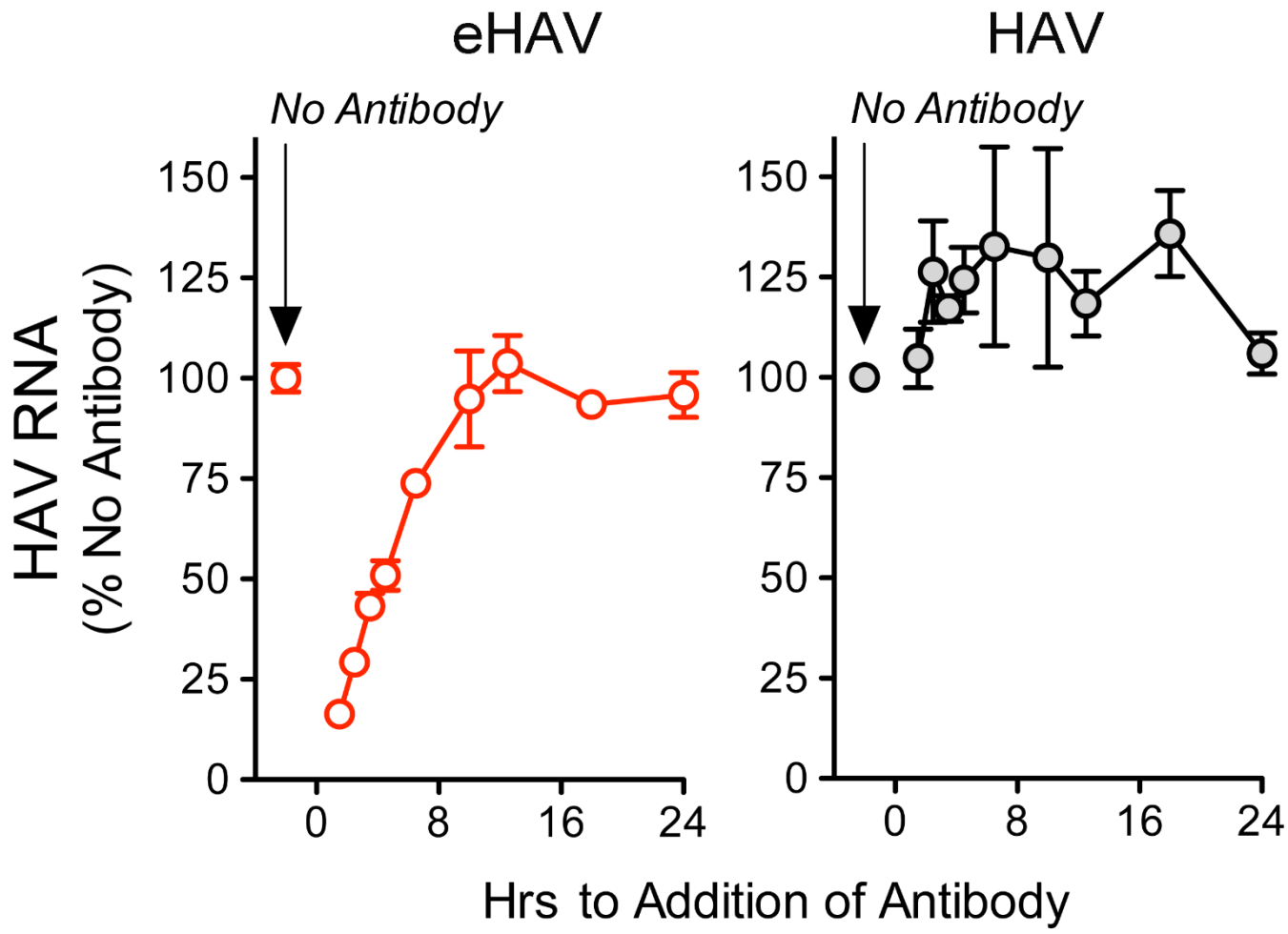
Chimpanzee
Plasma and Feces





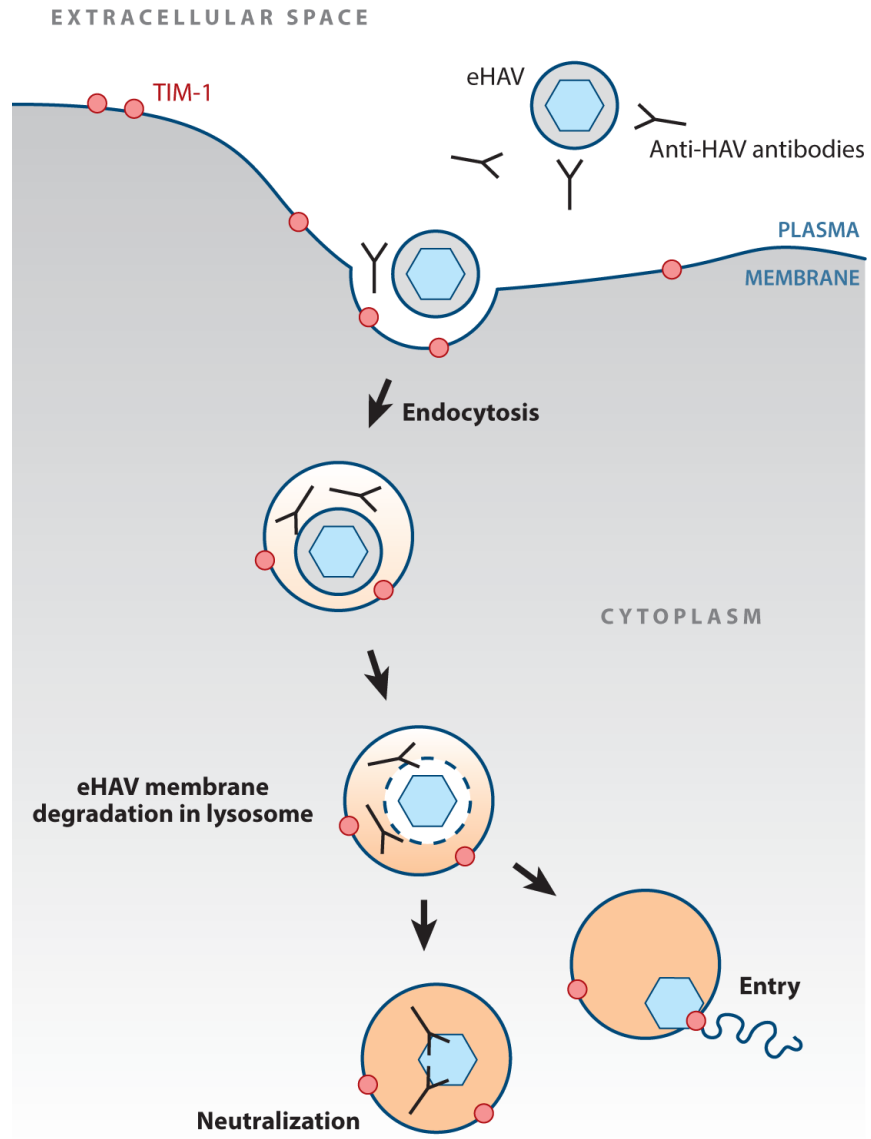
Z. Feng *et al.* Nature, 496:367-71, 2013

eHAV is Neutralized by Antibody Post-Endocytosis



Z. Feng *et al.* Nature, 496:367-71, 2013

Model for Post-Endocytic Neutralization of eHAV

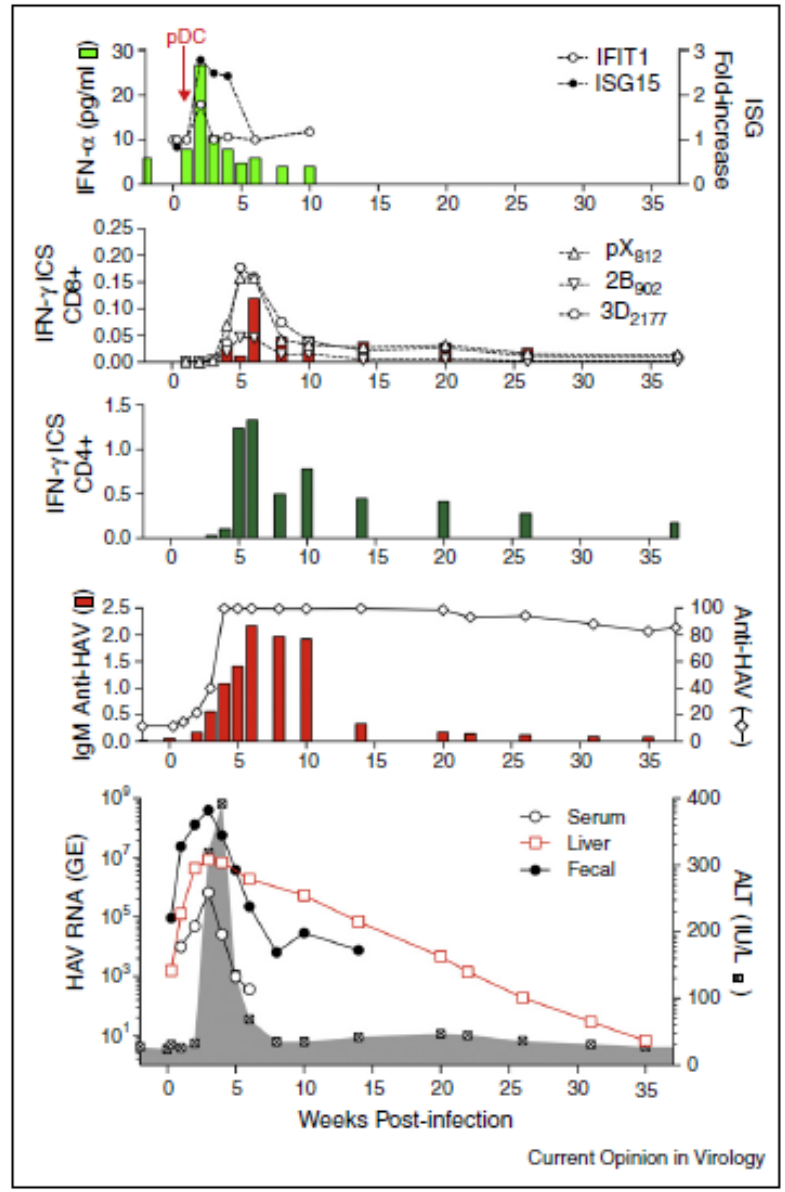


Z Feng and SM Lemon, *Ann Rev Virology* 1:26.1, 2014

Virologic and immunologic events in an acute HAV infection (Chimpanzee)

Pathogenesis: Is hepatitis A immune mediated?

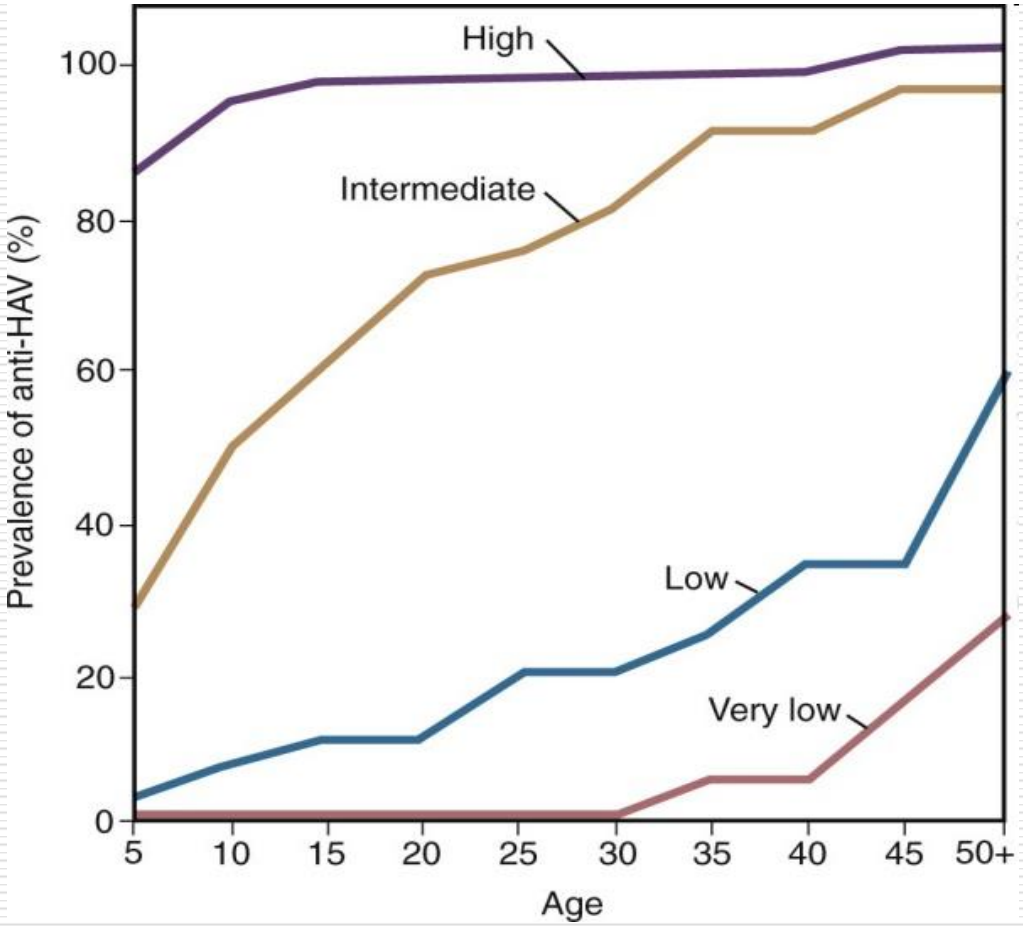
How are HAV infections controled.?



Walker et al, Current Opinions in Virology, 2015

WORLDWIDE PATTERNS of HAV ENDEMICITY

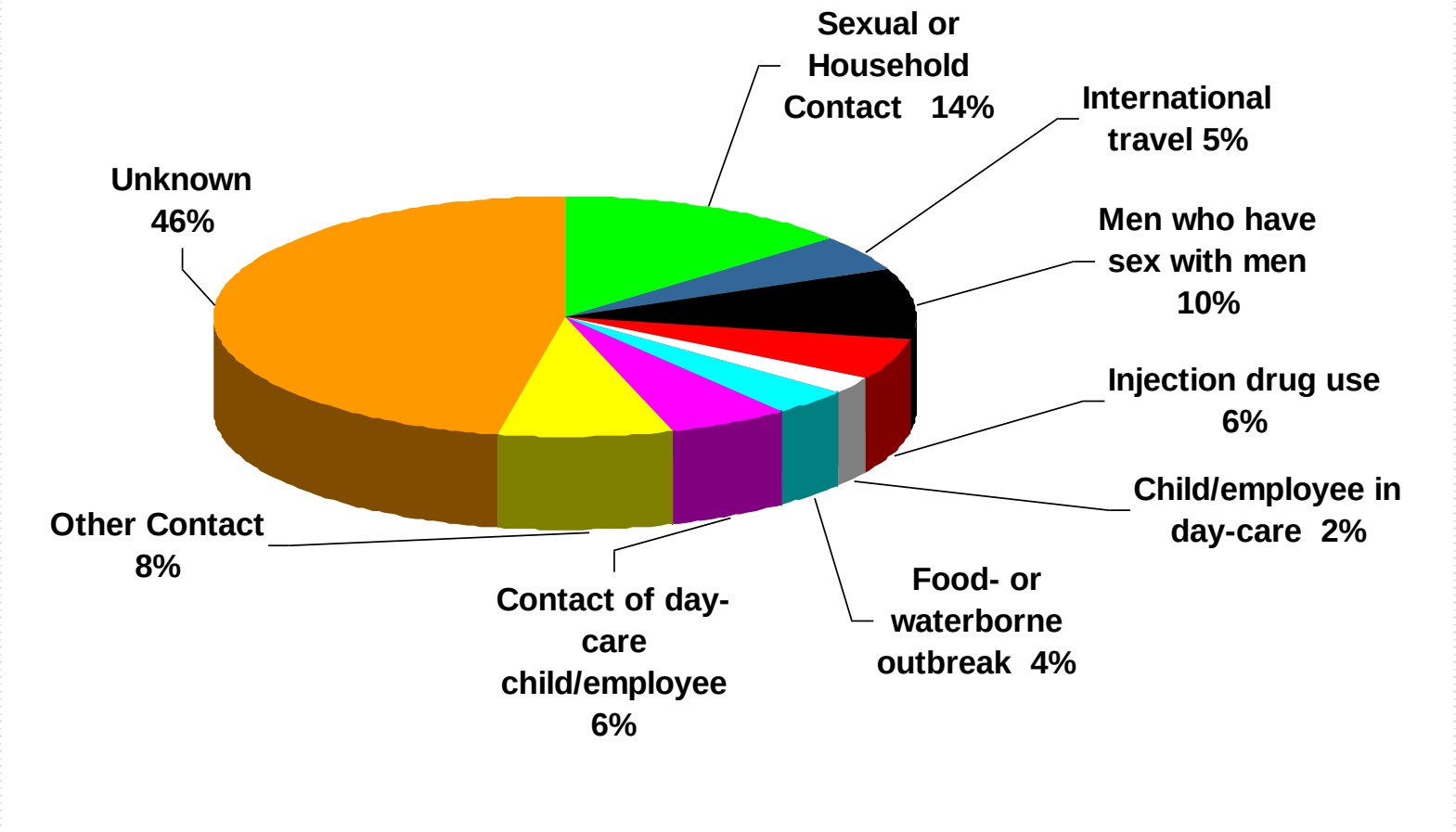
Age stratified prevalence of anti-HAV in different epidemiologic settings



GLOBAL PATTERNS OF HEPATITIS A VIRUS TRANSMISSION

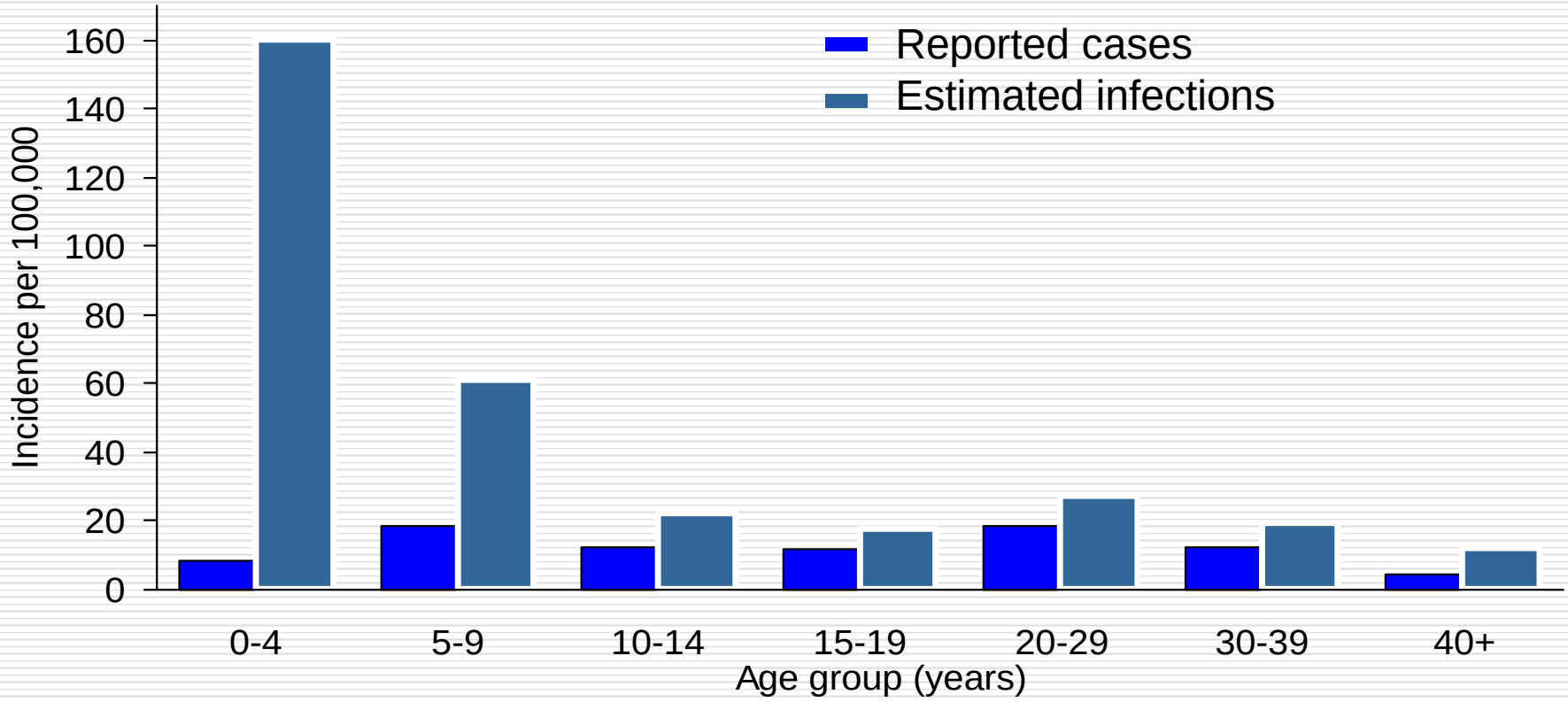
Endemicity	Disease Rate	Peak age of infection	Transmission Pattern
High	Low to High	Early childhood	Person to person Outbreaks uncommon
Moderate	High	Late childhood/ young adults	Person to person Food and water borne outbreaks
Low	Low	Adults	Person to person Food and water borne outbreaks
Very low	Very low	Adults	Travelers Outbreaks uncommon

RISK FACTORS ASSOCIATED WITH HEPATITIS A 1990-2000, UNITED STATES



Source: CDC

Age Distribution of Acute HAV Infections in the U.S.



Source: Armstrong & Bell, Pediatrics, 2002

Protection against hepatitis A

It’s the antibody, stupid!

(Paraphrased from Bill Clinton, 1992 Presidential campaign)

PREVENTING HEPATITIS A

- **Hygiene**
- **Sanitation**
- **Immune globulin (pre- and post-exposure)**
- **Inactivated Hepatitis A vaccine (pre- and post-exposure)**

Hepatitis A Prevention – Immune Globulin
largely replaced by vaccine

- Pre-exposure
  - travelers to intermediate and high HAV-endemic regions who cannot take HAV vaccine
- Post-exposure (within 14 days)

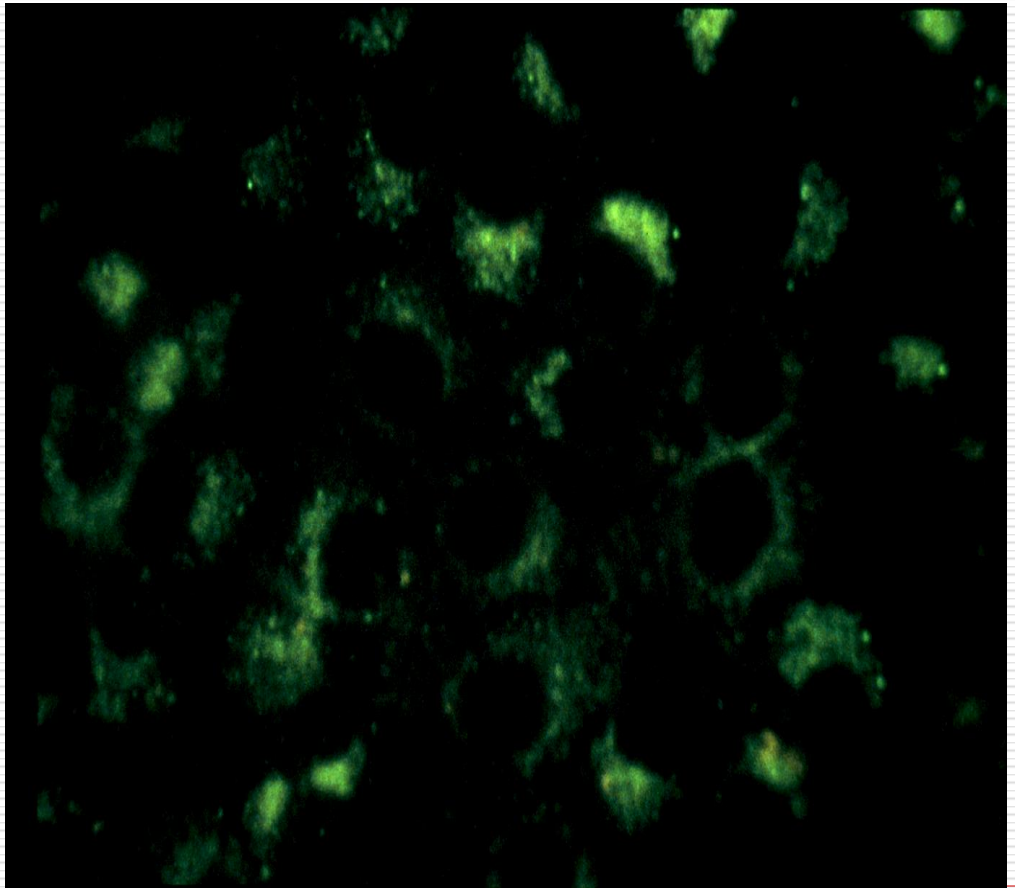
  Routine
  - household and other intimate contacts – vaccine now considered as good as IG

  Selected Situations
  - institutions (e.g., day care centers)
  - common source exposure (e.g., food prepared by infected food handler)

HAV Vaccine Principles

- One serotype
- Growth in cell culture
- Low level of serum antibody alone is protective

HAV in Cell Culture

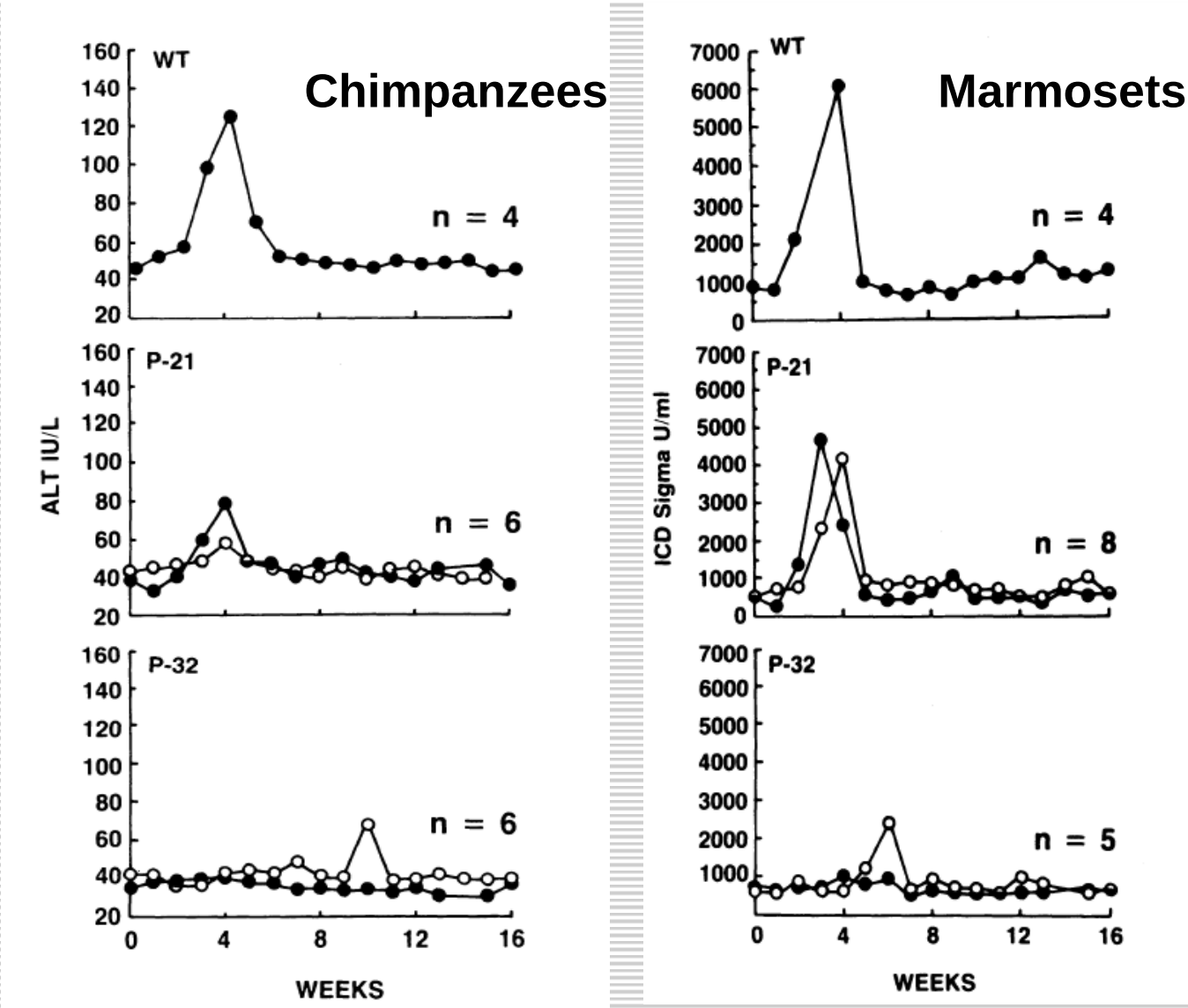


HAV in AGMK cells by Immunofluorescence

Characteristics of HAV in Cell Culture

- Primary isolation requires long incubation period
- Adaptation Through passage
- Host restriction to Primate cells + a few others
- HAV remains largely cell associated
- No cytopathic effect
- Virus establishes persistent infections

Attenuation of HAV after Serial Passage in 1º AGMK Cells



Karron et al., 1992

Characteristics of Live HAV Vaccine

- Proper attenuation is difficult to achieve
- Poor Response to oral administration
- Requires multiple i.m. or s.c. dose to achieve adequate immune response
- Antibody responses generally low but durable
- Risk of reversion to virulence?
- Cold chain requirement?

Two live HAV vaccines in use in China/India

Principles of killed HAV vaccine

- Produced in cell culture
- Virus attenuated in humans – safety factor
- Purified
- Inactivated by formalin
- Adjuvented – Alum - Virosomes
- Single dose provides at least short term immunity
- Two doses provide protection > 20 years

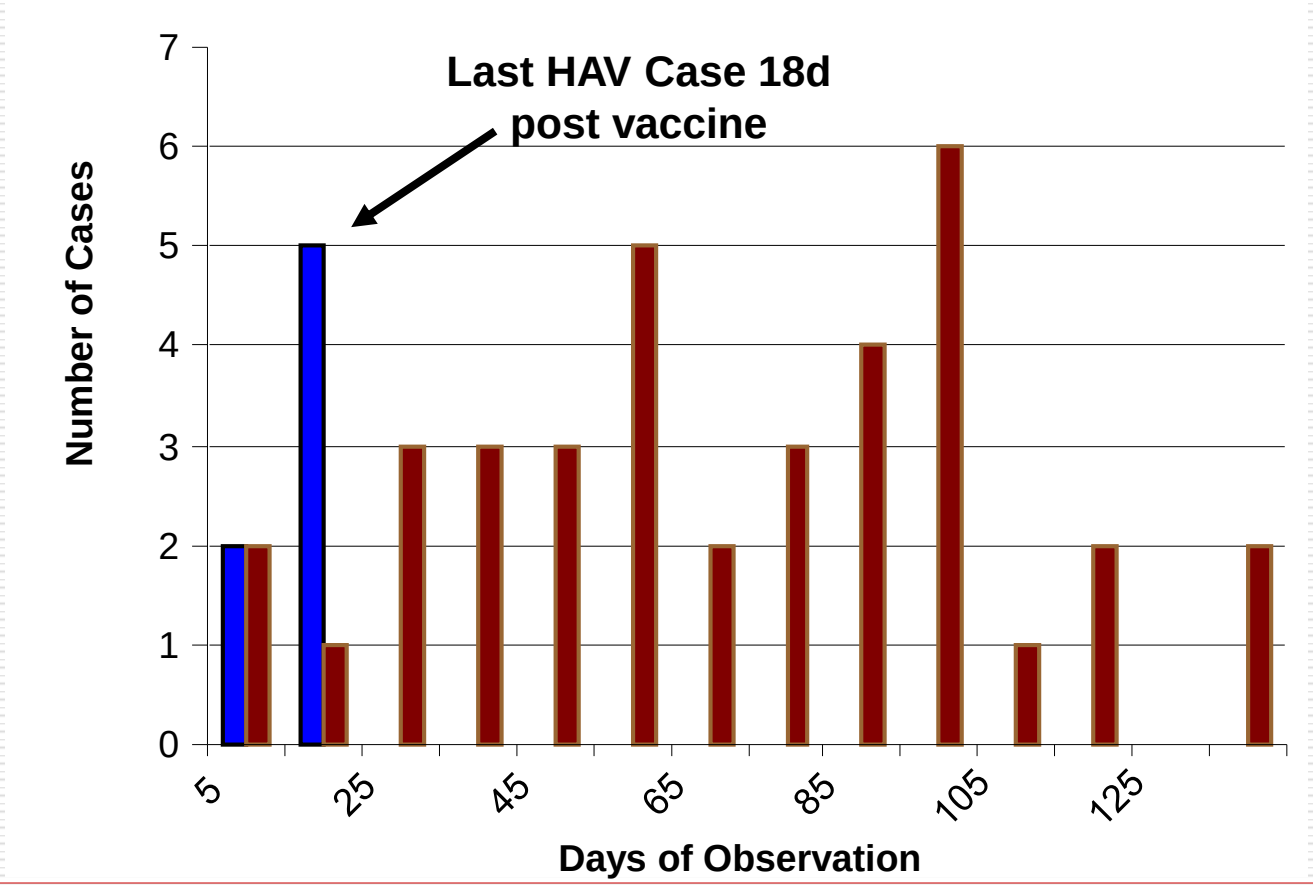
KILLED HEPATITIS A VACCINES

- **Highly immunogenic**
  - **97%-100% of children, adolescents, and adults have protective levels of antibody within 1 month of receiving first dose; essentially 100% have protective levels after second dose**

- **Highly efficacious**
  - **In published studies, 94%-100% of children protected against clinical hepatitis A after equivalent of one dose**

Efficacy of a Single Dose of HAV Vaccine (Merck)

HAV Vaccine n=519
Placebo n=518

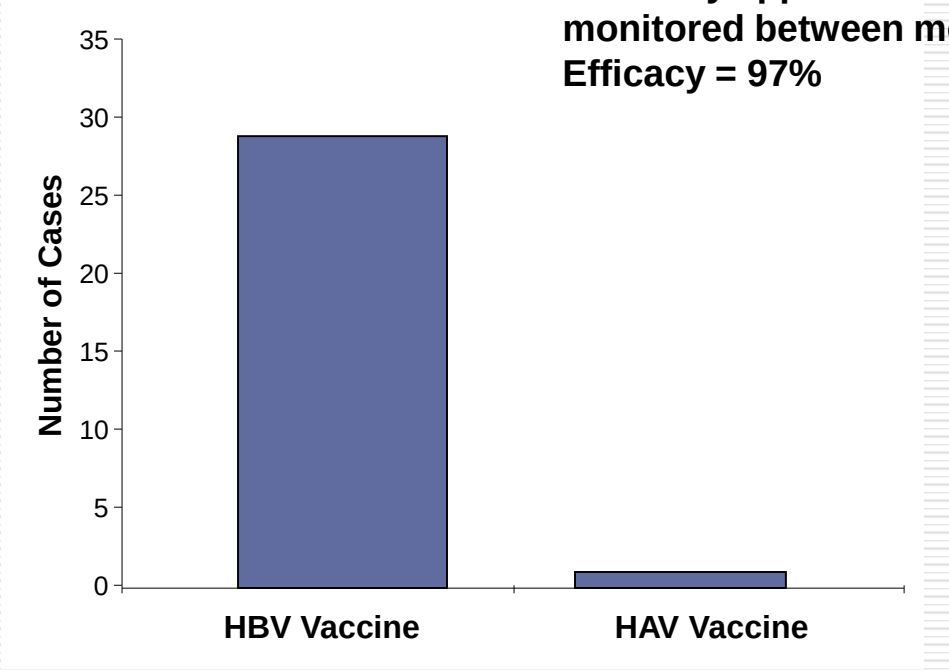


From Werzberger et al., 1992

Efficacy of a 2 Dose Inactivated HAV Vaccine (GSK)

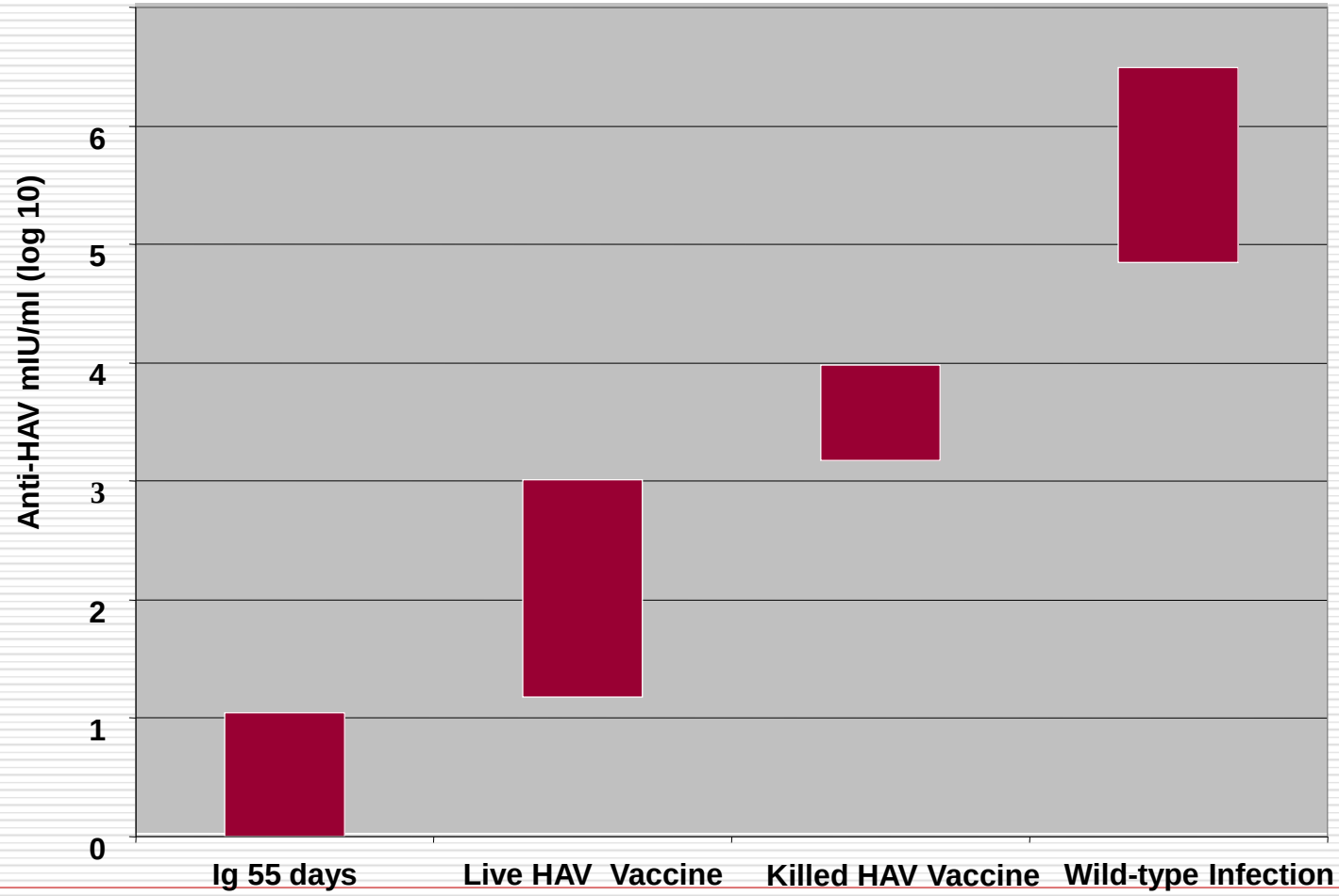
- n = 40,119 Thai schoolchildren

Clinically apparent cases were monitored between months 5-10
Efficacy = 97%



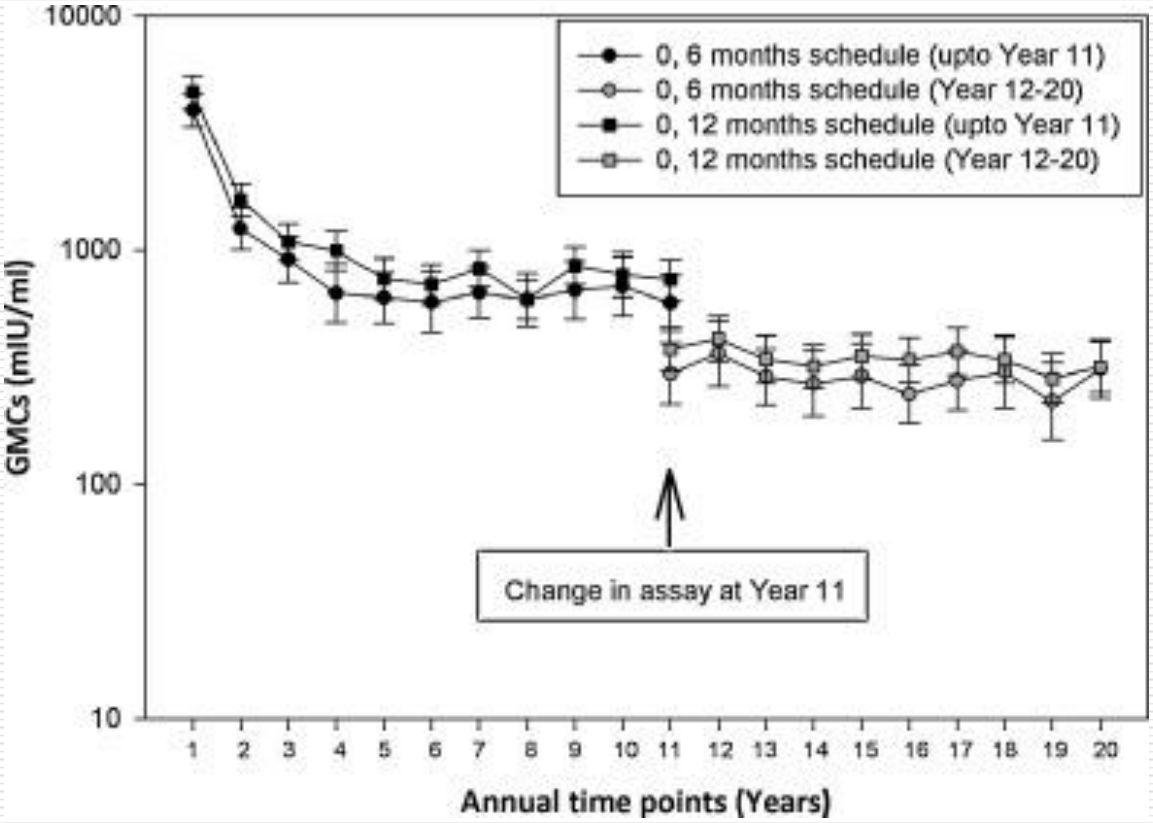
From Innes et al., 1992

HAV ANTIBODY TITERS



Lemon: Rev Med Virol, 1992

Durability of Vaccine Response



Theeten et al. Vaccine 2015

Worldwide HAV Vaccine Strategies

- **Developing Countries**
  - Probably no general use vaccine at this time
- **Transition Countries**
  - Focus vaccine primarily on children - universal
- **Developed Countries**
  - Mixed strategy for universal childhood vaccination, high risk individuals, high incidence areas emphasis on children, community outbreaks (children)
- **Highly Developed - Very low incidence countries**
  - High risk individuals ie. travelers

HA Vaccine U.S. - Initial Strategy 1996

- Children in regions with high rates of hepatitis A (e.g., Alaska Natives, American Indians)
- Persons at increased risk for infection
  - Travelers to intermediate and high HAV-endemic countries
  - Homosexual and bisexual men
  - Intravenous drug users
  - Persons with chronic liver disease (increased Health risk)

HA Vaccine U.S. - Modified Strategy 1999

- Children in regions with high rates of hepatitis A (e.g., Alaska Natives, American Indians)
- Children in communities, counties, states with consistently high disease rates
- Persons at increased risk for infection
  - Travelers to intermediate and high HAV-endemic countries
  - Homosexual and bisexual men
  - Intravenous drug users
  - Persons with chronic liver disease (increased Health risk)

HA Vaccine Strategy US: 2006 - Present

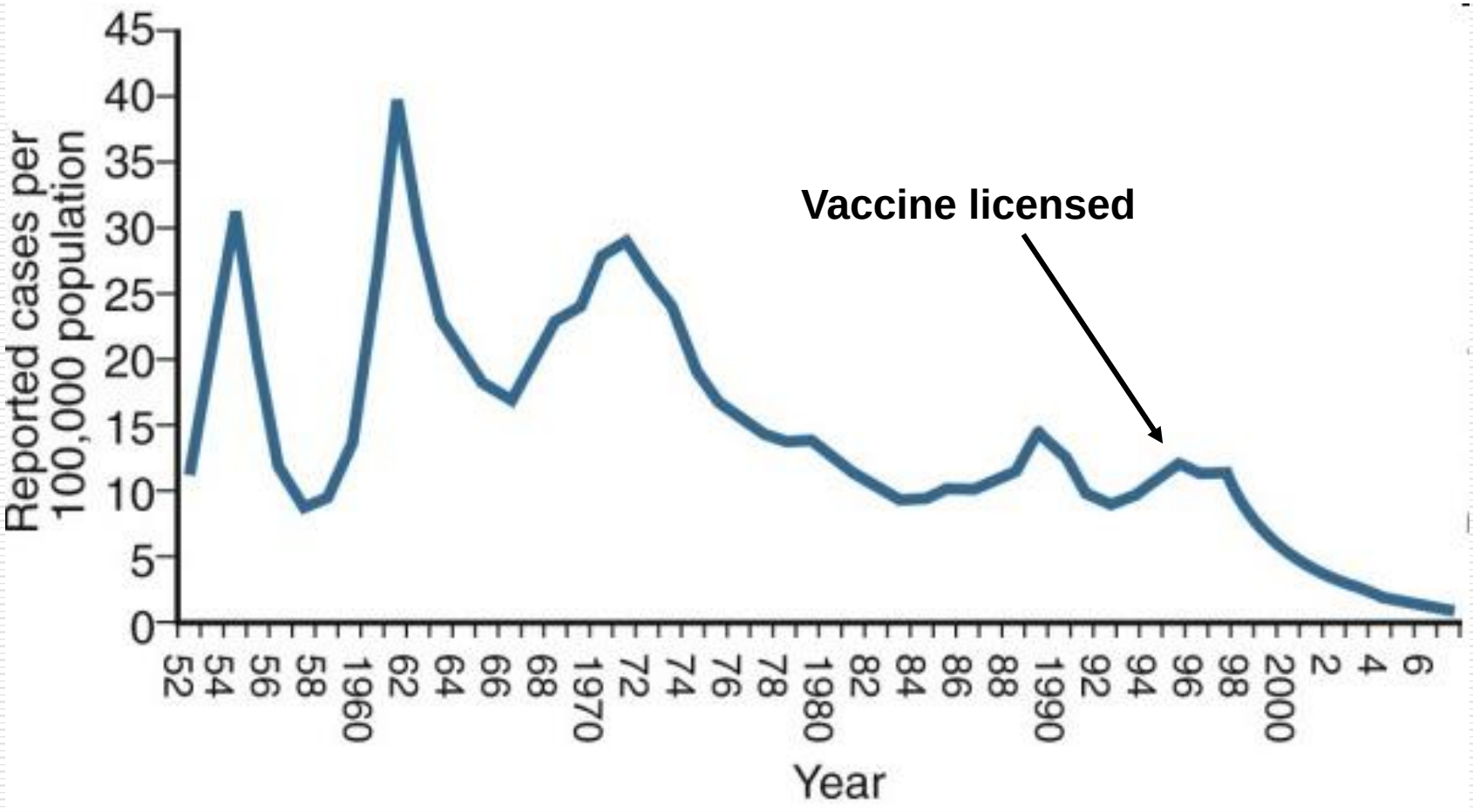
vaccine approved for 12 mo. old children

- Universal childhood vaccination at 12 mos
- Continue vaccination of high risk individuals

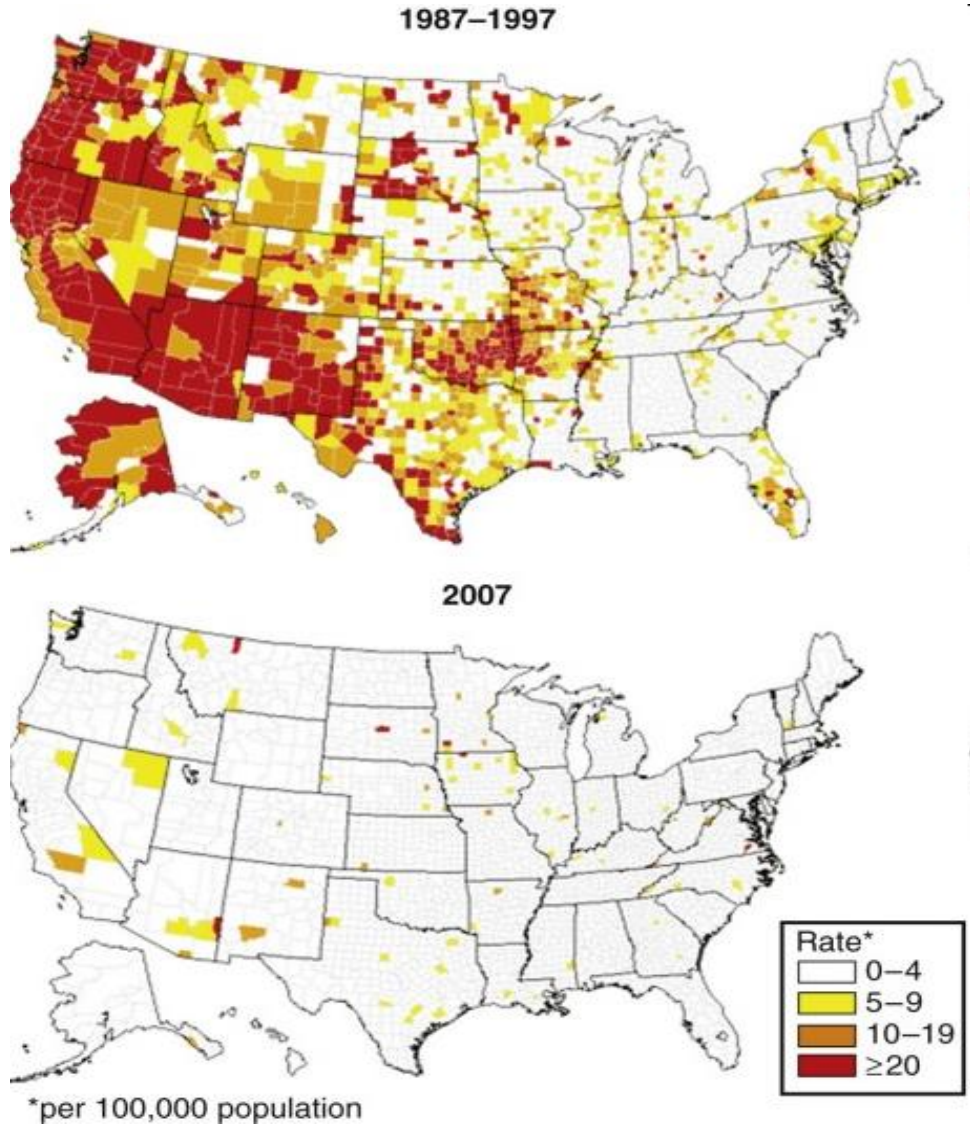
Universal Childhood Vaccination

- **Benefits**
  - established delivery system
  - vaccination before risk period
  - potential to interrupt transmission
- **Other issues & considerations**
  - immunogenicity in infants – maternal antibody
  - development of combination vaccines
  - duration of protection
  - cost-effectiveness

Hepatitis A Rates in the US: 1952-2007



Hepatitis A Rates by County in the US

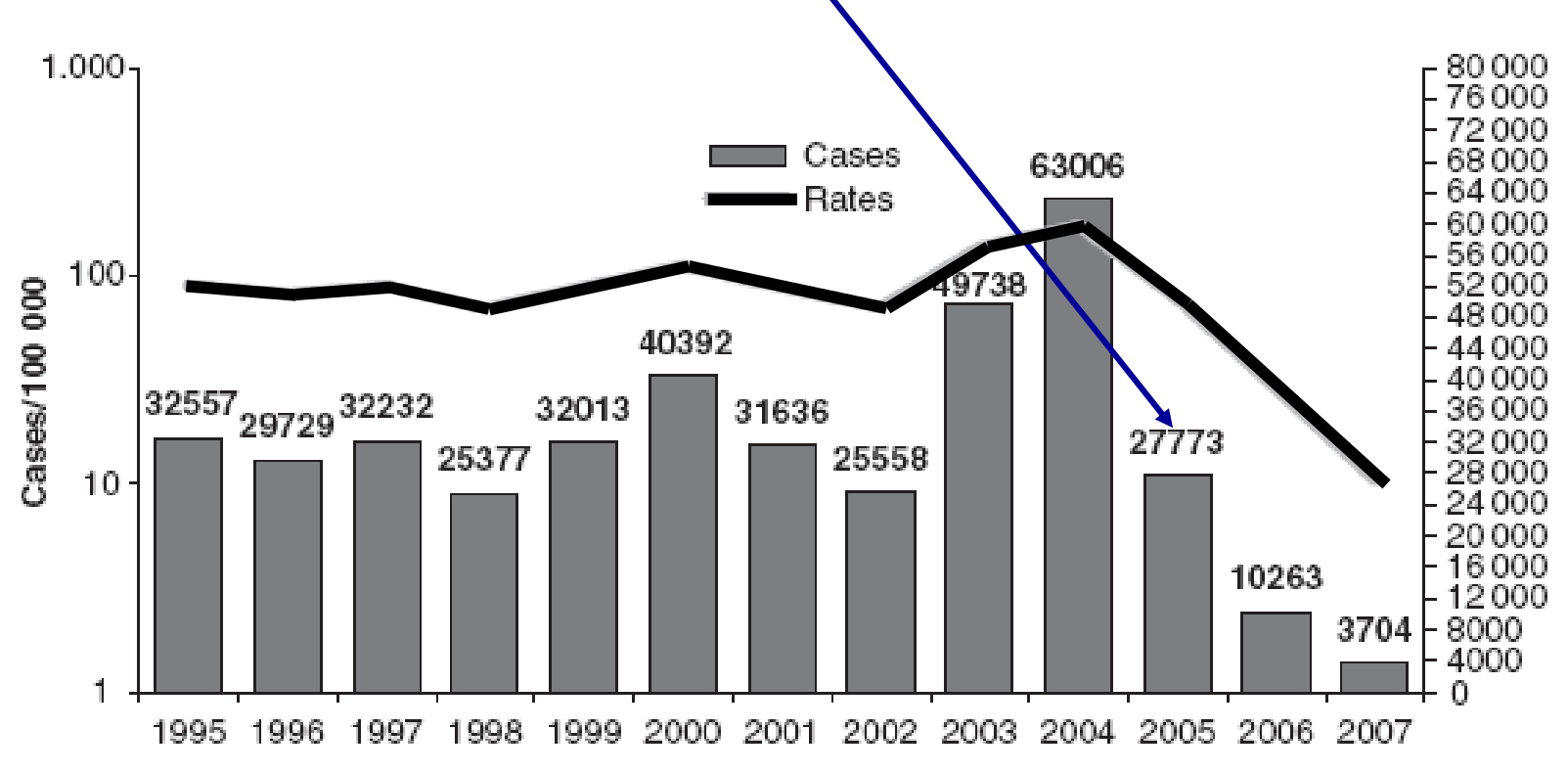


CDC data

Effect of Universal Childhood Vaccine in Argentina

Single dose HA vaccine given at 12 months

~95% Coverage Beginning July 2005



Vacchino, 2008

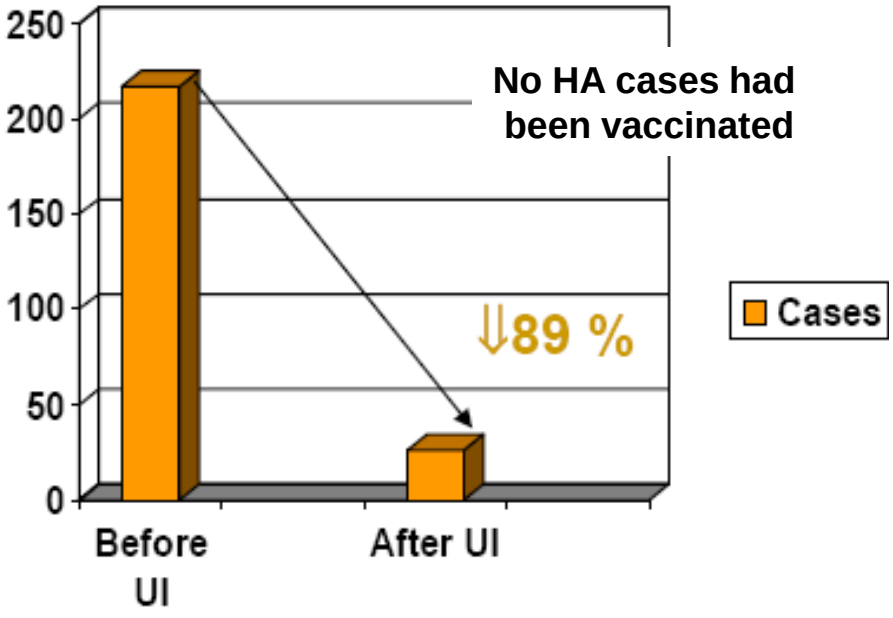
Change in HA Incidence by Age Group in Argentina After UI of 12 Month Old Children

Age (yrs)	Pre- UI (1998-2002)	2007	% decline
<1	32.2	6.1	81.2
1	67.9	11.5	83.1
2-4	201.3	26.1	87.1
5-9	248.8	28.2	88.7
10-14	108.6	17.9	83.6
15-49	20.6	4.4	78.8
50+	5.9	4.7	20.7
Overall	**88.5**	**10.2**	**88.0**

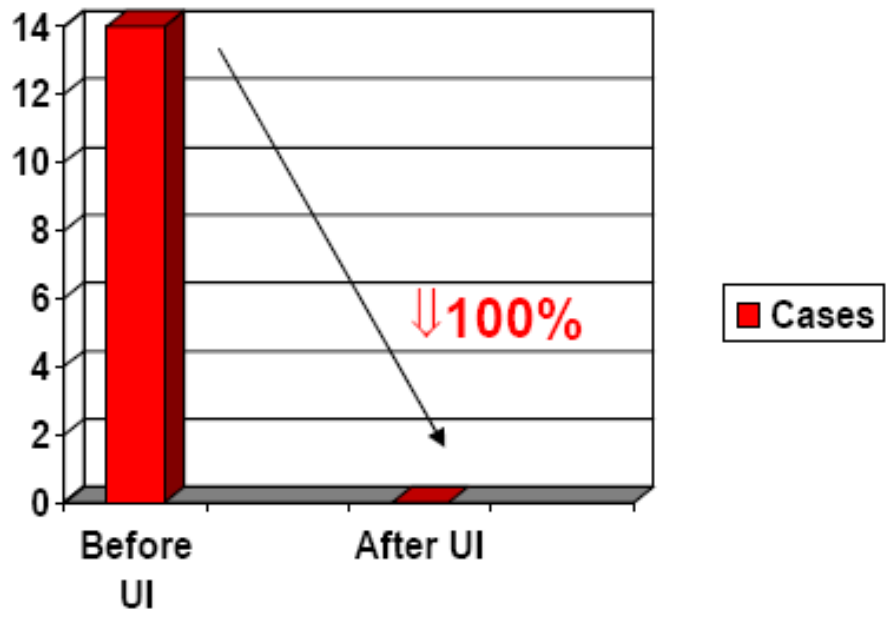
Vacchino, 2008

Argentina
Effect of UI on Rate of Severe Hepatitis A

Hospitalized



Fulimanant Hepatic Failure

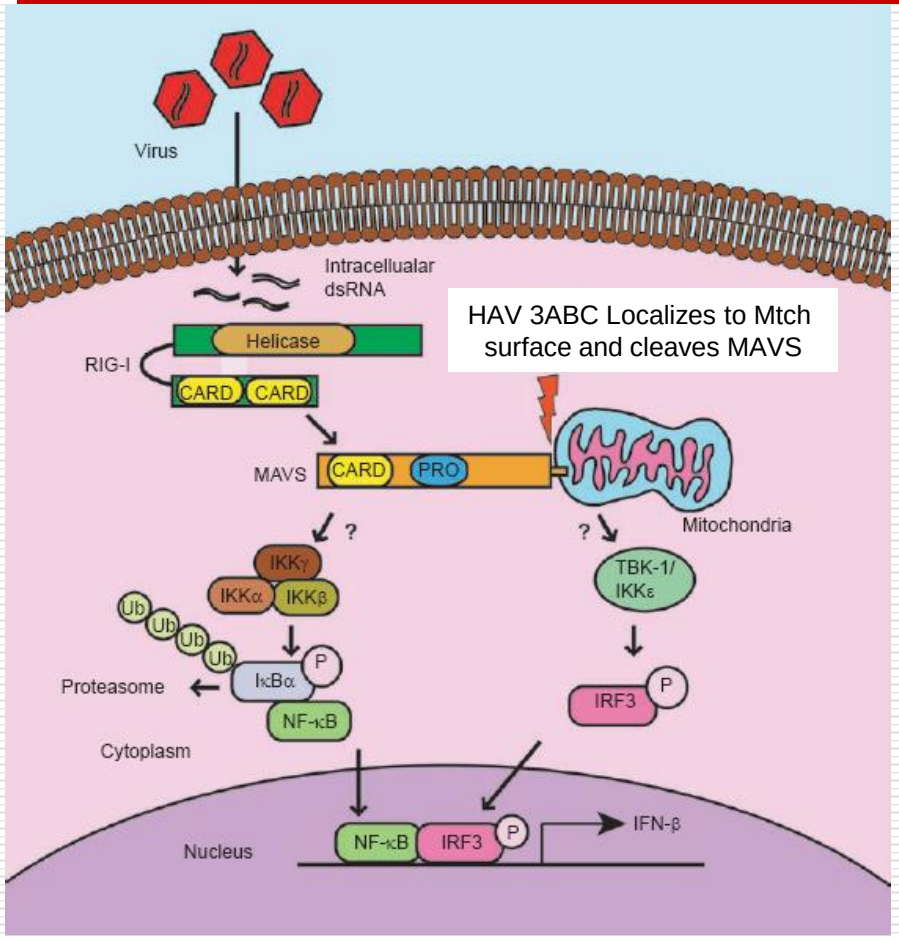


Pena et al., 2009

Summary

- HAV and HAV pathogenesis remain areas for study
- Serum antibody alone sufficient for protection
- HAV vaccines were first approved 22 years after the virus was identified
- Understanding of the epidemiology of HA has led to rational vaccine use strategies
- Childhood vaccination programs can have a profound impact on HA rates in the entire population
- HA can be controlled by vaccination and could potentially be eliminated

HAV Pathogenesis:
Disruption of IFN Signaling by HAV



From Seth et al, Cell Research, 2006

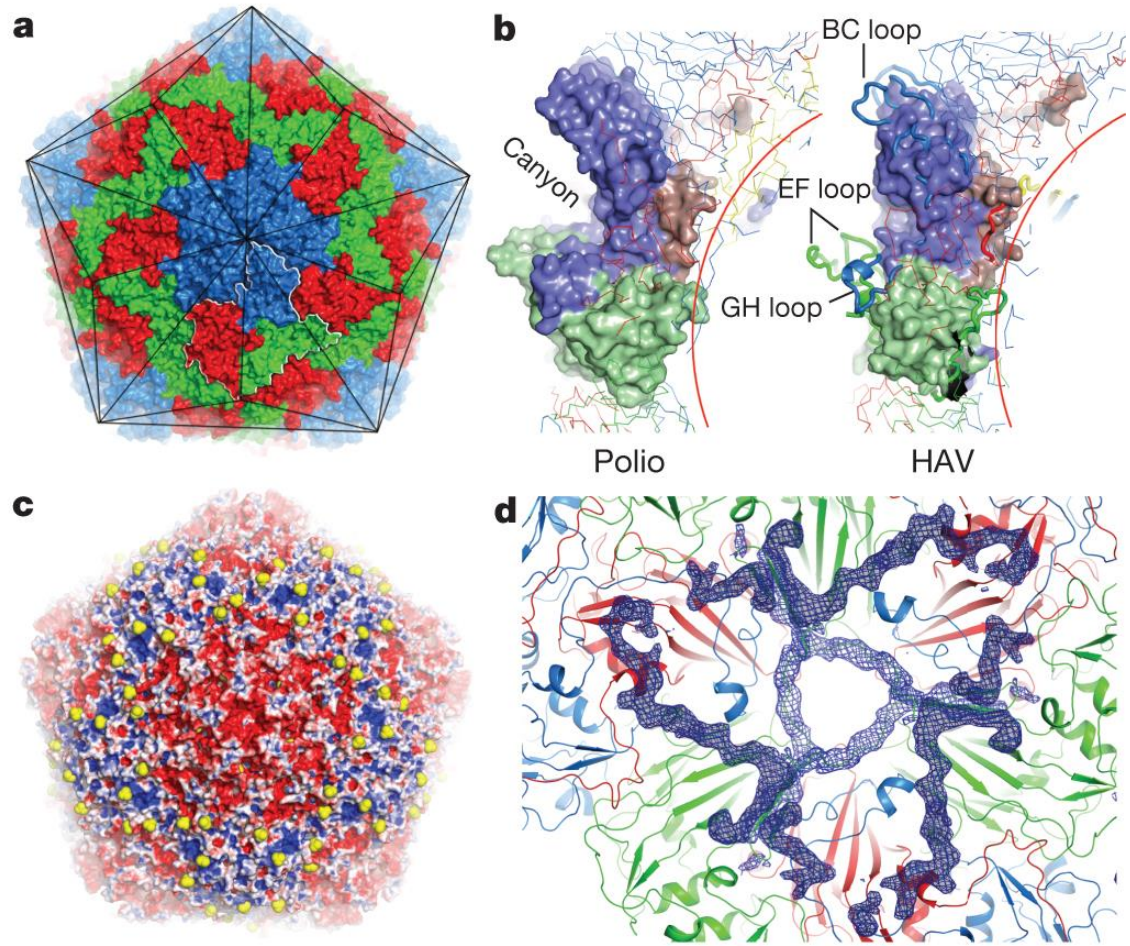
Yang et al., PNAS, 2007

HAV 3ABC protease precursor is localized to the mitochondrial surface through the transmembrane domain in 3A. The cysteine protease, $3C^{PRO}$ cleaves the **mitochondrial antiviral signaling** protein (MAVS) disrupting the interferon signaling pathway. Disruption of the IFN pathway may result in

a) the prolonged incubation period observed in HAV infections and

b) in the ability of the virus to establish persistent infections *in vitro*

Overall structure.

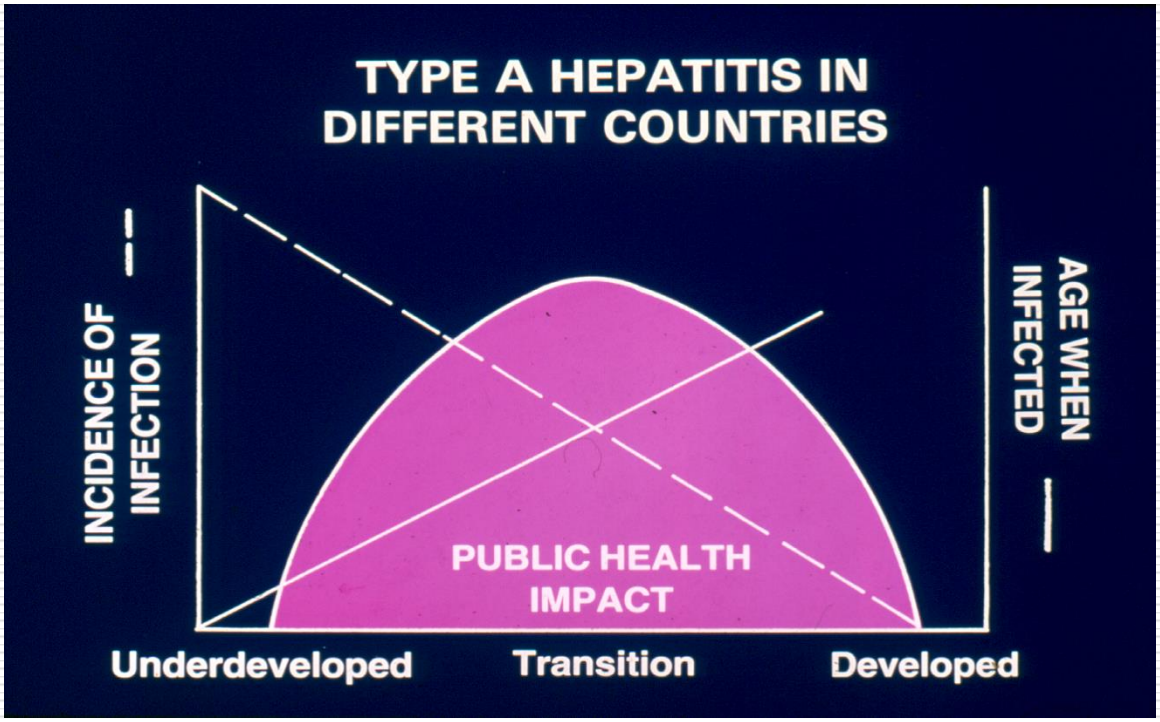


X Wang *et al. Nature* **000**, 1-4 (2014) doi:10.1038/nature13806

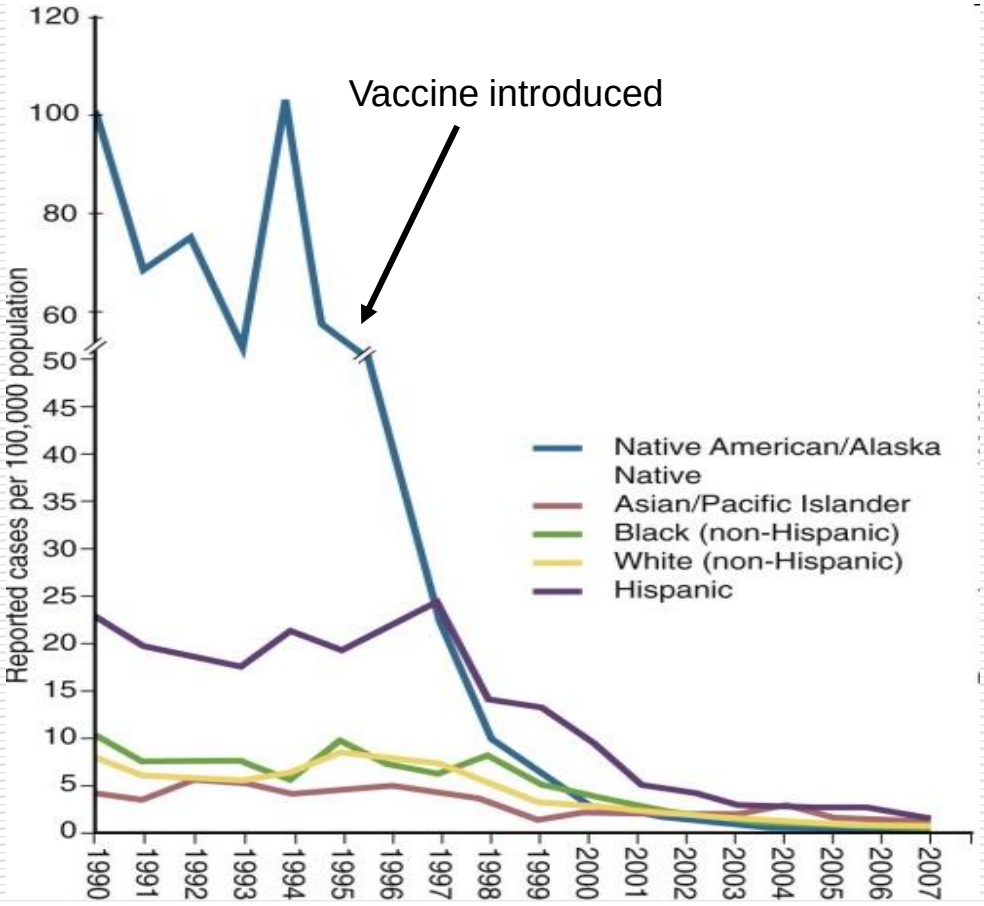
nature

Strategies for Use of HAV Vaccine

- Developing Countries
- Transition
- Developed World



HA Incidence in U.S. by Race and Ethnicity



Live attenuated HAV vaccine - Questions

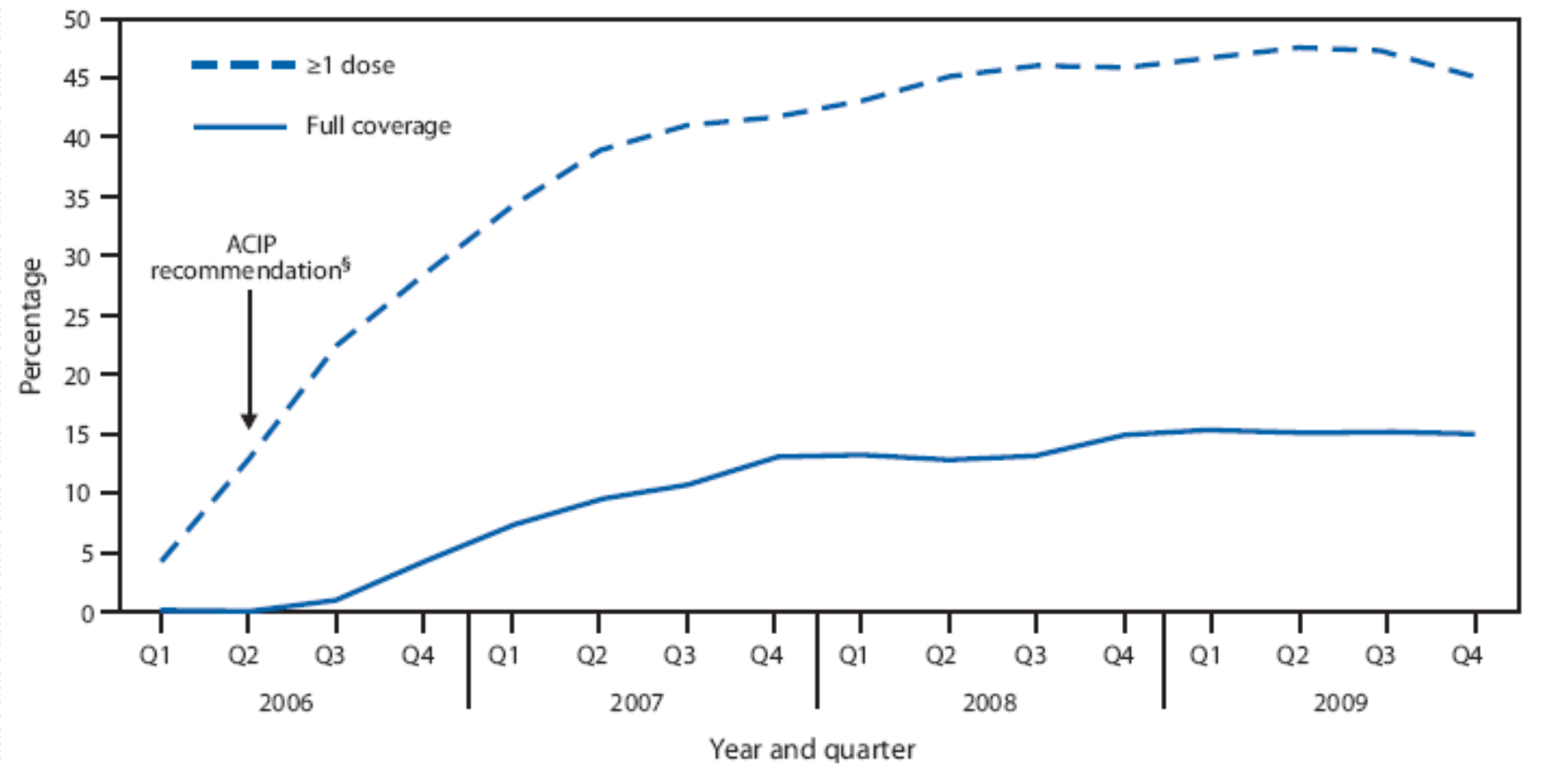
- Are there extrahepatic site of replication?
- What is the mechanism of hepatic injury; viral or immune mediated?
- Can limited replication produce an adequate immune response?
- Relevance of animal models to human attenuation?
- What degree of hepatic injury would be acceptable?

Clinical Manifestations of 8647 Hospitalized Patients 1988 Shanghai Epidemic (primarily 18-40 yo's)

Symptom	%	Clinical Findings	%	Complications	%
Jaundice	84	Hepatomegaly	87	Cholestasis	1.6-5.3
Weight loss	82	Splenomegaly	9	Upper gastrointestinal bleeding	0.5-1.2
Malaise	80	Skin rashes	3	Thrombocytopenic purpura	<0.1 (6 cases)
Fever	76	Mild edema	2	Guillain-Barr? syndrome	<0.1 (4 cases)
Nausea	69	Petechia	2	Pure red cell aplasia	<0.1 (3 cases)
Vomiting	47	Cardiac arrhythmias	0.8	Autoimmune hemolytic anemia	<0.1 (2 cases)
Abdominal pain	37			Transverse myelitis, optic neuritis	<0.1 (1 case each)
Arthralgia	6				

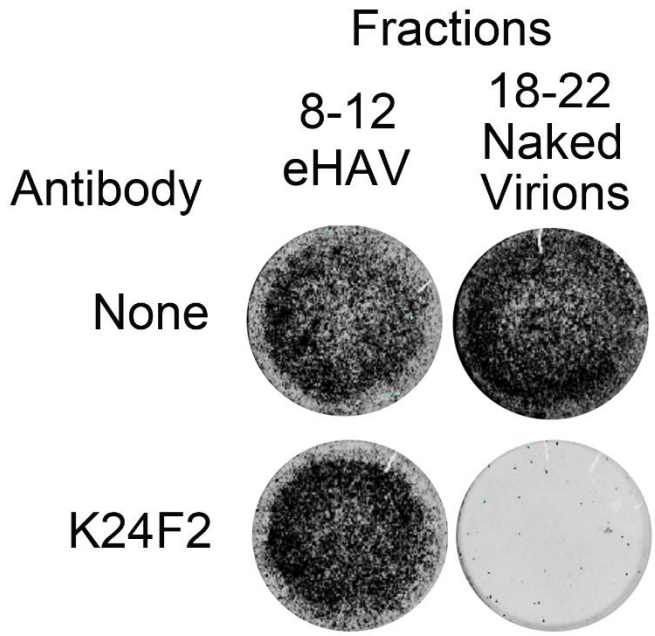
Yao G, 1991

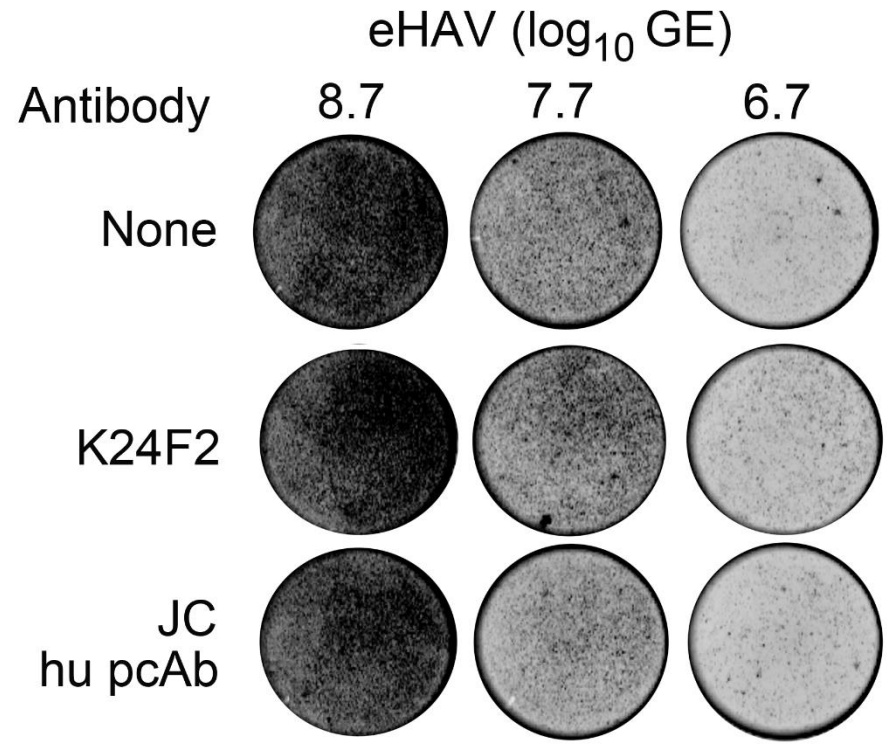
Mean HA Vaccine Coverage in Children 12-24 Months of Age



Information Systems Sentinal Sites, 2006-2009. MMWR, 2010

eHAV is Resistant to Neutralization by Anti-capsid mAbs and Polyclonal Post-convalescent Serum

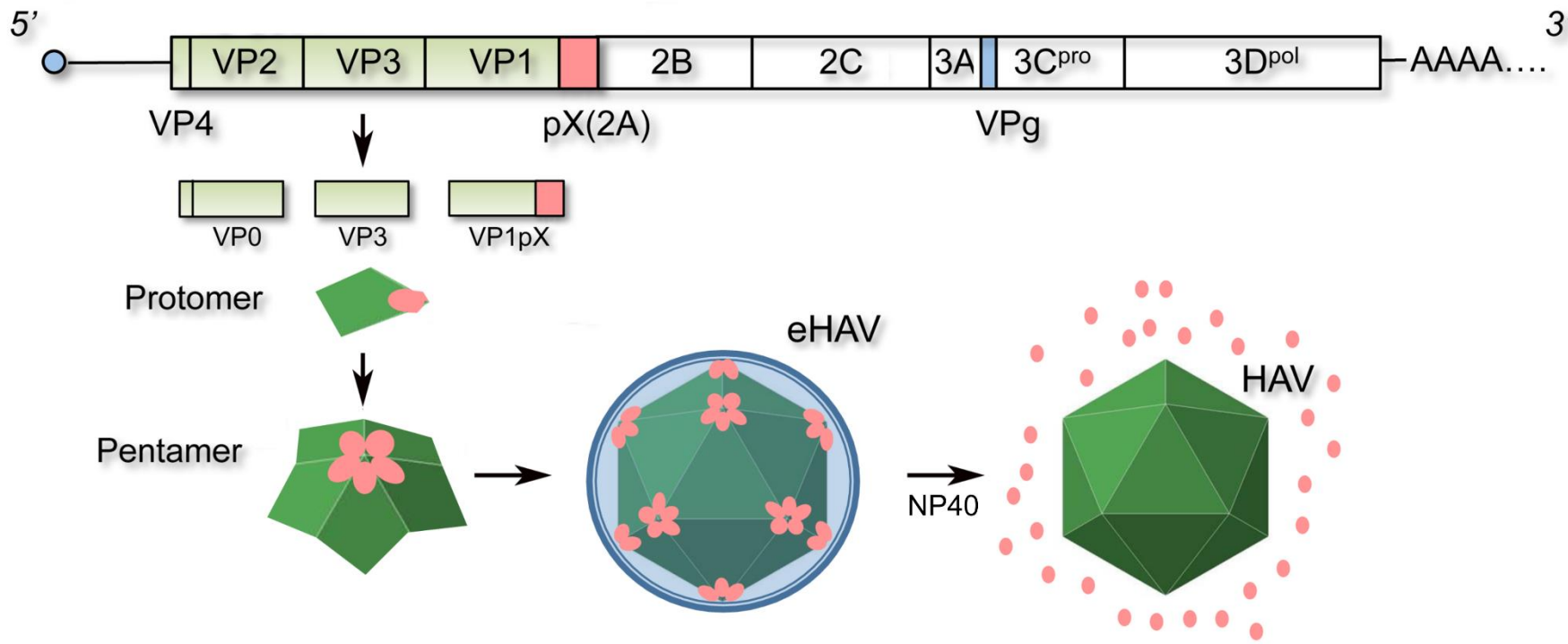




Infra-red Immunofluorescence Focus Reduction Assay

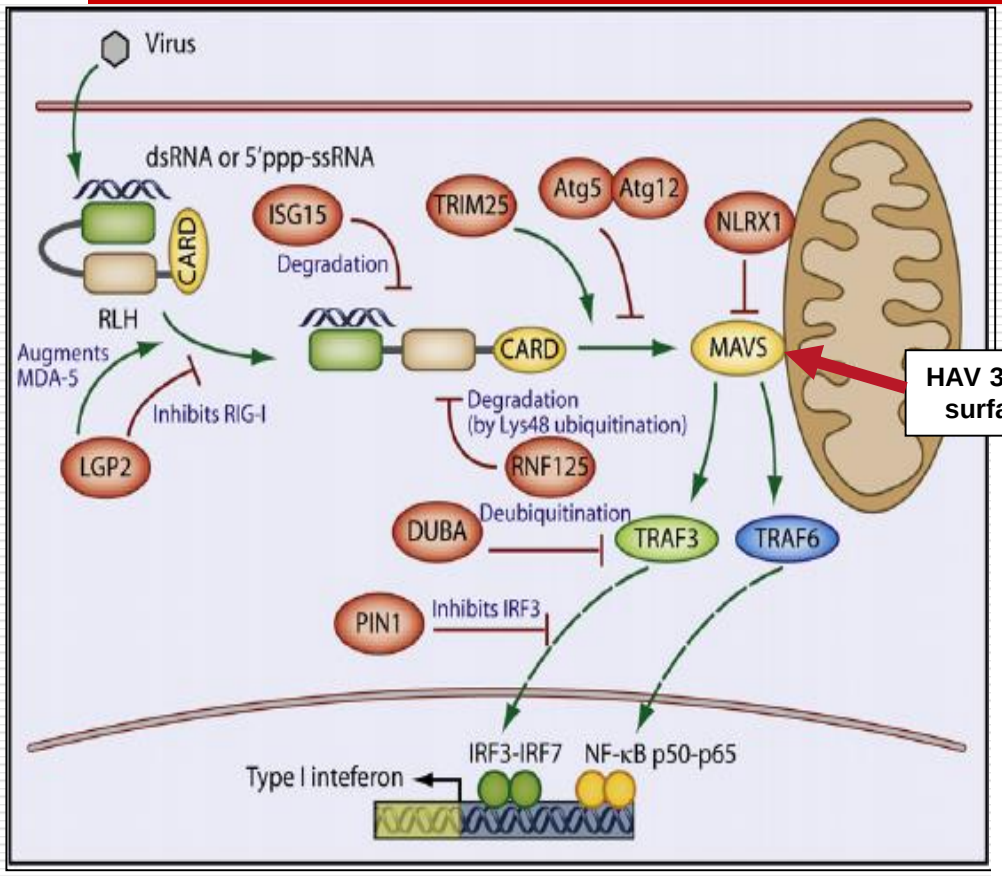
Z. Feng *et al.* Nature, 496:367-71, 2013

VP1pX Is Protected in eHAV Particles but Rapidly Degrades to VP1 upon Detergent Treatment



Z. Feng & S.M. Lemon, *Trends Microbiol* 2013

HAV Pathogenesis:
Disruption of IFN Signaling by HAV



From Moore and Ting, 2008

Yang et al., PNAS, 2007

HAV 3ABC Localizes to Mtch surface and cleaves MAVS

HAV 3ABC protease precursor is localized to the mitochondrial surface through the transmembrane domain in 3A. The cysteine protease, $3C^{PRO}$ cleaves the **mitochondrial antiviral signaling** protein (disrupting the interferon MAVS) signaling pathway. Disruption of the IFN pathway may result in

a) the prolonged incubation period observed in HAV infections and

b) in the ability of the virus to establish persistent infections *in vitro*