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HEV contamination in blood products and its safety

Experience with HEV to demonstrate the safety of plasma product

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Today's presentation

- **Prevalence of HEV in Japan**
- **Virus propagation and preparation**

- **Partitioning during ethanol fractionation.**
- **Heat inactivation.**
- **Removal by virus filters.**

- **Points to consider against HEV**

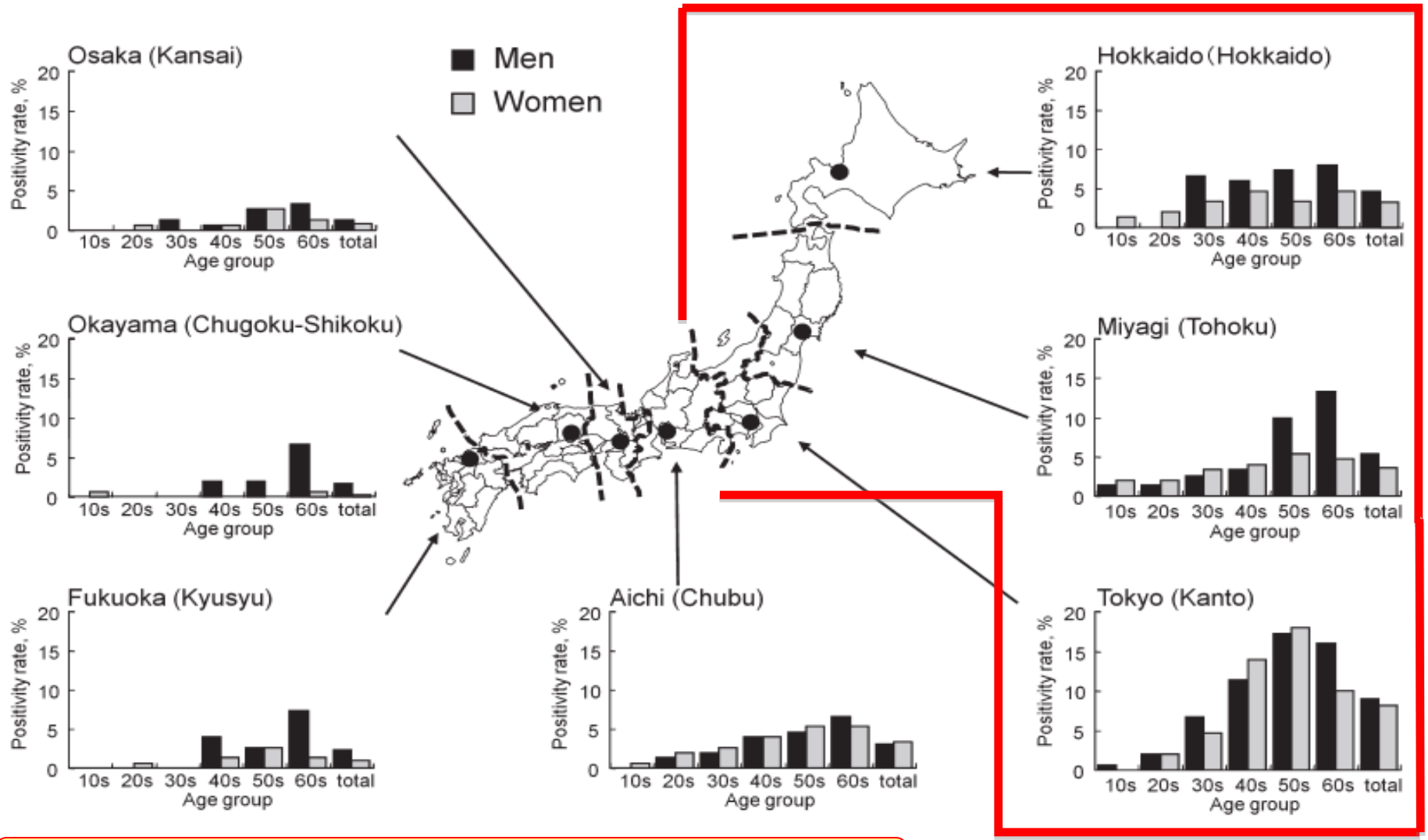
History of HEV research project in Benesis

2003	Started collecting information of HEV.
2004	A requirement for evaluation and enhancement of safety measures against B19, HAV, HEV and Prions was issued by MHLW Japan.
	HEV theme was added to collaborative research project with Osaka Univ.
2005	Rakuno Gakuen Univ joined the collaborative research project.
	Started virus hunting.
2007	Started NAT screening for source plasma of some products in Benesis.
2008	Data of HEV inactivation and removal were published.
2009	Reference package (4 HEV isolates) was provided to NIHS Japan.
2011	Convened an open seminar on HEV issues in Japan.

Hepatitis E Virus

Classification	Family; Hepeviridae Genus; Hepevirus
Pathogenicity	Viral hepatitis
Natural Route of Human HEV Infection	* Food-borne in developed countries * Water-borne in developing countries * Transfusion and Transplantation
Reservoirs	Pig, Wild Boar, Deer etc.
Serotypes/Genotypes	Serotypes: 1 Genotypes: 1 ~ 4 (Genotypes 1, 3 and 4 were found in Japan and major is 3)
Structural Characterization	Non-enveloped ssRNA virus Spherical viral particle, 27 ~ 34 nm in size
Resistance to Inactivations	Low pH: Yes Detergents: Yes Heat: ? (conditions for heat-stability are remained obscure)

Geographical prevalence of anti-HEV IgG in Japan



Eastern Japan was higher than western

Takeda H. et al., VoxSang, 2010: 99; 307-313

Positivity rates of HEV in donor plasma

Country	Rate	Reference
England	(1 : 7,040)	Ijaz S et al. VoxSang. 2012; 102: 272
Germany	1 : 4,525	Baylis SA et al. VoxSang. 2012; 103: 89-90
Sweden	1 : 7,986	
USA	<1 : 51,075	
Japan	1 : 8,415 (2005.1 – 2011.10)	In Hokkaido, by Japanese Red Cross [1)]
	1 : 18,782 (2007.7 – 2012.2)	Source plasma except in Hokkaido. By Benesis

[1)]: http://www.mhlw.go.jp/stf/shingi/2r98520000020cvw-att/2r98520000020de0.pdf

Significant reported post transfusion transmission cases in Japan

Donated year	HEV markers in donor plasma			Serious hepatitis E in recipient
	RNA	IgG	IgM	
2005	+	-	-	No
2005	+	-	-	No
2008	+	-	-	No
2008	+	+	+	No
2008	+	-	-	No

Anti-HEV IgG / IgM may have no-neutralizing- or weak- activity against HEV infection

Ref: Steering committee for blood operation in Japan, MHLW Japan.

Detection of HEV in pooled plasma

Source of Pools	Rate
Europe	3 / 34
Europe / North America	0 / 3
North America	1 / 4
Middle East	0 / 11
Southeast Asia	4 / 23

Baylis SA et al., VoxSang. 2012; 102: 182-183

Detection of HEV genome in plasma products

Products (Manufacturer)	Origin of plasma pools	Nano-filtration	B19	HAV	HEV
F-VII (A)	Central Europe, USA	35nm	1/3	0/3	0/3
F-VIII (B)	Central Europe, USA	NA	1/3	0/3	0/3
F-VIII (C)	Central Europe, USA	NA	1/3	0/3	0/3
F-VIII (D)	Central Europe, USA	NA	0/6	0/6	0/6
F-IX (E)	USA	NA	0/6	0/6	0/6
F-IX (F)	Central Europe, USA	NA	0/3	0/3	0/3
F-VIII/vWF (D)	Central Europe, USA	NA	3/4	0/4	0/4
F-VIII/vWF (E)	USA	NA	0/3	0/3	0/3
F-VIII/vWF (F)	Central Europe, USA	NA	0/3	0/3	0/3
APCC (A)	Central Europe, USA	35nm	4/8	0/8	0/8

Modrow S. et al., VoxSang. 2011; 00: 351-358

Virus hunting

Swine **Human** **NAT**

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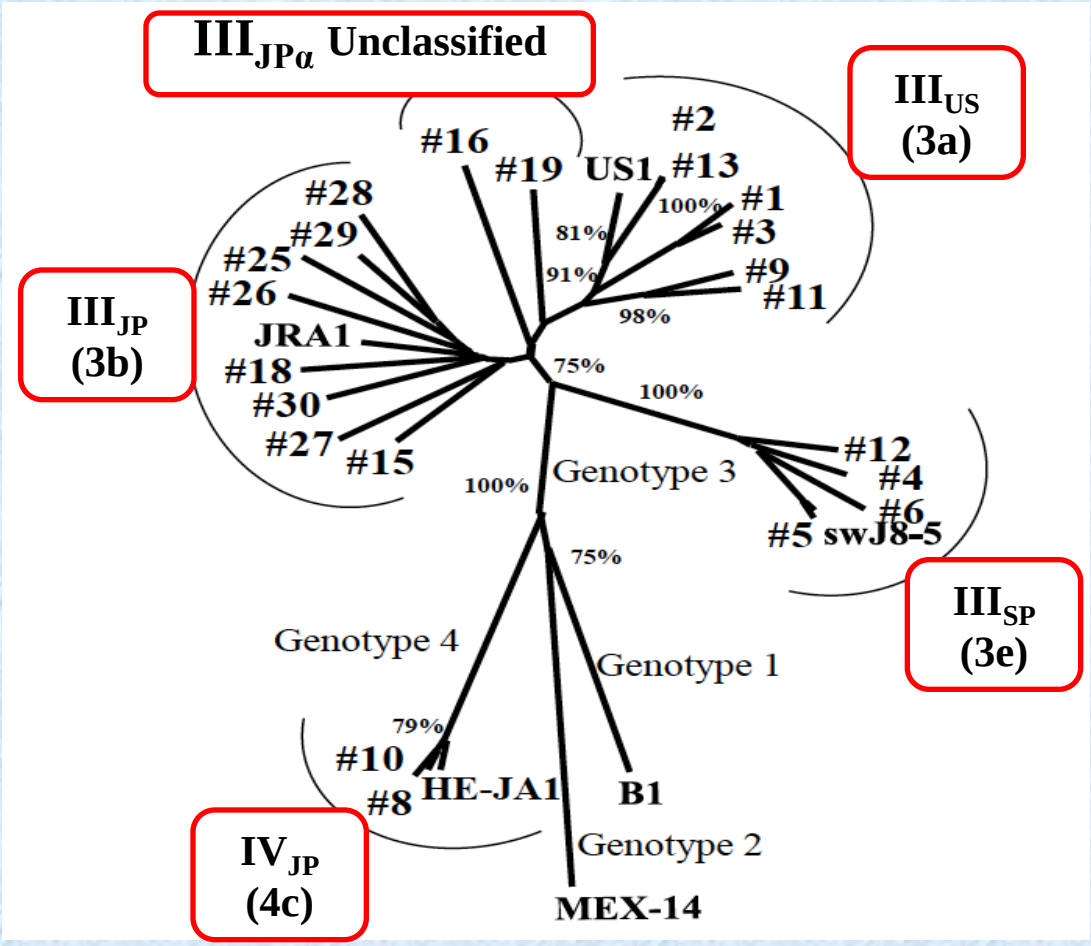
Phylogenetic analysis of HEV strains isolated from Japanese swine farms

HEV-RNA positive cases among 32 Japanese farms

Genotypes	Number of Farms
Not detected	5
$G3_{JP\alpha}$ Unclassified	2
$G3_{US}$	6
$G3_{JP}$	8
$G3_{SP}$	4
$G4_{JP}$	2
Not classified	5
Total	32



Genotype 3jp, 3sp, 3us and 4jp were isolated from swine feces in Japan

Sompong et al. Open Vet Sci J 2009; 3: 68-75.

HEV genome prevalence in donated plasma in Japan

Area	Duration	HEV Positive Ratio
Hokkaido [1] (Japanese Red Cross result)	2005.1 ~ 2011.10	224 / 1,884,849 (1 / 8,415)
Tokyo [2] (Japanese Red Cross result)	2006.5 ~ 2006.7	3 / 44,332 (1 / 14,777)
Except-Hokkaido* (Benesis result)	**2007.7 ~ 2012.2**	**16/ 300,504 (1 / 18,782)**

*Obtained from source plasma for limited products in Benesis. Source of plasma in Japan could not be determined precisely, but Hokkaido could be excluded from these donations.

[1] http://www.mhlw.go.jp/stf/shingi/2r98520000020cvw-att/2r98520000020de0.pdf

[2] http://www.mhlw.go.jp/shingi/2006/08/dl/s0823-4c02.pdf

Properties of HEV positive donor plasma

Number	1	2	3	4	5	6	7	8	9
HEV Genome (Log copies/mL)	7.22	4.79	4.64	3.60	4.14	2.34	3.34	<1.69	3.46
Genotype /Cluster	III JPα	III US	III US	III JP	III JP	III JP	III JP	NA	III SP
IgG	–	++	–	+	–	–	–	+	–
IgM	–	+	–	–	–	–	–	–	–

Number	10	11	12	13	14	15	16	17	18
HEV Genome (Log copies/mL)	4.99	3.38	3.48	(1.35)	4.57	3.56	3.93	3.96	2.60
Genotype /Cluster	III JP	III JP	III US	III US	III US	III JP	III US	III US	III US
IgG	–	–	–	+++	–	+	–	+	+
IgM	–	–	–	–	–	–	–	+	++

#1-16:Jananese domestic plasma. #17-18: Plasma from USA

Propagation, preparation and cell based infectivity assay of HEV for inactivation/removal studies

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HEV partitioning, inactivation and removal during manufacturing process of plasma products

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Partitioning of HEV with / without SD treatment during ethanol fractionation II+III

	$G3_{JP}$ (swine, feces)	huG3 (human, serum)	
Detergent;	Yes (Triton x100 / SD / SD)	No	Yes (SD / SD)
Before	6.0 / 7.0 / 7.2	6.5 / 6.4* / 6.4*	6.4* / 6.5*
Supernatant (Removal)	4.1 / 4.0 / 5.3 (1.9 / 3.0 / 1.9)	6.1 / 6.5* / 6.4* (0.4 /0.0 /0.1)	5.6* / 5.7* (0.8 / 0.8)
Precipitate (Removal)	5.7 / 7.0 / 7.0 (0.2 / 0.0 / 0.2)	5.8 / 6.0* / 5.8* (0.7 / 0.4 / 0.6)	6.4* / 6.4* (0.0 / 0.1)

Amounts of HEV were determined by PCR

*: HEV in serum was substituted with PBS after ultracentrifugation.

HEV derived from swine feces tends to partition in the precipitate fraction whereas no partitioning is observed for human HEV.

Partitioning of HEV with NaDCA treatment during ethanol fractionation II+III

	$G3_{JP}$ (swine, feces)	huG3 (human, serum)		
		Na Deoxycholic acid		
Detergent;	**(Triton x100 / SD / SD)**	**1%**	**0.01%**	**0%**
Before	**6.0 / 7.0 / 7.2**	**6.5**	**5.9**	**6.4/6.4**
Supernatant (Removal)	**4.1 / 4.0 / 5.3 (1.9 / 3.0 / 1.9)**	**3.3 (3.2)**	**5.7 (0.2)**	**6.5/6.4 (0.0/0.0)**
Precipitate (Removal)	**5.7 / 7.0 / 7.0 (0.2 / 0.0 / 0.2)**	**6.4 (0.1)**	**4.9 (1.0)**	**6.0/5.8 (0.5/0.6)**

Amounts of HEV were determined by PCR

***: HEV in serum was substituted with PBS after ultracentrifugation.**

Lipids attached to HEV particles seemed to affect partitioning ability during fraction II+III

Partitioning of HEV with SD treatment during ethanol fractionation IV

Detergent;	$G3_{JP}$ (swine, feces)	huG3 (human, serum)	
	Yes (Triton x100 / SD / SD)	No	Yes (SD / SD)
Before	6.0 /7.0 /7.1	6.6 /6.5 /6.7	6.5 /6.5
Supernatant (Removal)	5.8 /6.8 /6.8 (0.2 /0.2 /0.3)	4.3 /5.9 /5.6 (2.3 /0.6 /1.0)	4.9 /5.1 (1.6 /1.5)
Precipitate (Removal)	5.4 /6.6 /6.7 (0.7 /0.4 /0.4)	6.2 /6.3 /6.5 (0.4 /0.1 /0.1)	6.6 /6.7 (-0.1 /-0.2)

~~Amounts of HEV were determined by PCR~~

*: HEV in serum was substituted with PBS after ultracentrifugation.

HEV derived from feces shows no partitioning whereas partitioning to precipitate fraction seen for human HEV.

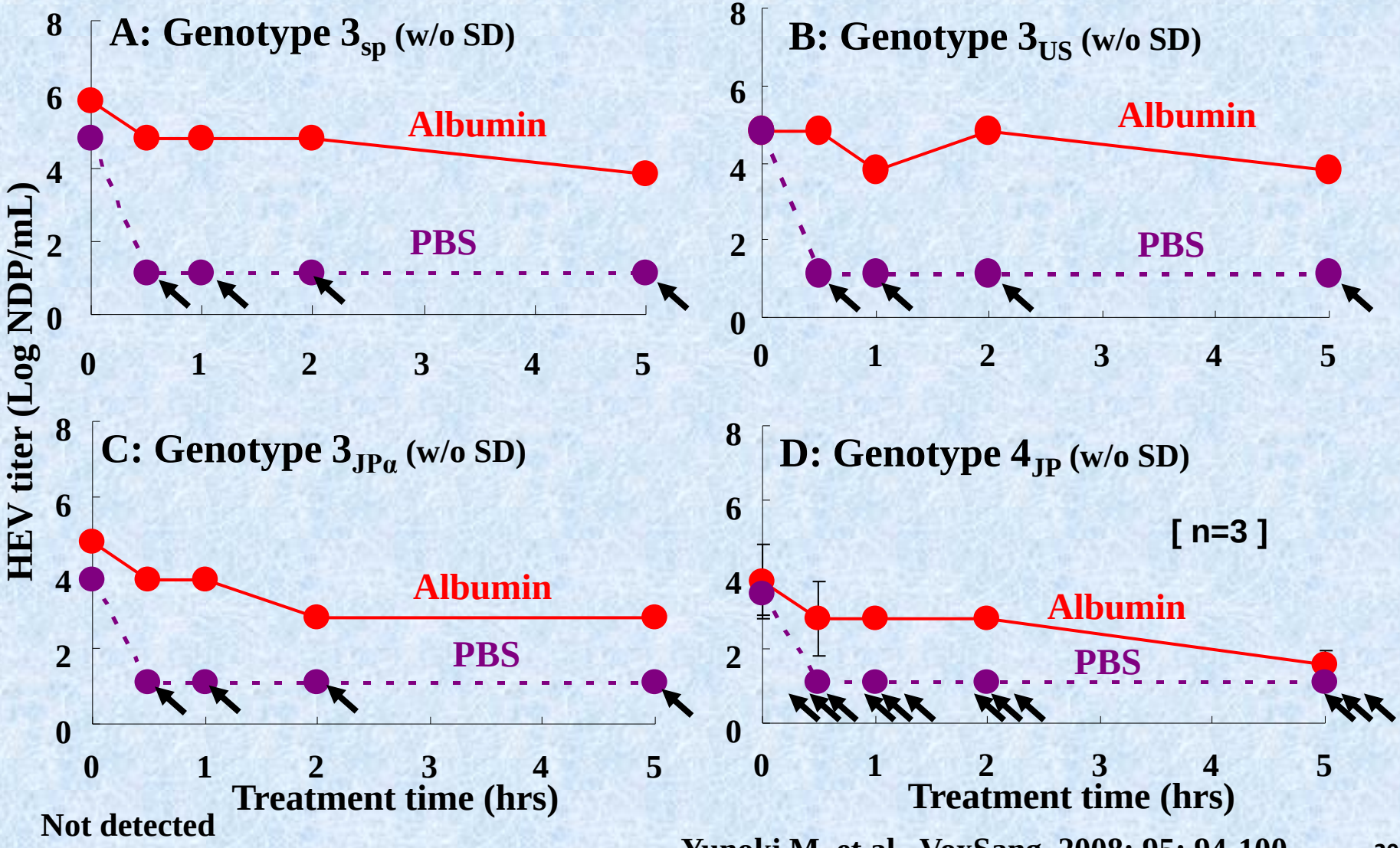
HEV reduction during ethanol fractionation of albumin preparation

Fractionation Step	Model Virus		Relevant Virus/Origin			
			B19	HAV	HEV	
	CPV	EMC	Human Plasma	Cul.Sup	Swine Feces	Human Serum*
I	0.0	0.0	0.0	0.0	0.0	0.0
II+III	≥4.3	2.4	2.9	3.3	2.3	0.0
IV	4.1	5.6	2.5	2.2	0.0	1.3

*: without detergent treatment

Partitioning property of HEV during ethanol fractionation is not reproducible.

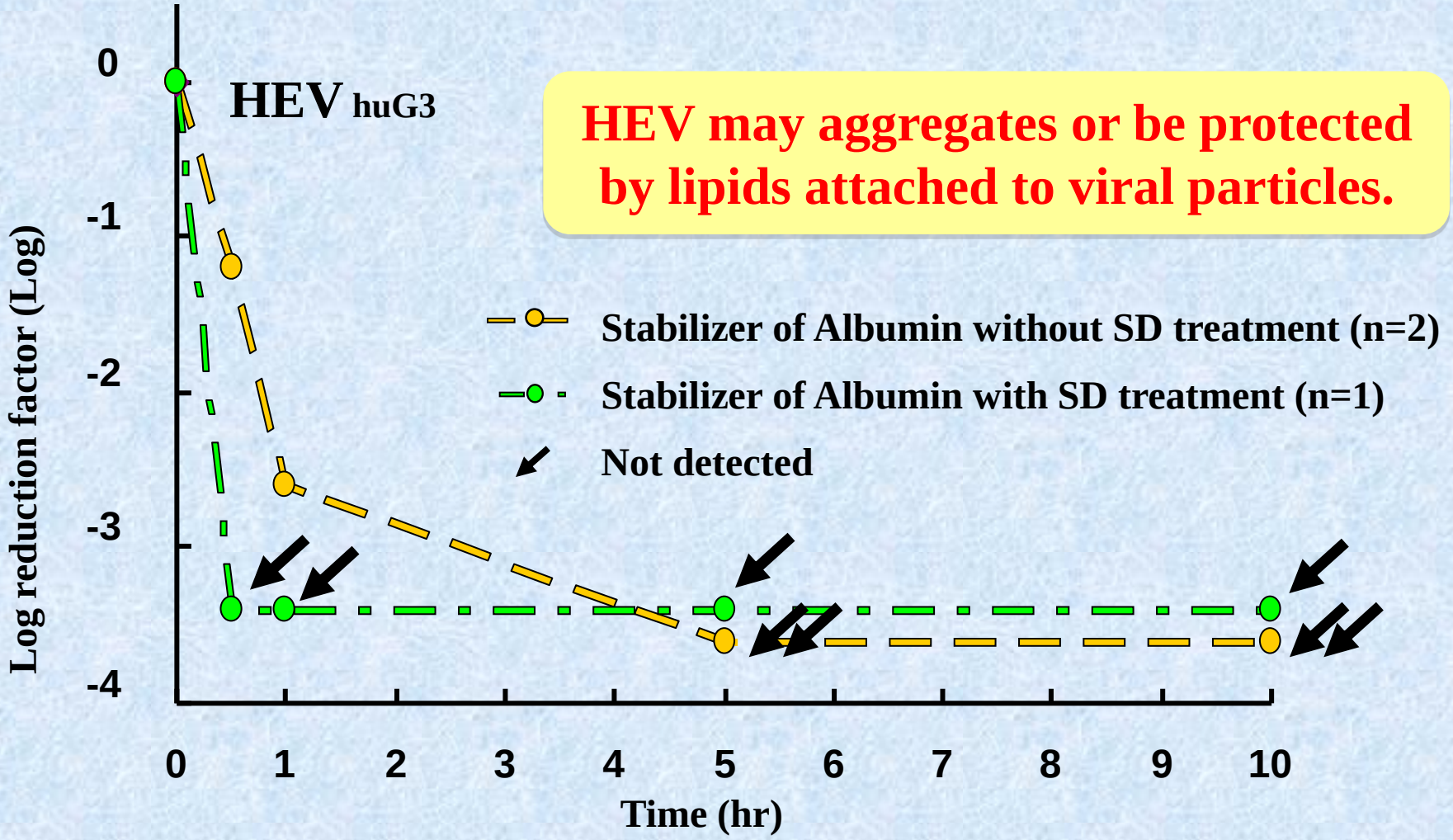
Inactivation kinetics of four HEV isolates in albumin during 60ºC liquid-heating



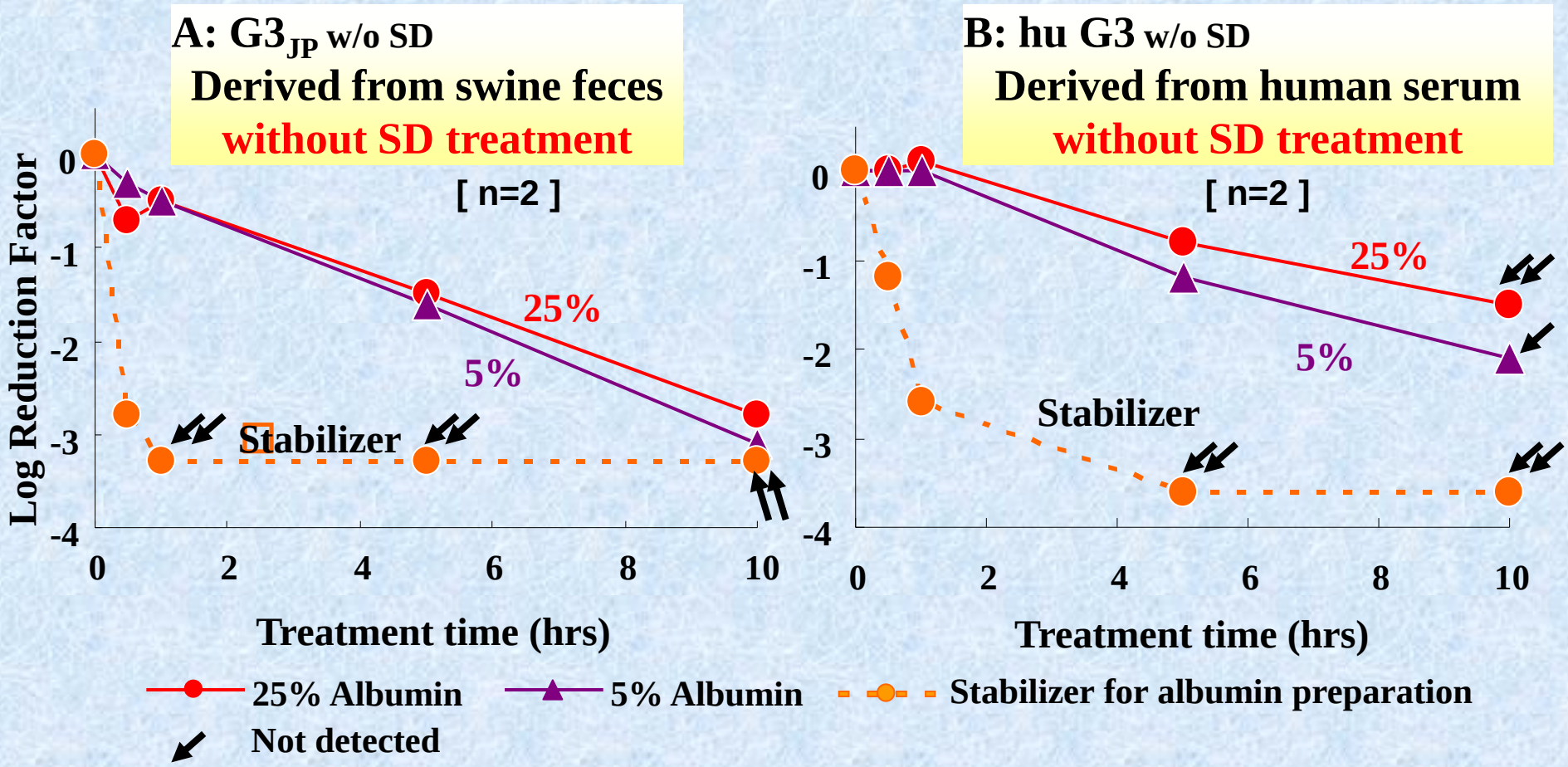
Not detected

Yunoki M. et al., VoxSang. 2008; 95: 94-100

Change of kinetics pattern of HEV derived from human serum after SD treatment

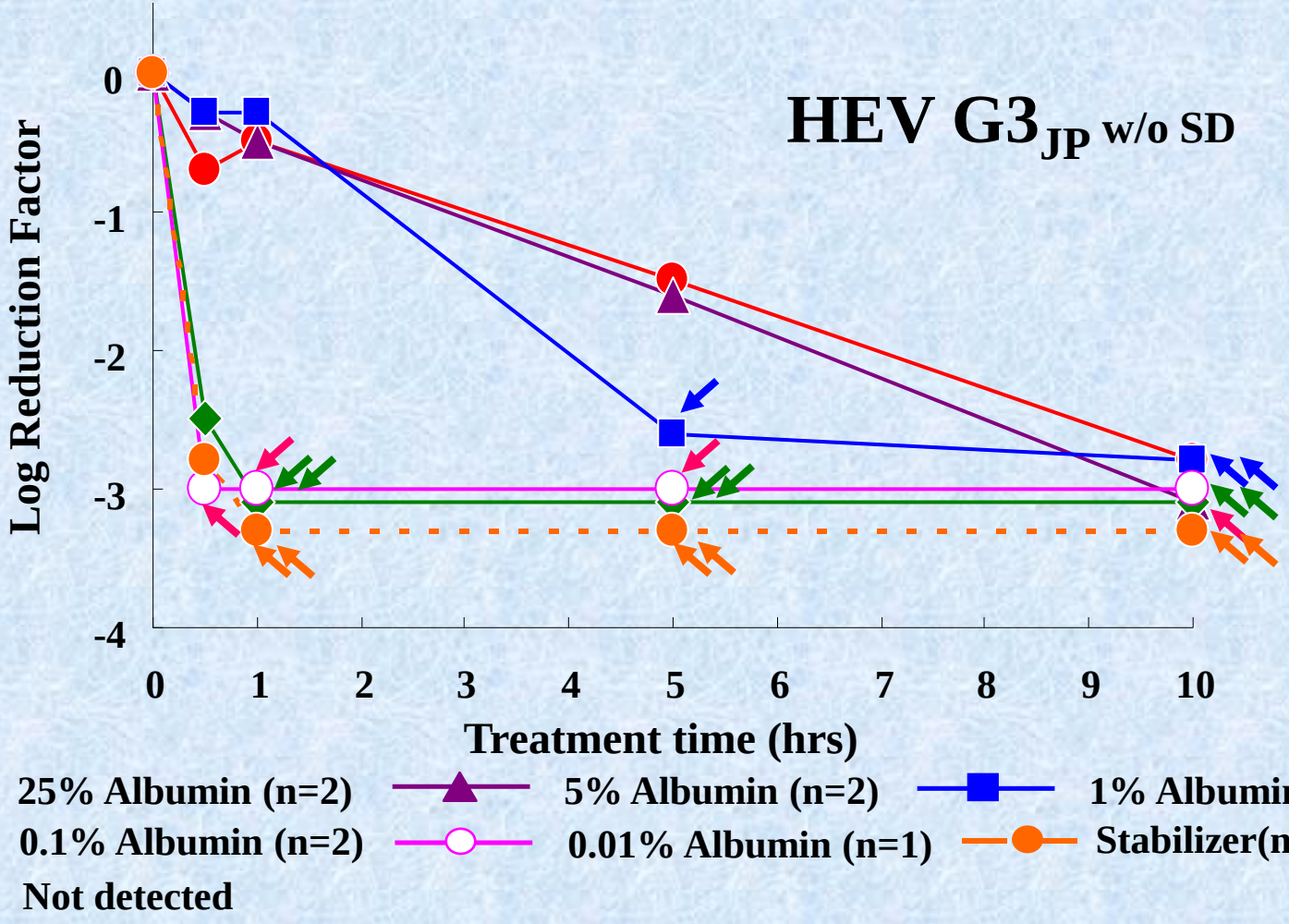


Difference of inactivation kinetics between two types of HEV isolates during 60ºC liquid-heating



HEV in serum tends to more resistant against heat inactivation in first phase period

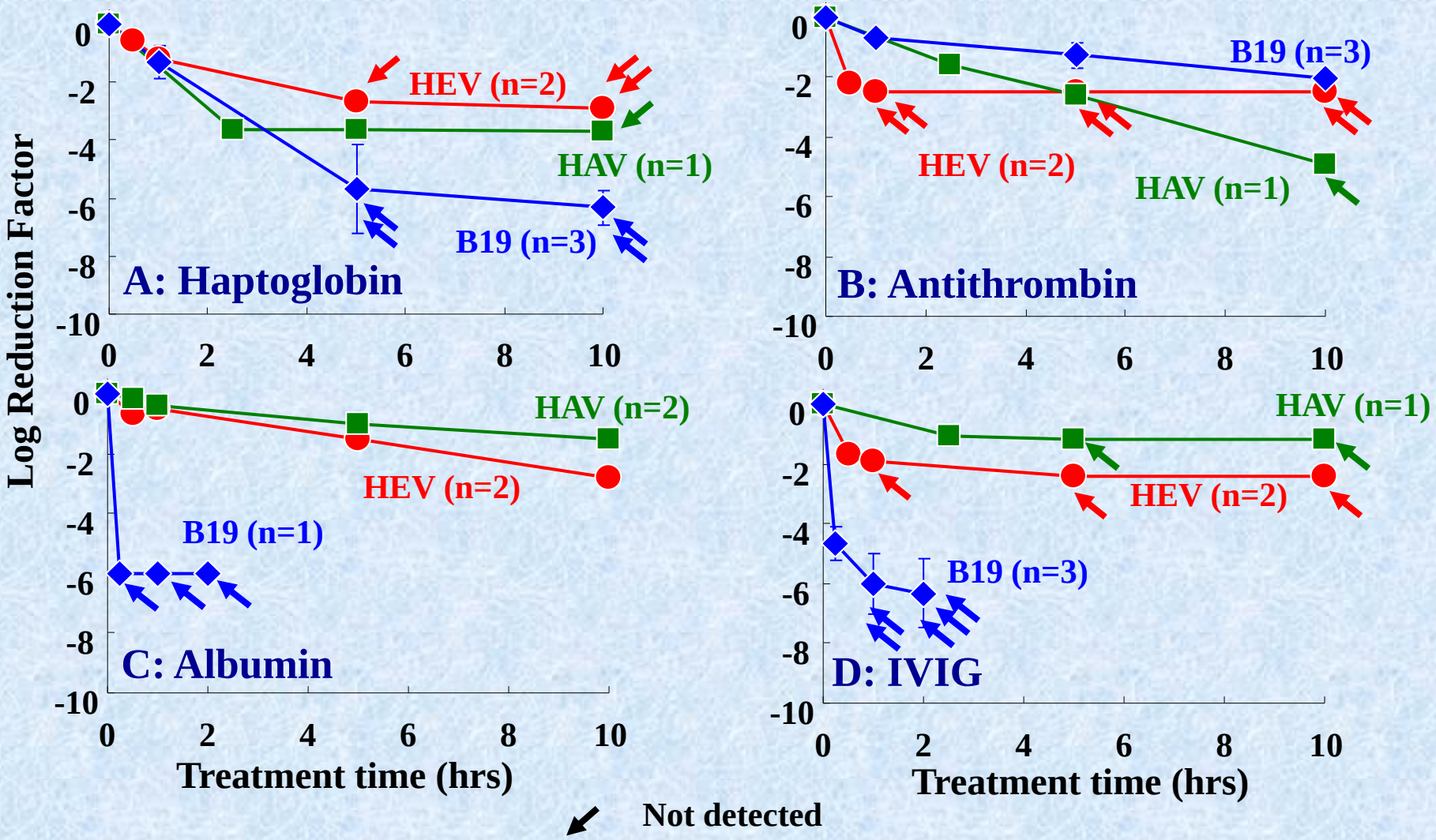
Different inactivation kinetics of HEV during 60ºC liquid-heating in different concentration of Albumin



Heat stability of HEV was dependent on concentration of albumin

Inactivation kinetics of B19, HAV and HEV during 60ºC liquid-heating in plasma products

(All viruses were prepared without SD)



↙ Not detected

HEV inactivation by low pH

	Infectious titers		
	PBS	pH 3.0	pH 2.5
0 hr		(4.6)*	
5 hrs	4.8	4.6	3.8
Log reduction	0.0	0.0	0.8

*: Theoretical value was used.

1. Infectious virus titer was indicated as log non detectable end-point per mL.
2. HEV G3jpα derived from swine feces with SD treatment was used

HEV was seemed to stable under low pH condition at least pH 2.5 for 5 hrs.

HEV removal by virus removal filters

Load material	Planova Filter	HEV in PBS			
		3jpα	3us	3sp	4jp
Detargent treatment, 0.22μm, 0.1μm filtrate	P-75 (73±2 nm)	(6.2/4.7) 1.5	(8. 9/7.5) 1.4	(8.2/6.9) 1.3	(7.6/6.3) 1.3
P-75 filtrate	P-35 (35±2 nm)	(6.2/4.8) 1.4	(6.9/<3.3) ≥3.6	(6.4/3.8) 2.6	(5.6/4.5) 1.1
	P-20 (19±2 nm)	(6.2/<3.3) ≥2.9	(6.9/<3.3) ≥3.6	(6.4/<3.2) ≥3.2	(5.6/<3.0) ≥2.6
	P-15 (15±2 nm)	(6.2/<3.3) ≥2.9	(6.9/<3.3) ≥3.6	(6.4/<3.2) ≥3.2	(5.6/<3.0) ≥2.6

Condition of filtation
P-75N: Planova 75: 0.001m^2 module, 50kPa, RT
P-35N: Planova 35: 1cm long single module, 50kPa, RT
P-20N: Planova 20: 1cm long single module, 50kPa, RT
P-15N: Planova 15: 1cm long single module, 50kPa, RT

Upper column: Log genome amounts (Before / Filtrate) of HEV were determined by PCR.
Lower column: Log reduction factor

Yunoki M.,et al., , VoxSang (2008) 95, 94-100, Benesis Corp.

Removal of HEV in Albumin and Fibrinogen preparations by virus removal filtrations

Product	Filter	Model virus		Relevant Virus (Origin)		
		CPV	EMC	B19 (Human plasma)	HAV (Culture sup)	HEV (Swine feces)
Albumin	P-15 (15±2 nm)	2.5	≥4.7	5.3	≥3.9	≥3.5
Fibrinogen	P-35 (35±2 nm)	0.0	2.4	0.0	0.0	3.2
	P-20 (19±2 nm)	2.5	≥6.7	1.9	2.2	≥3.9

- Virus amount of CPV and EMC were measured by infectivity assay whereas B19, HAV and HEV were measured by PCR.
- The results were shown as LRF, mean of two experiments.

Points to consider against HEV contamination in plasma products

- **HEV demonstrated non-reproducible partitioning during ethanol fractionation.**
- **We should pay attention when evaluating ethanol fractionation with HEV.**
- **HEV seems to low pH resistant (Not inactivated pH 2.5 for 5hr).**
- **HEV demonstrated heat resistance during inactivation in albumin.**
- **HEV could be removed by 19 and 15 nm filters.**
- **Inactivation (heating) and removal (virus filtration) could be an effective measure against HEV contamination.**

Research Collaborators

Benesis Corp.

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