VIRUS TRANSMISSION BY BLOOD PRODUCTS: DETECTION OF INFECTIOUS HEPATITIS A VIRUS IN FACTOR VIII CONCENTRATES BY EXPERIMENTAL INFECTION OF TAMARINS

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HAV Transmissions by Factor VIII Concentrates (solely S/D-treated)

Country	Year	Hepatitis A-Cases	Source of Plasma	Reference
→ Italy	1989 1990 1991 1992	3 10 33 6	USA	Lancet 339:819 (1992) Ann. Intern. Med. 120:1-7 (1994)
Germany	1988 1990 1991 1992	4 2 2 2		Lancet 340:1231 (1992) Vox Sang. 67(S1):39-46 (1994)
→ Ireland	1992	17	Ireland	Lancet 340:1466 (1992)
Belgium	1992	6	Belgium	Lancet 341:179 (1993)
South Africa	1993/94	9 3(6)		Hepatology 22:1363-1367 (1995)
USA	1995	3	USA	MMWR 45:29-32 (1996)
UK	1996	(4) 3	USA	not published
Germany	1997	6	USA	J. Med. Virol. 57:91-99 (1999)

S/D: solvent/detergent method (TNBP/Tween 80 or Triton X-100) Horowitz et al. , Transfusion 25:516-522 (1985)

→ animal studies (were not successful)

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Impact of NAT Tests on Virus Safety: HBV and HAV Investigation on Production Pools*

Manufacturer	HBV DNA (07/92-06/96) N_{pos}/N_{total}	HAV RNA (01/96-06/98) N_{pos}/N_{total}
A	0/25	0/1
B	1/52	0/6
C (Pasteurization)	0/191 ←	2/132
D (S/D)	1/14	0/43 ←
E	1/26	
F	0/4	0/4
G		0/19
H		0/6
Whole	3/312	2/211

*Pool size can considerably differ between the manufacturers

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Impact of NAT Tests on Virus Safety: HAV

1997:

- HAV infections in 7 hemophilic patients (6 developed acute hepatitis A)
- recipients of a factor VIII batch (manufacturer D; virus inactivation: S/D treatment)
- plasma pools: screening "HAV RT/PCR-negative"
- PEI: HAV RT/PCR (retrospective studies)

Pools	HAV PCR-pos (VP3)	Factor VIII	HAV PCR-pos (VP3)
A	7 / 11	Lot 1	6 / 17
B	0 / 8		

Pool A: about 6×10^2 geq/ml

Anti-HAV-Negative Patients Treated with the Incriminated Factor VIII Lot

Patient	Age at the begin of infusion	Administered factor VIII units	First Infusion (1996)	Symptoms of Disease (1997)	First Detection of IgM anti HAV (1997)	Date of serum collection for PCR (1997)
P1	44	12,000	DEC 01	JAN 18	JAN 20	FEB 20
P2	35	60,000	NOV 21	JAN 08	JAN 13	FEB 19
P3	60	44,000	NOV 26	JAN 03	JAN 13	FEB 20
P4	46	120,000	NOV 27	JAN 17	JAN 17	FEB 28
P5	25	60,000	DEC 05	JAN 13	JAN 20	APR 07
P6	21	4,000 (bolus infusion)	DEC 02	mid DEC(96)	MAR 20	MAR 20
P7	43	17,000	DEC 06	none	na	APR 01
P8	43	2,000	JAN 06(97)	none	na	na
P9	24	18,000	DEC 20	none	na	na

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HAV Sequence Analysis: Percentage Homology of VP3 and VP1/2A[1]

VP3 / VP1/2A	Plasma pool #a	Factor VIII #1	Factor VIII #2	Patient 1	Patient 2	Patient 3	Patient 4	Patient 6	Patient 7	PCR Ctrl
Plasma pool #a		100.0	100.0	ND[2]	100.0	100.0	ND	100.0	100.0	94.3
Factor VIII #1	100.0		100.0	ND	100.0	100.0	ND	100.0	100.0	94.3
Factor VIII #2	100.0	100.0		ND	100.0	100.0	ND	100.0	100.0	94.3
Patient 1	100.0	100.0	100.0		ND	ND	ND	ND	ND	ND
Patient 2	100.0	100.0	100.0	100.0		100.0	ND	100.0	100.0	94.3
Patient 3	100.0	100.0	100.0	100.0	100.0		ND	100.0	100.0	94.3
Patient 4	100.0	100.0	100.0	100.0	100.0	100.0		ND	ND	ND
Patient 6	ND	ND	ND	ND	ND	ND	ND		100.0	94.3
Patient 7	100.0	100.0	100.0	100.0	100.0	100.0	100.0	ND		94.3
PCR Ctrl	89.5	89.5	89.5	89.5	89.5	89.5	89.5	ND	89.5	

[1]VP3 (nt 2067-2188); VP1/2A (nt 2979-3264)

[2]ND = not done;

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Animal Study Performance

- **Animals:** 3 tamarins (*Saguinus fuscicollis)* were caged individually, but with acoustic contact each other.
- **Factor VIII product:** Concentration of 84 vials (each 1,000 IU factor VIII; 10 ml after reconstitution) with UC by using 30% sucrose cushion.
- **Inoculation:** Each animal received an equivalent of 28,000 IU of factor VIII of the implicated lot by i.v. injection without adjuvants. The animals were observed up to 132 days p.i.
- **Follow-up parameters:**

  Faeces (collected daily): HAV antigen (EIA); HAV RNA (RT/PCR).

  Sera (collected weekly): Liver transaminases ALT & GGT; antiHAV EIA (IgG+IgM); IgM antiHAV EIA; HAV RNA (RT/PCR).

Indicators of HAV Infection in *Saguinus fuscicollis* (I)

Animal #2							
	Serum					Faeces	
Days p.i.	ALT	GGT	HAV antibodies		HAV RNA	HAV antigen	HAV RNA
	(n-fold of baseline)		IgG/IgM	IgM	VP1/2A		VP3
-30	1.03	0.40	neg	neg	nd	nd	nd
14	0.78	1.03	nd	nd	pos	neg	nd
25	nd[1]	nd	nd	nd		nd	pos
27	nd	nd	nd	nd			pos
28	3.67	1.21	neg	neg	pos		pos
31	nd	nd	nd	nd			pos
35	7.67	2.42	nd	nd	pos		pos
38	nd	nd	nd	nd		neg	pos
42	4.85	5.67	pos	pos	pos	neg	nd
56	1.56	1.52	pos	pos	nd	nd	nd
63	1.24	2.06		nd	neg	nd	nd
70	1.33	1.45	pos	neg	nd	nd	nd

[1]nd: not done.

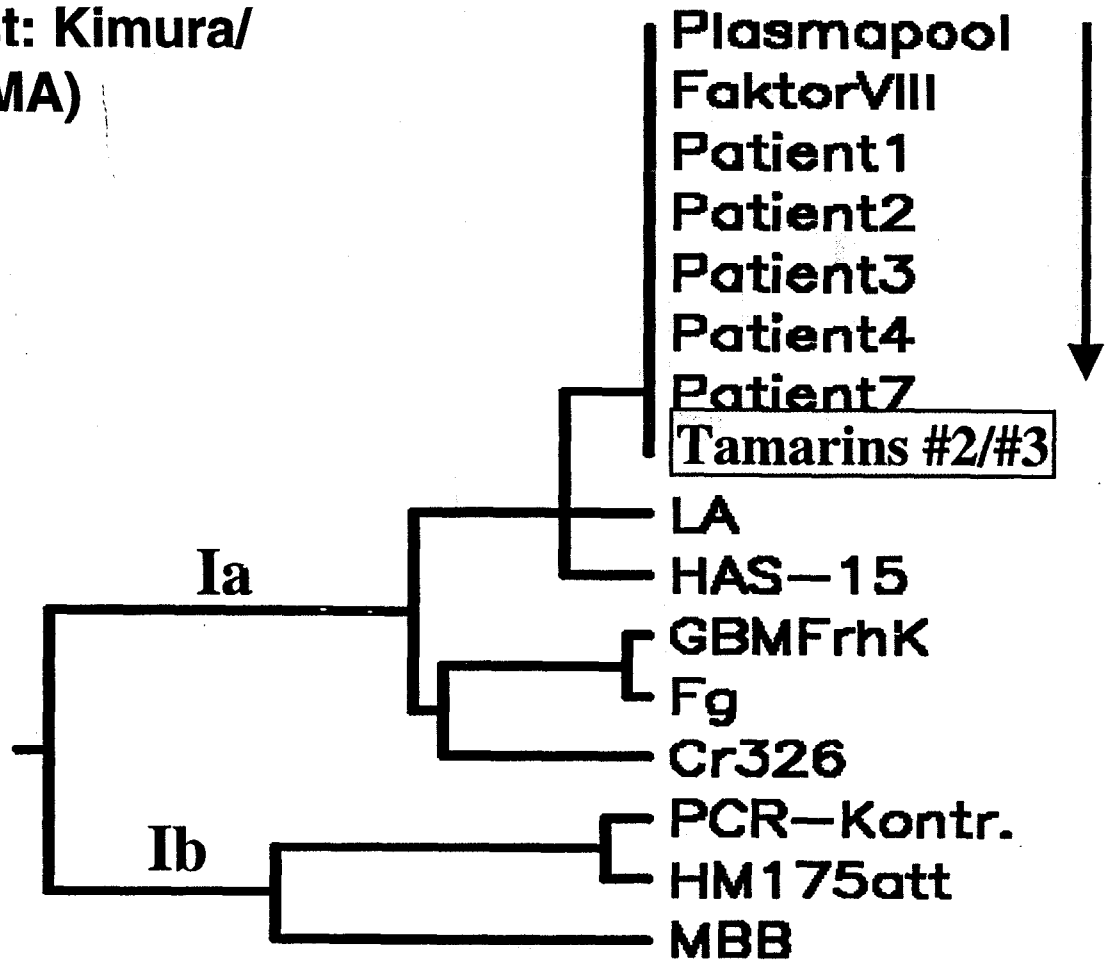
Indicators of HAV Infection in *Saguinus fuscicollis* (II)

Animal #3								
	Serum					Faeces		
Days p.i.	ALT	GGT	HAV antibodies		HAV RNA	HAV antigen	HAV RNA	
	(n-fold of baseline)		IgG/IgM	IgM	VP1/2A		VP3	VP1/2A
-30	0.73	0.94	neg	neg	nd	nd	nd	
14	1.77	1.18	nd	nd	neg	neg	nd	
24	nd[1]	nd	nd	nd	nd	neg	neg	neg
25	nd	nd	nd	nd	nd	neg	pos	pos
27	nd	nd	nd	nd	nd		neg	neg
28	2.56	1.41	neg	neg	neg	nd	neg	
31	nd	nd	nd	nd	nd	neg	pos	nd
35	1.77	0.94	nd	nd	neg		pos	pos
38	nd	nd	nd	nd	nd	nd	pos	pos
42	1.65	1.53	neg	neg	neg	neg	nd	
56	1.44	0.50	neg	neg	nd	nd	nd	
63	1.29	0.74	nd	nd	neg	nd	nd	
70	1.59	1.12	neg	neg	nd	nd	nd	

[1]nd: not done.

Genetic Relationship between HAV Strains of Genotype I

VP1/2A region (nt 2979-3264)
PHYLIP (dnadist: Kimura/ neighbor: UPGMA)



Impact of NAT Testing on Enhancing Viral Safety

Virus	"window" titer	NATdetection limit	pool size	NA extraction
HBV (DNA)	$\leq 10^3$ geq/ml	100 geq/ml	single donation/ maxi-pool + virus concentration?	proteolysis + chaotropic reag.
HCV (RNA)	10^{7-8} geq/ml	100 geq/ml	100 (96)	chaotropic reagent
HAV (RNA)	10^6 geq/ml (10^5 ID/ml?)	1,000 geq/ml	mini-pool (100)/ maxi-pool + virus concentration	chaotropic reagent

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Summary

- The animals received an equivalent of 28,000 IU of factor VIII of the implicated lot i.v.
- Tamarin #1 died 30 days p.i. (no signs of HAV infection).
- Tamarin #2 showed a classical HAV infection.
- Tamarin #3: HAV antigen and RNA detectable only in faeces.
- For the 1st time detection of infectious HAV in a coagulation factor concentrate by experimental infection of susceptible animals (*Saguinus fuscicollis).*
- Complete identity of the HAV sequences: animals and chain of infection plasma pool/plasma product/patient

Impact of NAT Tests on Virus Safety: HAV

Final proofs for HAV transmission

- 100 % HAV sequence identity (VP1/2A, VP3) between plasma pool, factor VIII lot and recipients[1]
- singular sequence; unique HAV strain (genotype IA)[1]
- Cluster of HAV infection traced to a contaminated plasma pool
- HAV titre in plasma donor: approx. 10^6 geq/ml[1]
- HAV experimentally transmitted to tamarins by factor VIII lot 1
- again 100 % HAV sequence identity (VP1/2A)

[1]Chudy et al., J. Med. Virol. 57:91-99 (1999)

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