

Supplementary material

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Additional data about molecular assays

Screening of ratHEV samples were carried out using real-time RT-qPCR with the primers and probes described previously^{1,2} and listed in Table S1. They were performed in final volumes of 50µl containing 20µl of the One Step PrimeScript III RT-qPCR Mix (Takara Bio, Shiga, Japan), 17.75µl of RNase-DNase free water, 0.75µl of forward (20µM), 0.75µl of reverse (20µM), 0.75µl of probe (20µM) and 10µl of RNA. As positive control, we used RNA from a rodent liver sample previously tested as ratHEV positive in our laboratory (GenBank accession number: OR282813). Positive liver samples by RT-qPCR were then sequenced by nested RT-PCR using the primers depicted in Table S1. Samples were tested serially from nested RT-PCR-1 to 4 until positive results were obtained. The first round of nested RT-PCR was conducted using the One Step PrimeScript III RT-PCR Kit (Takara Bio, Shiga, Japan) using the same positive control than for RT-qPCR, as well as volumes for primers, master mix and RNA as described but adding 18.5 µl of RNase-DNase free water. The second round was carried out with the premixed 2× solution containing Taq DNA Polymerase, dNTPs and Reaction Buffer (Promega, Madison, WI, USA). The volumes for each reaction were: 25µl of master mix, 15µl of RNase-DNase free water, 2.5µl

of each primer (20µM each) and 5µl of template., whereas the second was carried out with the premixed 2X solution of Taq DNA Polymerase, dNTPs and Reaction Buffer (Promega).

Table S1. List of primers and probes, when applicable, for each molecular assay

PCR	ID	Ref	Primers (5'-3')		Probe (5'-3')
			Forward	Reverse	
RT-qPCR	qPCR-1	1	CTTGTTGAGCTYTTCTCCCCT	CTGTACCGGATGCGACCAA	TGCAGCTTGTC TTTGARCCC
RT-qPCR	qPCR-2	2	CCACGGGGGTTAATACTGC	CGGATGCGACCAAGAAACAG	CGGCTACCGCC TTTGCTAATGC
Nested RT-PCR	seq PCR -1	3	1 st PCR: CTTGGTTYAGGGCCATAGAG 2 nd PCR: TTYAGGGCCATAGAGAAGGC	1 st PCR: CAGCAGCGGCACGAACAGCA 2 nd PCR: ACAGCAAAAGCACGAGCACG	-
Nested RT-PCR	seq PCR -2	-	1 st PCR: TTTGCTAATGCTCAGGTGGT 2 nd PCR: CCTYTGCAGCTTGTCTTTGA	1 st PCR: CATDCCATGAGCACGCAT 2 nd PCR: GTGCAAAAGGAAAGATCAG	-
Nested RT-PCR	seq PCR -3	4	1 st PCR: CCTYTGCAGCTTGTCTTTGA 2 nd PCR: CTGTTTCTTGGTCGCATCCG	1 st PCR: CATDCCATGAGCACGCAT 2 nd PCR: GTGCAAAAGGAAAGATCAG	-

Additional data about results

Table S2. Molecular results of positive animals for rat hepatitis E virus.

ID	qPCR-1*	Ct qPCR-1	qPCR-2*	Ct qPCR-2	GenBank Accesion Number	Faeces global qPCR	qPCR-1	Ct qPCR-1	qPCR-2	Ct qPCR-2
1	+	20.50	+	20.29	PP216692	+	+	22.48	+	25.37
2	-		+	39.17		-	-		-	
3	-		+	39.87	PP507100	-	-		-	
4	-		+	41.08	PP507101	-	-		-	
5	-		+	36.96	PP256190	-	-		-	
6	+	16.43	+	19.02	PP216688	+	+	23.28	+	26.08
7	+	33.24	-		PP256186	+	+	33.31	+	37.44
8	+	30.73	+	35.46	PP507098	-	-		-	
9	+	31.45	+	35.16	PP256187	+	+	39.92	-	
10	+	33.06	+	37.28		-	-		-	
11	+	30.81	+	36.50	PP256188	+	+	42.92	-	
12	-		+	38.03	PP256189	-	-		-	
13	+	11.05	+	15.61	PP216689	+	+	21.32	+	25.36
14	+	26.99	+	32.07	PP216690	+	+	34.09	-	
15	+	24.47	+	29.48	PP216691	+			+	38.13
16	+	31.44	+	37.10	PP507099	-	-		-	
17	+	34.70	+	38.64		-	-		-	
18	+	32.57	+	32.95	PP216698	-	-		-	
19	+	17.21	+	18.11	PP216693	+	+	19.48	+	22.61
20	-		+	37.40	PP256193	+	+	34.84	+	41.07
21	-		+	34.53	PP216699	+	+	35.92	+	39.27
22	+	33.79	+	33.69	PP216700	-	-		-	
23	-		+	37.06	PP256191	+	+	34.34	+	36.95
24	-		+	38.16	PP216694	-	-		-	
25	+	30.09	+	30.10	PP216695	+	+	29.80	+	32.10
26	+	24.82	+	25.04	PP216696	+	+	25.97	+	31.09
27	-		+	39.92	PP256192	+	-		+	38.10
28	+	32.27	+	32.95	PP216697	+	+	32.61	+	35.55
29	+	22.75	-		PP471864					

ID	qPCR-1*	Ct qPCR-1	qPCR-2*	Ct qPCR-2	GenBank Accession Number	Faeces global qPCR	qPCR-1	Ct qPCR-1	qPCR-2	Ct qPCR-2
30	-		+	39.37		+	+	44.65		
31	-		+	39.76	PP507102	-	-		-	
32	+	36.3	-		PP256196	+	+	35.63	-	
33	-		+	40.54		+	+	36.03	-	
34	+	36.4	+	38.75		-	-		-	
35	-		+	39.40	PP471853	-	-		-	
36	-		+	38.18		+	+	36.60	-	
37	-		+	39.54						
38	+	36.51	+	38.91	PP256207	-	-		-	
39	+	26.79	-		PP471854	+	+	29.61	-	
40	+	33.02	-		PP083440	+	+	36.56	+	38.49
41	+	27.96	+	31.13		+	+	33.73	+	36.67
42	+	27.29	+	32.56	PP083425	+	+	28.80	+	33.76
43	+	13.30	+	19.90	PP083427	+	+	18.86	+	21.72
44	+	28.69	+	32.60	PP216701	+	+	32.72	-	
45	+	33.86	+	36.07	PP216702	-	-		-	
46	+	36.41	-			-	-		-	
47	+	28.45	-			-	-		-	
48	+	28.07	+	34.86	PP216703	+	+	33.19	-	
49	+	25.26	+	33.51	PP216704	+	+	35.48	-	
50	+	25.27	-		PP216705	-	-		-	
51	+	27.38	+	32.01	PP216706	+	+	36.94	-	
52	+	29.10	+	34.27	PP083428	+	+	34.25	+	36.61
53	+	28.83	+	36.05	PP083441	+	+	35.99	-	
54	+	27.20	+	31.54	PP083429	+	+	32.45	+	41.10
55	+	28.27	+	32.86	PP083430					
56	+	27.18	+	35.50	PP083431	+	+	35.88	-	
57	+	14.74	+	33.53	PP083442					
58	+	23.40	+	20.95	PP083432					
59	+	13.26	+	29.41	PP083433	-	-		-	

ID	qPCR-1*	Ct qPCR-1	qPCR-2*	Ct qPCR-2	GenBank Accession Number	Faeces global qPCR	qPCR-1	Ct qPCR-1	qPCR-2	Ct qPCR-2
60	+	13.27	+	19.08	PP083434					
61	+	30.51	+	19.07	PP083435	+	+	21.09	+	23.33
62	+	28.60	+	37.82	PP083436					
63	+	28.27	+	34.66	PP083443	-	-		-	
64	+	29.86	+	34.10	PP083444	+	+	38.42	-	
65	+	30.02	+	37.21	PP101769	-	-		-	
66	+	31.32	+	36.46	PP083445	-	-		-	
67	+	32.52	+	38.33	PP083446	+	+	35.81	-	
68	+	30.25	-		PP083447	-	-		-	
69	+	29.41	+	36.73	PP083448	-	-		-	
70	+	32.92	+	38.03	PP083449	-	-		-	
71	+	30.65	+	39.27	PP101770	-	-		-	
72	-		+	37.69	PP083450	-	-		-	
73	+	35.47	-		PP083451	-	-		-	
74	-		+	39.27	PP083452	-	-		-	
75	+	34.66	-		PP083453	-	-		-	
76	+	25.31	+	30.76	PP083437	+	+	40.52	+	41.44
77	+	34.53	+	43.74	PP101760					
78	+	34.23	+	36.74	PP216707	+	+	36.49	-	
79	+	31.44	+	36.57	PP256197	+	+	34.54	+	41.58
80	-		+	40.27		-	-		-	
81	+	33.44	+	39.51	PP256198	+	-		+	38.86
82	+	35.71	-		PP996732	+	+	36.07	-	
83	+	35.01	-		PP256199	+	+	34.35	-	
84	+	32.25	-		PP256200	-	-		-	
85	+	35.04	-			-	-		-	
86	+	34.67	-		PP507107	-	-		-	
87	+	35.07	+	37.68		-	-		-	
88	+	35.92	-		PP256202	-	-		-	
89	-		+	38.35	PP101761					
90	+	38.14	-		PP101771	-	-		-	
91	-		+	40.18	PP101762	-	-		-	

ID	qPCR-1*	Ct qPCR-1	qPCR-2*	Ct qPCR-2	GenBank Accession Number	Faeces global qPCR	qPCR-1	Ct qPCR-1	qPCR-2	Ct qPCR-2
92	+	34.49	-			-	-		-	
93	+	33.75	+	36.50	PP216708					
94	-		+	38.58	PP256203	-	-		-	
95	+	38.86	-		PP256204	-	-		-	
96	+	30.24	+	34.35	PP101763	+	+	40.14	-	
97	+	34.35	-		PP101764	+	+	36.87	-	
98	+	39.95	-		PP507109	+	-		+	39.46
99	+	35.53	+	36.30	PP507110	-	-		-	
100	+	19.91	+	22.44	PP216710	+	+	23.98	+	26.29
101	+	31.16	+	34.63	PP507112	-	-		-	
102	+	36.67	-		NA [†]	-	-		-	
103	-		+	38.86	NA [†]	-	-		-	
104	+	35.09	+	38.41	PP507113	+	+	26.86	+	30.36
105	+	36.32	+	38.20	PP507114					
106	+	20.10	+	23.08	PP216711	+	+	23.72	+	26.13
107	+	39.99	+	40.21		-	-		-	
108	+	18.50	+	20.06	PP471856	-	-		-	
109	+	30.49	+	36.00	PP471857	-	-		-	
110	+	34.88	+	38.96	PP471858	-	-		-	
111	+	29.18	+	32.66	PP471859	-	-		-	
112	+	37.67	-			-	-		-	
113	+	17.70	+	19.84	PP471860	+	+		+	
114	-		+	39.32	PP471861					
115	-		+	36.47	PP471862	-	-		-	
116	-		+	40.91	PP471863	-	-		-	
117	+	35.60	-		PP256205	-	-		-	
118	+	32.49	+	38.1	PP256206	-	-		-	
119	-		+	39.23	PP471850	-	-		-	
120	-		+	39.39		-	-		-	
121	+	37.39	+	36.77	PP256194	-	-		-	
122	-		+	26.13	PP471852					
123	+	36.13	-		PP256195	-	-		-	

ID	qPCR-1*	Ct qPCR-1	qPCR-2*	Ct qPCR-2	GenBank Accession Number	Faeces global qPCR	qPCR-1	Ct qPCR-1	qPCR-2	Ct qPCR-2
124	+	22.05	+	29.29		+	+	32.21	+	37.23
125	+	36.01	-			+	+	33.77	-	
*Sixty-nine (55.2%) out of the 125 animals were positive by both qPCRs in liver tissue. NA [†] : These samples were confirmed by sequencing but these were not submitted to GenBank due to low quality										

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