The Development of Chronic Hepatitis in Rabbits Experimentally Infected with HEV Isolate from Rabbit

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October 7 2014

Hepatitis E Virus (HEV)

Characteristics

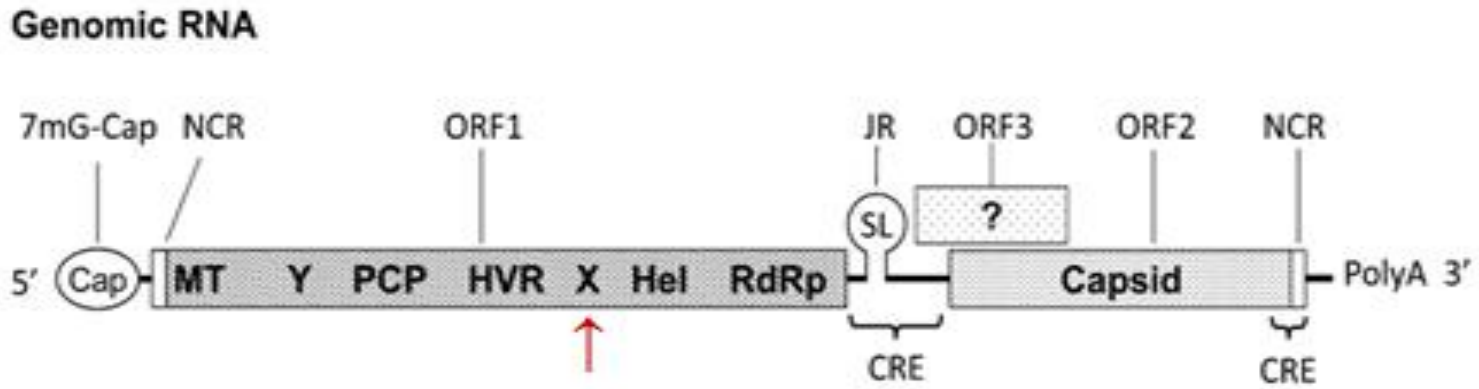
- **Faecal-oral transmission:** primarily through contaminated water and undercooked pork.
- **Self-limited disease:** Fulminant form of Hepatitis E in few patients
- **High mortality (~20%) in pregnant woman ?** 1~3%;
- **Chronic hepatitis and cirrhosis in immunocompromised patients (SOT and HIV patients)**
- **Poor prognosis in patients with chronic liver diseases and HEV superinfection:** Most cases of severe HEV represent “acute on chronic”

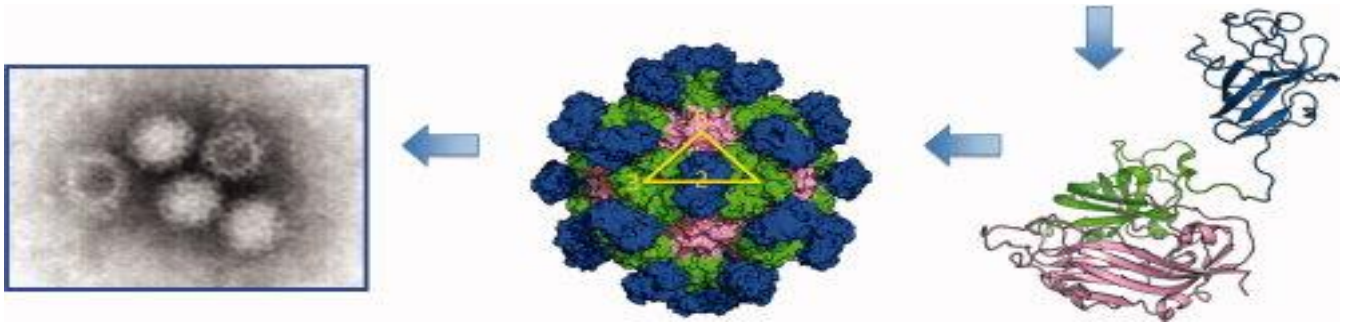
Milestones

- **1983, Balayan MS:** Demonstration of enterically transmitted non-A non-B hepatitis (ET-NANBH).
- **1990, Reyes GR:** Cloning and sequencing of HEV genome.
- **1998, Meng XJ:** Identify the first HEV strain of animal origin (Swine HEV) ; **Zoonosis**.
- **2004, ICTV:** HEV was classified in the new genus Hepevirus in the family Hepeviridae.
- **2010, China:** The world’s first recombinant hepatitis E vaccine.
- **2012, Emerson SU:** Adaptation of a Genotype 3 Hepatitis E Virus to Efficient Growth in Cell Culture

HEV: Virology

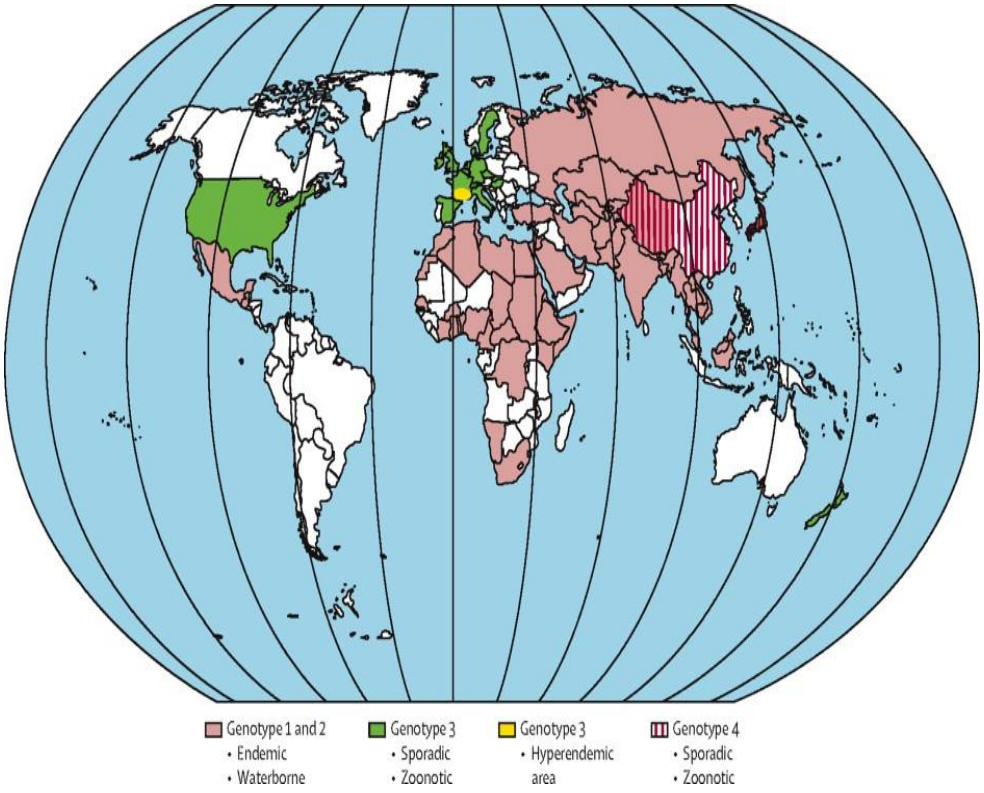
Positive sense, single-stranded RNA non-enveloped virus
HEV genome ~7.2kb, 3 ORFs
Virion: 32~34nm icosahedral,



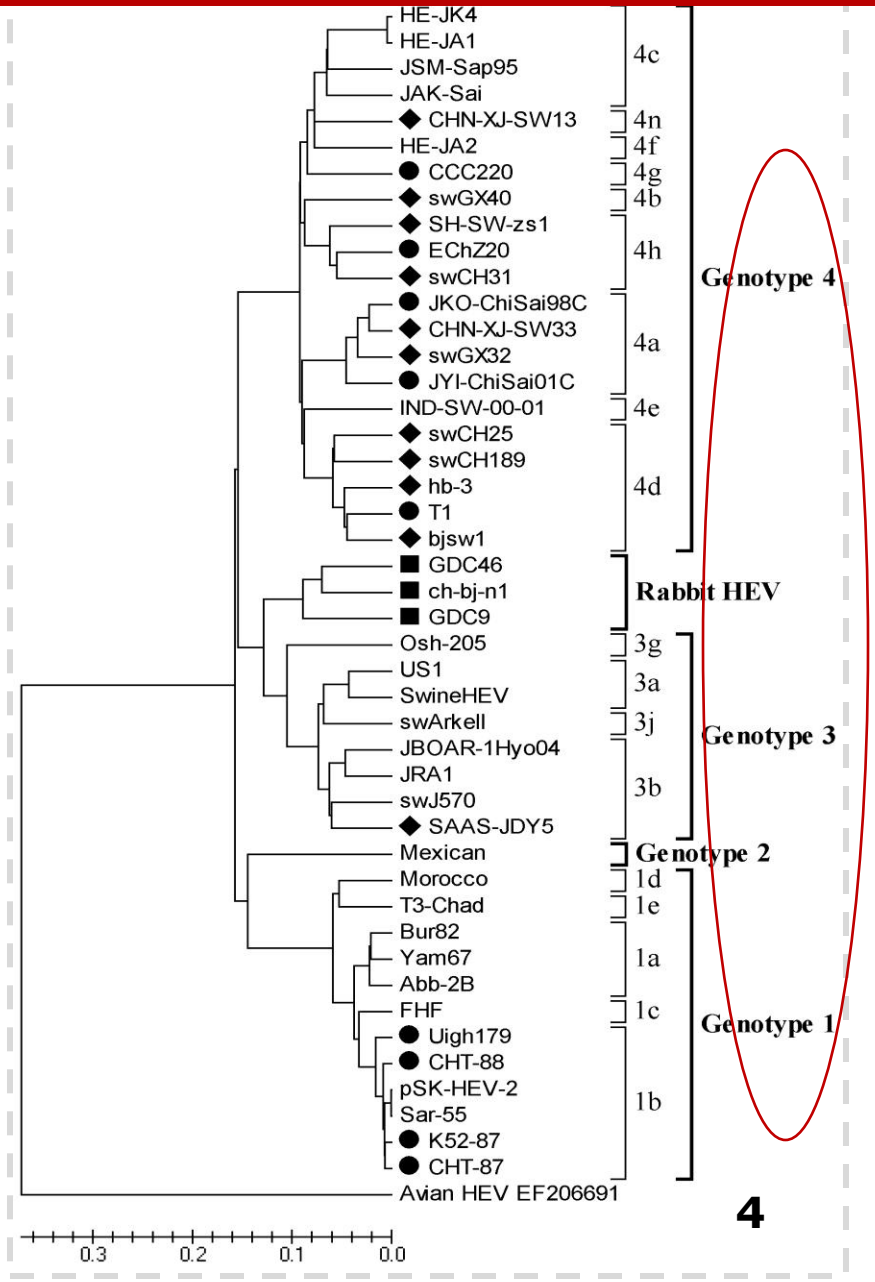


Aggarwal R et al, Hepatology. 2011

Genotype and distribution

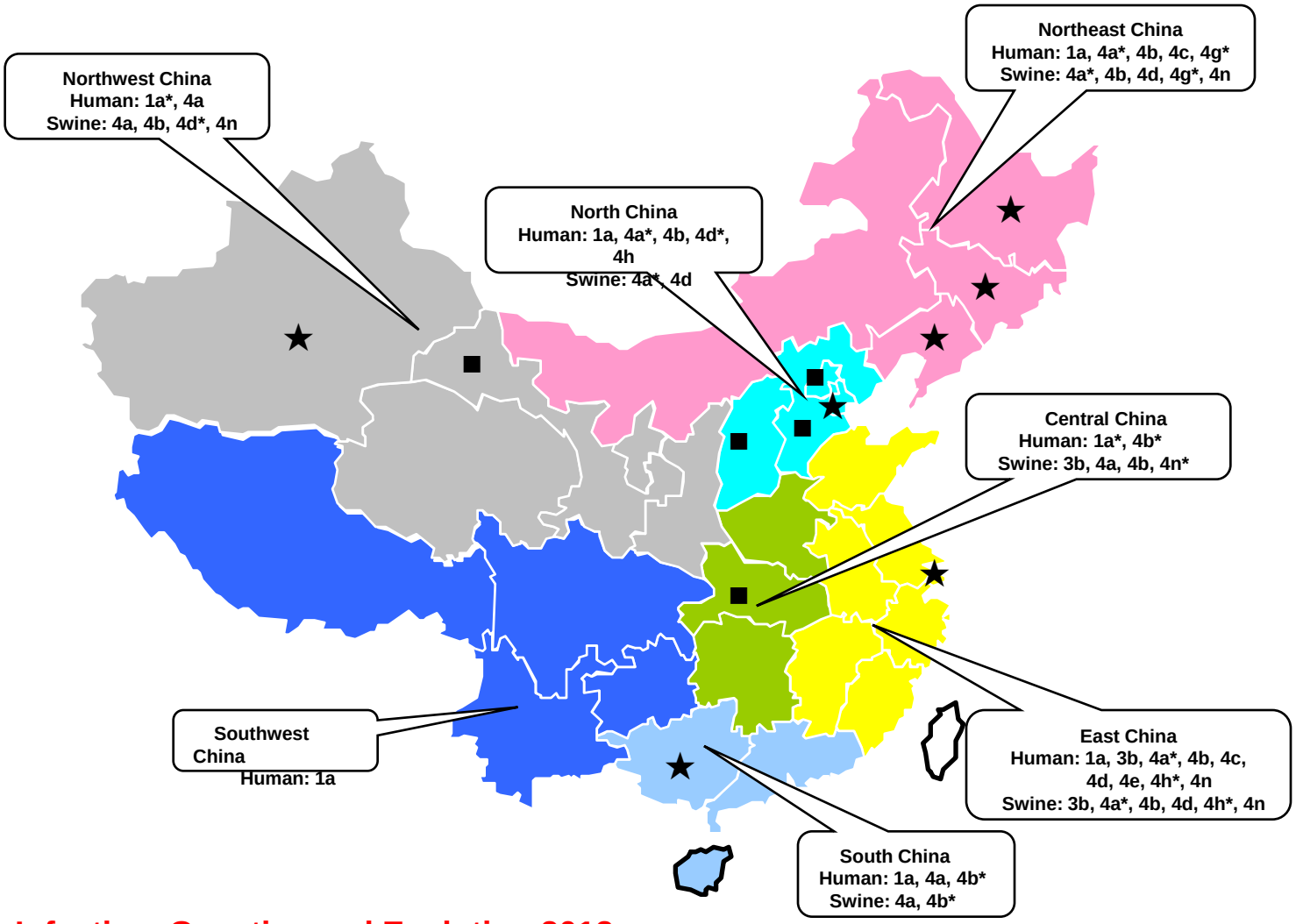


Kamar N et al. Lancet .2012



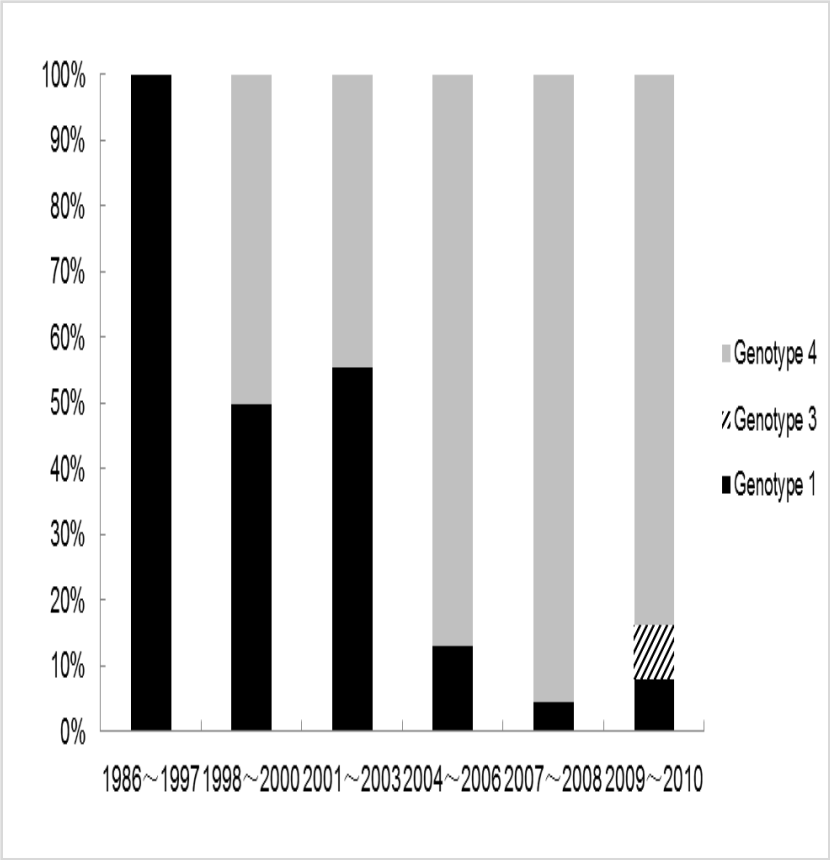
HEV: Epidemiology in China

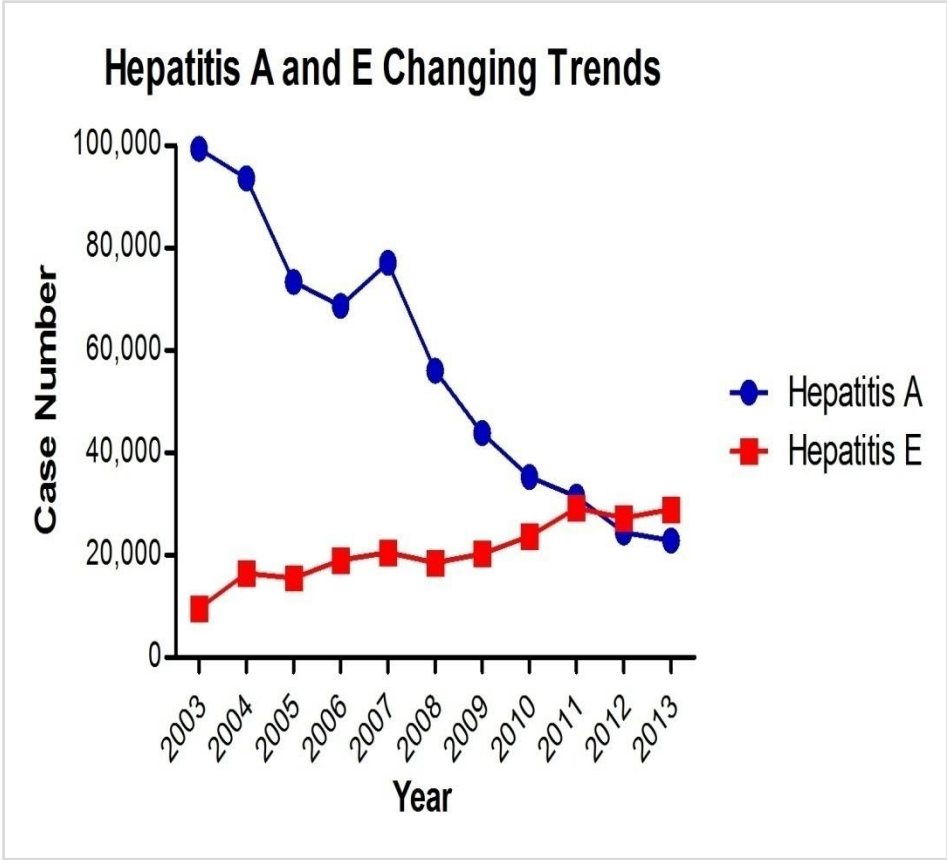
Temporal distribution of genotypes of human HEV isolates in Mainland China (1986-2011)



Liu P et al. Infection, Genetics and Evolution.2012

Incidence of hepatitis E in China





Liu P et al. Infection, Genetics and Evolution.2012

Data from China CDC

HEV: Zoonosis and animal reservoirs













Out-group

Human HEV (G1, G2, G3, G4)



?



Rabbit HEV

- First isolated from China in 2009;

The epidemiology of rabbit HEV around the world

Nation	Area	Year	Rabbit Species	Sample for detection	Anti-HEV antibody (%)	HEV RNA (%)
China	Gansu	2009	Rex	Serum	57.30	7.50
	Beijing	2010	Rex	Serum/feces	54.62	6.96
	Jilin	2011	Rex	Serum	9.42	0.00
	Beijing	2011	New Zealand White	Serum	42.10	1.90
	Hebei	2011	Rex	Serum	7.30	1.30
	Shanxi	2011	Japanese White, Chinchilla	Serum	7.70	1.90
	Hubei	2011	Rex	Serum	53.40	11.60
	Zhejiang	2011	Rex	Serum	10.70	0.00
	Guangxi	2011	Rex	Serum	3.03	0.00
	Inner Mongolia	2012	Rex	Serum	57.30	71.60
USA	Virginia	2011	Californian, Flemish, Lop, Mini Rex, New Zealand, Salitan, etc	Serum/feces	36.50	22.00
France	Western France	2012	Farmed, Wild	Bile (farmed), Liver tissue(Wild)	Un-detected	7.00(farmed) 23.00(wild)

Liu L et al. Chin J Viral Dis, 2014

JVH

Journal of Viral Hepatitis, 2010 doi:10.1111/j.1365-2893.2010.01341.x

Study on prevalence and genotype of hepatitis E virus isolated from Rex Rabbits in Beijing, China

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Received February 2010; accepted for publication March 2010

SUMMARY. A novel genotype of hepatitis E virus (HEV) isolated from rabbits is reported. The aim of this study was to confirm and further investigate the prevalence of the novel HEV genotype in rabbits in China. Sera and faecal samples were collected from farmed rex rabbits in Beijing, China. All serum samples were tested for anti-HEV antibody by EIA. Both the serum and the faecal samples were evaluated for detection of HEV RNA using a nested RT-PCR assay. The nucleotide sequences of rabbit HEV were then analysed, and sequence homology of rabbit HEV compared against human HEV genotypes 1–4, and avian HEV. Results: The prevalence of positive serum anti-HEV from rex rabbits was 54.62% (65/119). The detection rate of HEV RNA using ORF2 primers was 6.96% (8/115) amongst rabbit faecal samples. All eight amplicons shared 98.3–100% nucleotide homology with each other and had identities of 75.8–78.6%, 73.9–75.0%, 77.5–81.0%, 74.2–78.6% and 54.8–57.6% with the corresponding regions of genotypes 1–4 and avian HEV, respectively. Phylogenetic analysis showed that the eight sequences formed one individual branch and were on the same branch with GDC9 and GDC46, both of which were reported to be a novel genotype of HEV isolated from rabbits. The conclusion is that this study provides further information about HEV infecting rabbits, which may be a new animal host of HEV, as well as genetical evidence of a new mammalian genotype of HEV.

Keywords: antibody, genotype, hepatitis E virus, rabbit.

INTRODUCTION

Hepatitis E virus (HEV) is a small, nonenveloped, single-strand, positive-sense RNA virus that is transmitted via the faecal–oral route, primarily through contaminated water supplies. Cases in which HEV infection has been identified after ingestion of meat from HEV-infected deer or swine have been reported, and the HEV sequence isolated from the meat identical to ones that were isolated from the patients, definitively demonstrating zoonotic transmission of HEV [1,2]. HEV, which was previously referred to as an enterically transmitted non-A, non-B hepatitis virus, is the causative agent of acute self-limited or fulminant hepatitis E and is considered to be endemic in many developing countries where sanitation conditions are suboptimal [3,4]. A study showed that hepatitis E accounts for a significant proportion of enterically transmitted form of viral hepatitis in humans [5,6] and poses an important public health problem in both developing countries and some industrialized countries, including the United States and several European countries [7]. In epidemics, disease morbidity mainly affects young adults, with a relatively high mortality of up to 25% in infected pregnant women [8].

HEV is classified under the family Hepeviridae consisting of four recognized major genotypes that infect humans and other animals [9]. Genotypes 1 and 2 HEV are restricted to humans and are often associated with large outbreaks and epidemics in developing countries with poor sanitation conditions [10]. Genotypes 3 and 4 HEV infect humans, pigs and other animal species and are responsible for sporadic cases of hepatitis E in both developing and industrialized countries [11–13]. The avian HEV associated with hepatitis-splenomegaly syndrome in chickens is genetically and antigenically related to mammalian HEV and likely represents a new genus in the family [14,15]. Various species of animals, such as non-human primates, rodents, swine, cows and horses, have been found with a high prevalence of anti-HEV antibody since Meng [16] isolated HEV from swine in 1997.

Abbreviations: HEV, hepatitis E virus; RT-nPCR, nested reverse transcription polymerase chain reaction; ORF, open reading frame; EIA, enzyme immunoassay.

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The rabbit HEV isolate from fecal samples with the detection rate of HEV RNA was 6.96%.

Geng JB et al. JVH. 2010

Phylogenetic analysis of the full genome of rabbit hepatitis E virus (rbHEV) & molecular biologic study on the possibility of cross species transmission of rbHEV

Table 4
Specific amino acid substitutions in the ORF1 region among 94 HEV isolates form different genotypes.

Group	Genotype	Conserved amino acids and their sites[a]															
		40	62	83	591	708–716	931	1061	1151	1256	1287	1298	1438	1445	1454	1573	1578
Nonzoonotic group	HEV-1	H	F	S	N	VDAVS	L	A	S	F	L	V	A	Q	D	E	P
	HEV-2	H	F	S	N	ITDTP	L	A	S	F	L	V	A	Q	D	E	P
Zoonotic group	HEV-3	R	L	A	V	SGFSSDFSP	P	S	A	Y	M	L	E	P	E	D	R
	HEV-4	R	L	A	V	SGFSSCFSP	P	S	A	Y	M	L	E	P	E	D	R
Rabbit HEV	Novel type	R	F	A	V	SGFSSDFSP	L/P	S	A	Y/F	M	L	Q/P	P	E/G	D	R

[a] The amino acid positions are in accordance with CHN-XJ-SW13 (GU119961).

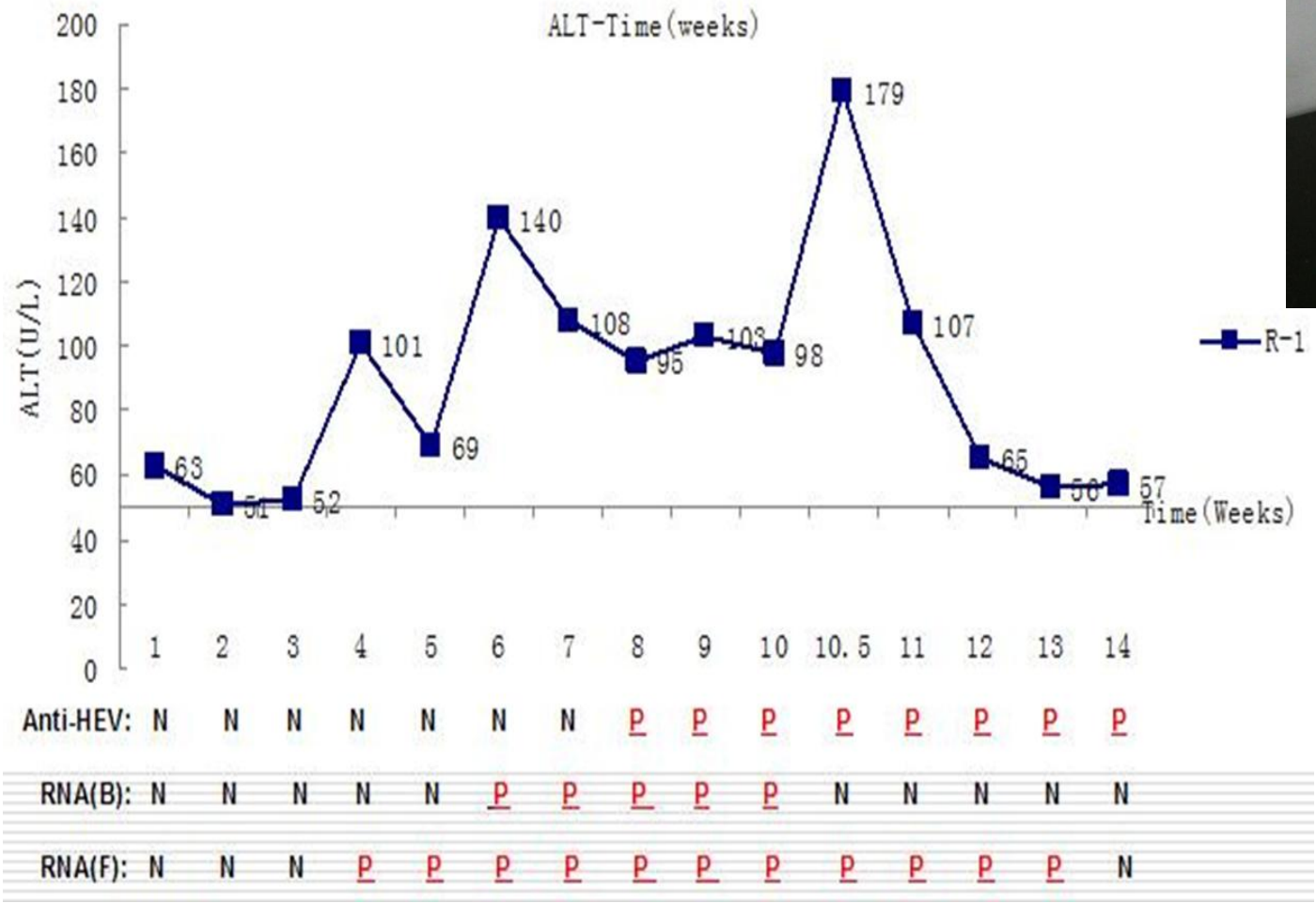
Table 5
Specific amino acid substitutions in the ORF2 region among 94 HEV isolates form different genotypes.

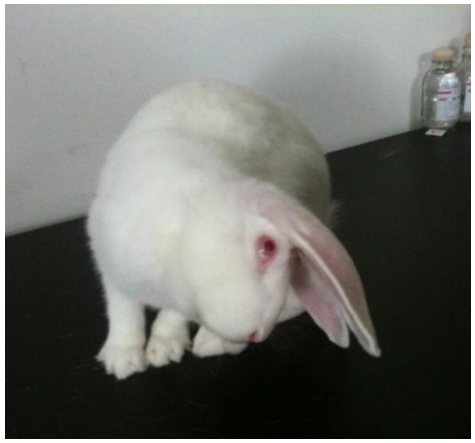
Group	Genotype	Sample number	Conserved amino acids and their sites[a]						
			19	105	131	497	506	511	613
Nonzoonotic group	HEV-1	21	P	A	H	S	V	S	A
	HEV-2	1	P	A	H	S	V	S	A
Zoonotic group	HEV-3	30	A	S	P	T	M	T	G
	HEV-4	39	A	S	P	T	M	T	G
Rabbit HEV	Novel genotype	3	A/V	S	P	T	M	T	G

[a] The amino acid positions are in accordance with CHN-XJ-SW13 (GU119961).

Geng JB et al. Infect Genet Evol. 2011

Pathogenecity of Hepatitis E Virus from Rabbits

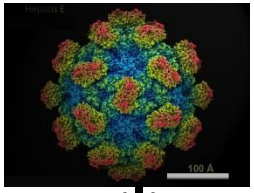




Hepatitis E virus and neurologic disorders

Kamar N,et al. Emerg Infect Dis. 2011

Extrahepatic Replication of HEV in Experimentally Infected Rabbits





PBS

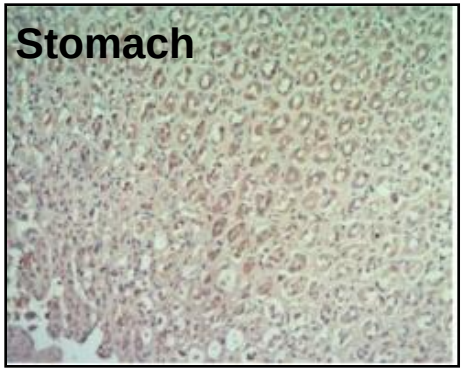


Liver, stomach, small intestine, kidney, spleen, heart, brain, bladder and lung

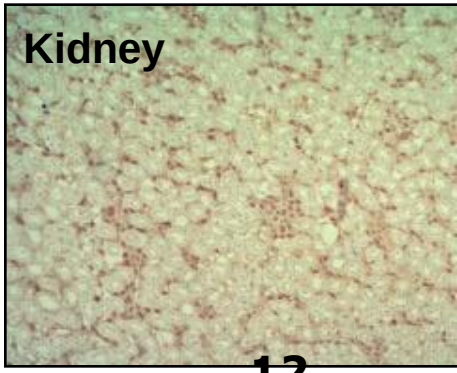


(+) and (-) HEV RNA



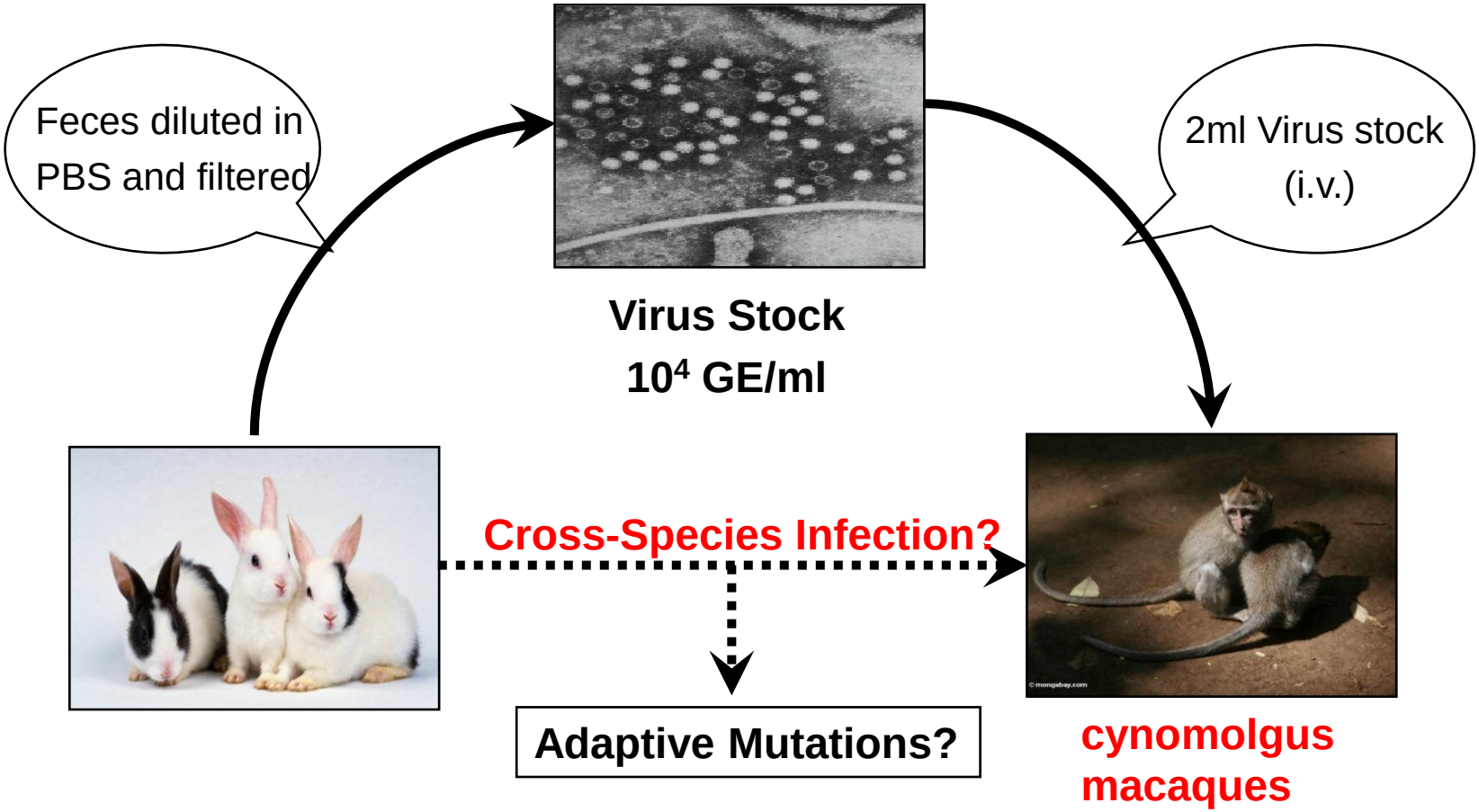






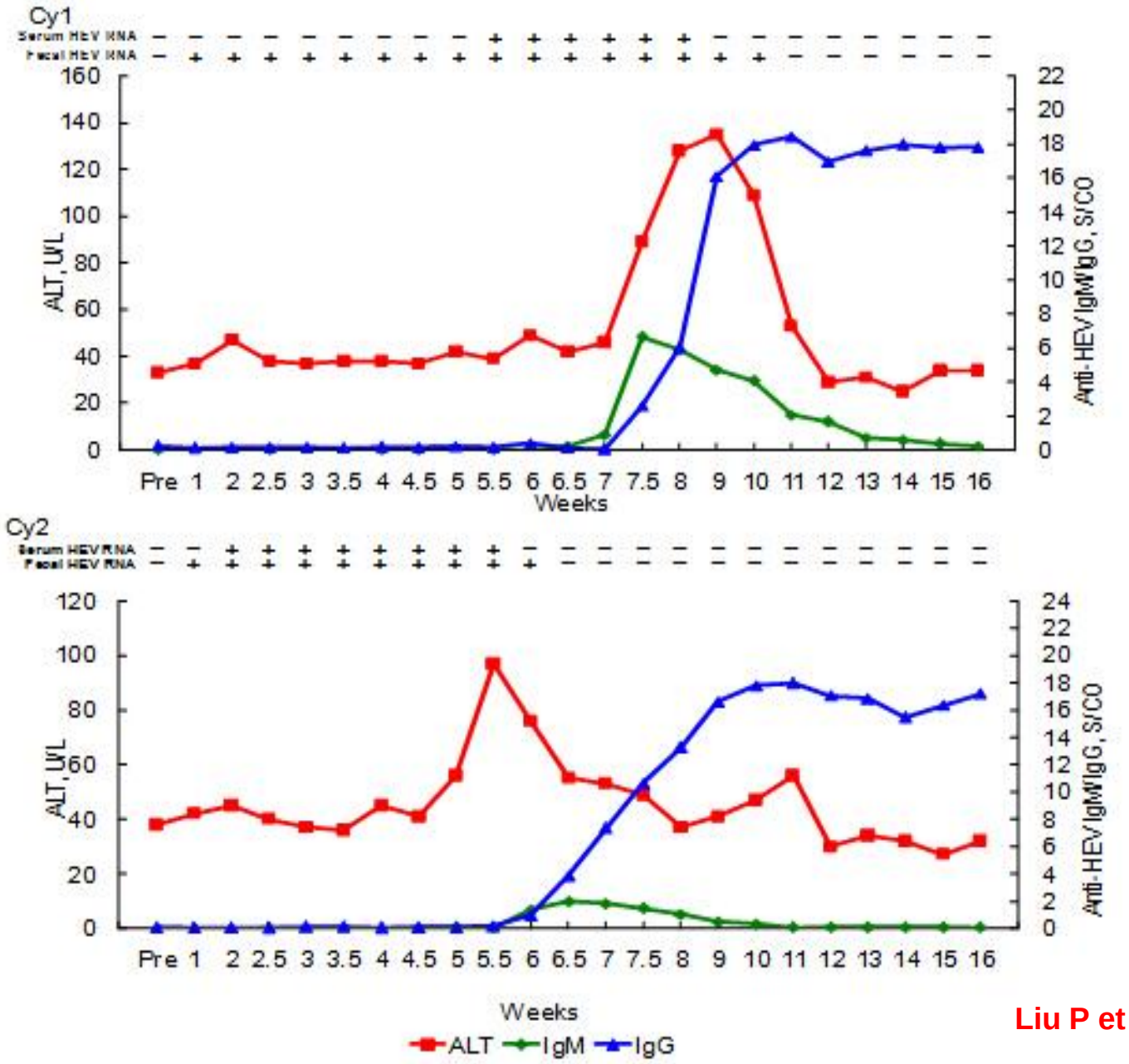
Liu P et al, Emerg Infect Dis 2013; Han J et al, Plos one 2014.

Experimental Infection of Monkeys with Rabbit HEV



- **Prior to inoculation:** Anti-HEV antibodies and HEV RNA were negative，ALT were normal (0-40 U/L);
- **Sample Collection**: Blood and Feces (twice a week), monitor for 16 weeks；
- **ALT** in serum: Hepatitis (≥twice the pre-challenge ALT)；

Cross-species transmission of HEV to cynomolgus macaques

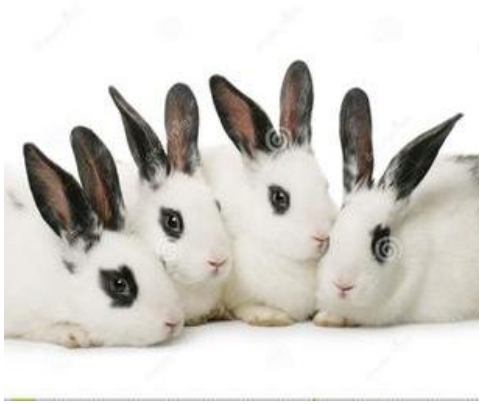


Comparing the complete genome sequence of HEV passed in the monkys with that of the inoculum showed 99.8% nucleotide identity.

Liu P et al, Emerg Infect Dis 2013

Pathogenesis study of rHEV: Chronicity

SPF Rabbits Infected with Rabbit Hepatitis E Virus Isolate Experimentally Showing the Chronicity of Hepatitis



Group 1 was control including 2 rabbits inoculating with PBS, C1&C2.

PBS



rHEV

Inoculating a homologous rabbit HEV isolate(**Group 2** including 4 rabbits R1—R4)



sHEV

Inoculating a heterologous swine HEV isolate (**Group 3** including 4 rabbits S1—S4)

Pathogenesis study of rHEV: Chronicity

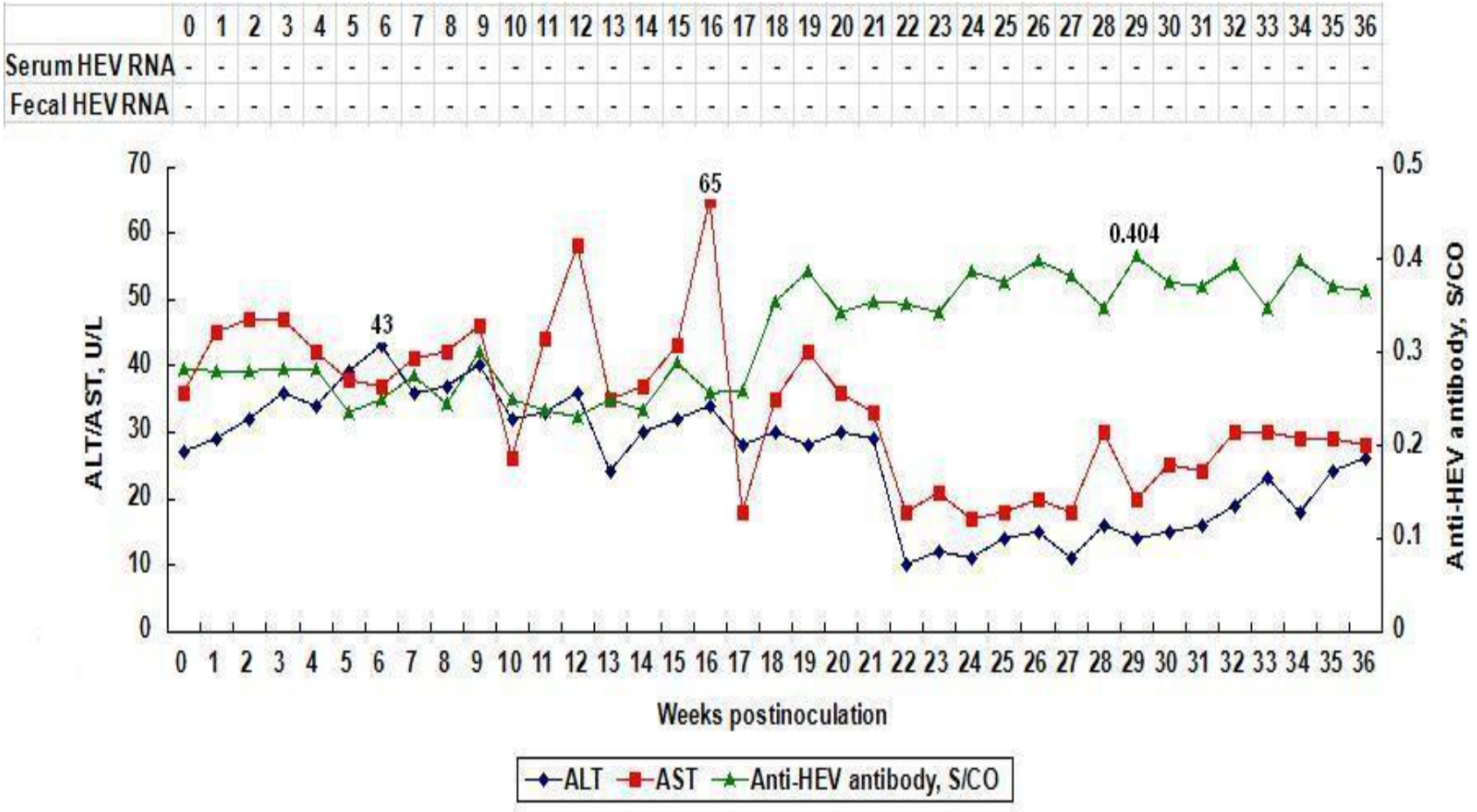


Figure 1. Dynamic seroconversion of anti-HEV, HEV RNA,ALT and AST observed in rabbits.

Rabbit **C1** in group 1 inoculated with sterile PBS.

Pathogenesis study of rHEV: Chronicity

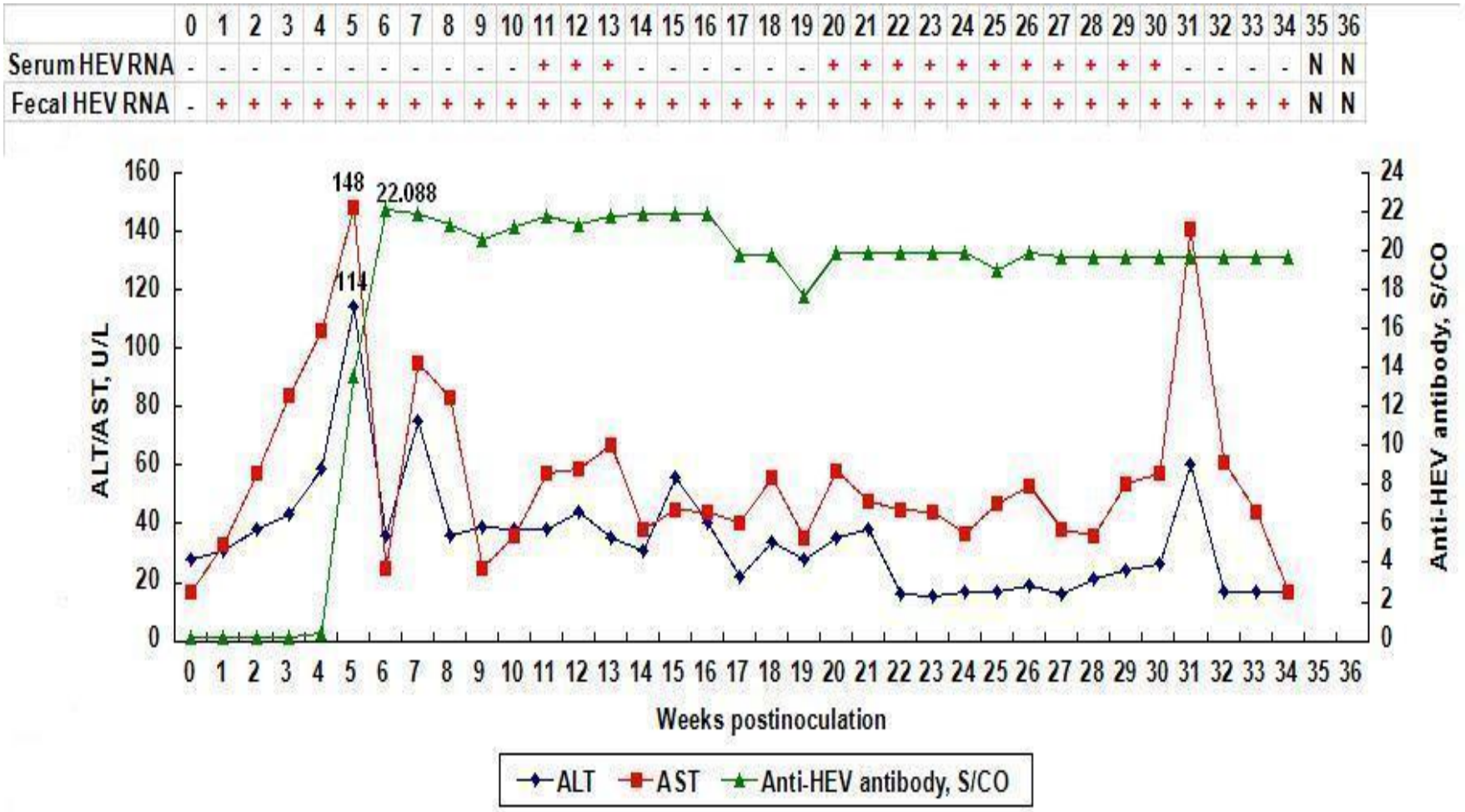


Figure 2. Dynamic seroconversion of anti-HEV, HEV RNA,ALT and AST observed in rabbits.

Rabbit **R3** in group 2 1inoculated with rabbit HEV strain.

Pathogenesis study of rHEV: Chronicity

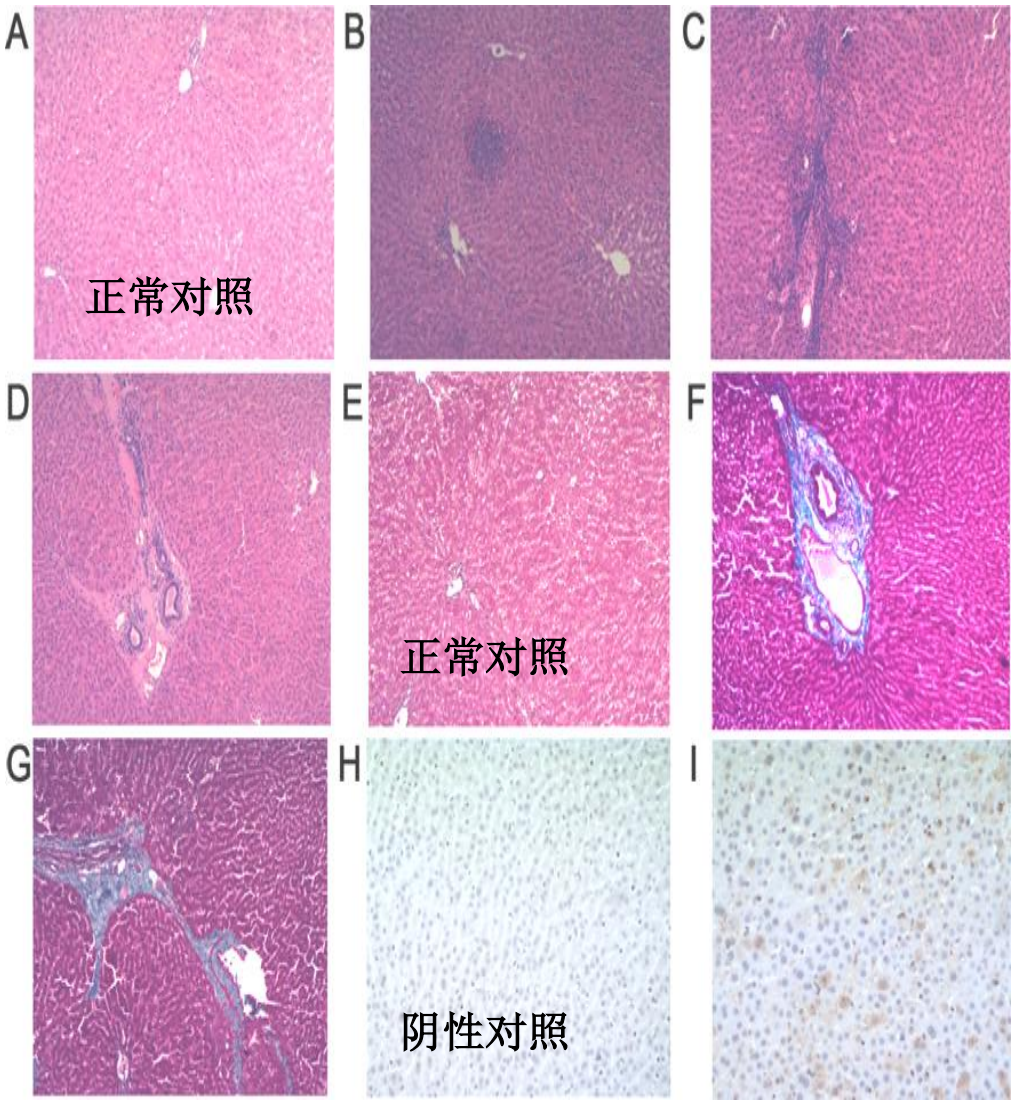


Figure 3. Liver histology.

A–D (H & E stain, original magnification, 610),
E–G (Masson's trichrome stain, 610),
H–I (Immunohistochemistry, 640).

(A) Liver section from a control rabbit with no visible pathological signs of HEV infection.

(B)-(C) Lymphocytes distributed focal or scattered in hepatic lobule, the inflammatory cells gathered along blood vessel walls.

(D) Chronic inflammatory cells infiltrate the portal area, blood vessel walls thickening associated with fibrosis, local hyaline degeneration.

(E) No histopathological changes with minimal staining limited to areas immediately adjacent to portal structures.

(F) Artery wall thickening associated with moderate to severe fibrosis.

(G) More advanced portal and periportal fibrosis with short fibrous septa.

(H) Negative immunohistochemistry result for HEV antigen in liver sections from the control rabbits.

(I) Positive results for HEV antigen in liver sections of experimental groups.

Pathogenesis study of rHEV: Chronicity

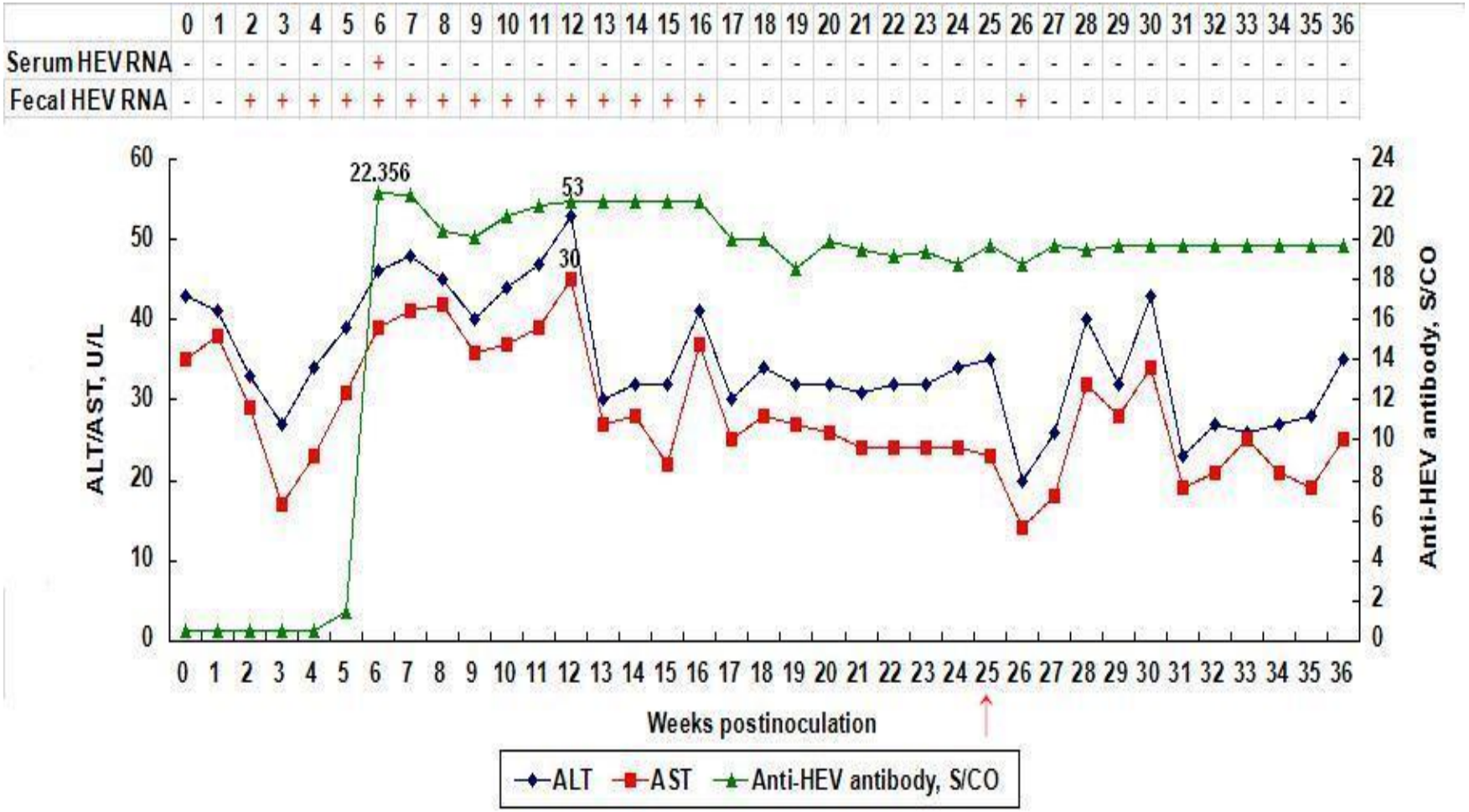


Figure 4. Dynamic seroconversion of anti-HEV, HEV RNA,ALT and AST observed in rabbits.

Rabbit **S4** in group 3 inoculated with a genotype 4 swine HEV at 0wpi, and rabbit HEV at 25wpi.

Note: ↑ indicates group 3 rabbits were inoculated with rabbit HEV strain at 25wpi (when recovered from initial infection).

Pathogenesis study of rHEV: Chronicity

Table 1. Detection of HEV RNA in fecal/serum samples collected weekly from rabbits.

Group	Rabbit ID	Positive(+) or Negative(-) in Fecal/Serum Samples at Indicated Wpi																																					
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1a	C1	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
PBS	C2	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
2b	R1	-/-	+/-	+/-	+/-	+/+	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	-/-	-/-	+/-	+/-	+/-	+/-	+/+	-/-	+/-	+/-	+/-	+/-	N/N	N/N	N/N	N/N	N/N
rHEV	R2	-/-	+/-	+/-	+/-	+/-	+/-	+/-	+/+	+/-	+/-	+/-	+/-	+/-	+/-	+/+	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/+	+/-	+/+	+/-	+/+	+/+	+/-	+/-	N/N
	R3	-/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/+	+/+	+/+	+/-	+/-	+/-	+/-	+/-	+/-	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/-	+/-	+/-	+/-	N/N	N/N
	R4	-/-	+/-	+/+	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	-/-	+/-	-/-	+/-	-/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/+	+/+	+/-	+/-	+/-
3c	S1	-/-	-/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	-/-	-/-	+/-	+/-	-/-	+/-	+/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
sHEV	S2	-/-	+/-	-/-	+/-	-/-	+/-	-/-	+/-	+/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+/-	+/-	+/-	+/+	+/-	+/-	+/-	+/-	+/-	+/-	+/-
	S3	-/-	+/+	+/-	+/-	+/-	-/-	+/-	-/-	+/-	+/-	-/-	+/-	+/-	+/-	+/-	+/-	-/-	+/-	-/-	+/-	-/-	-/-	-/-	-/-	-/-	-/-	+/-	+/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	S4	-/-	-/-	+/-	+/-	+/-	+/-	+/+	+/-	+/-	+/-	+/-	+/-	-/-	+/-	+/-	-/-	+/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-

HEV Replication in Extrahepatic Tissue of Infected Rabbits

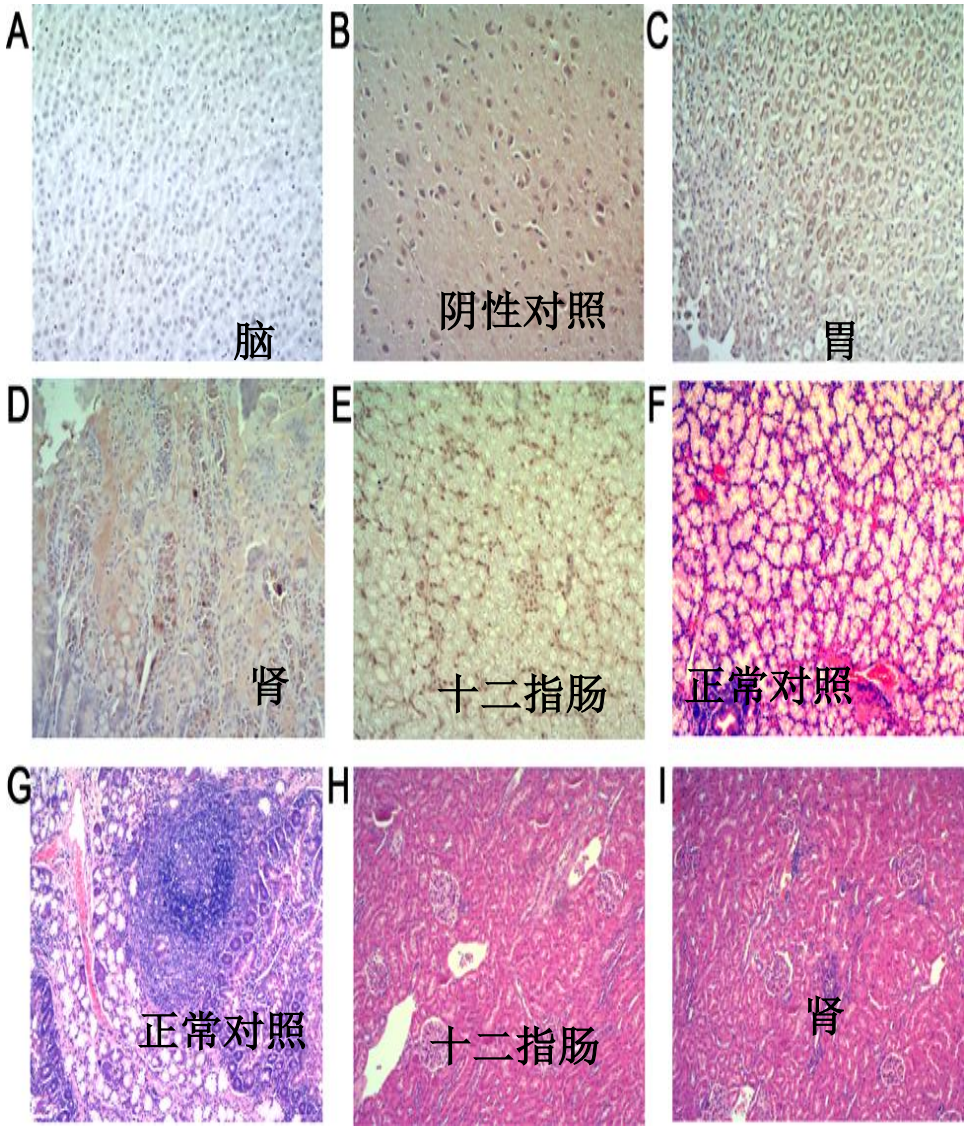


Figure 5. Extrahepatic tissue histology.

A–E (Immunohistochemistry, 640),

F–I (H & E stain 610).

(A)Negative immunohistochemistry result for HEV antigen in extrahepatic tissue sections from the control rabbits.

(B)–(E) Positive results for HEV antigen in brain, stomach, duodenum and kidney.

(F) Duodenum section from a control rabbit with no visible pathological signs of inflammation.

(G) A large number of lymphocytes infiltrate mucosal interstitial, focal lymph follicles formed in duodenum sections.

(H) Kidney section from a control rabbit with no visible pathological signs of HEV infection.

(I) Multifocal lymphocytes and mononuclear cells infiltrate in renal interstitial.

Summary

In summary, on rabbit HEV study, our group has provided the first experimental evidences on the following aspects:

- 6.96% detection rate of HEV RNA amongst rabbit fecal samples meaning another important reservoir of HEV
- Phylogenetic analysis of the whole genome showing the potential possibility of rabbit HEV cross-species transmission.
- Rabbit HEV transmissible to cynomolgus macaques suggesting that rabbits may be a new source of human HEV infection
- SPF rabbits experimentally infected with a homologous rabbit HEV isolate developed signs of chronic hepatitis providing the laboratory evidence of chronic hepatitis E
- Both positive and negative-stranded HEV RNA and HEV antigen expression detected in extrahepatic tissues indicating the existence of HEV replication in many tissues in addition to the liver



- Based on our studies, Rabbit HEV possesses strong pathogenecity and possibility to infect human, and rabbit can be used as animal model to further investigate HEV.

Acknowledgements

This work was partially supported by the National Science Foundation of China (Grant No. 81271827)

and Specialized Research Fund for the Doctoral Program of Higher Education (Grant No.20120001110098)

Thanks!

