

Supplementary Materials:

Table S1. Piscihepevirus isolations from salmonids in Canada from 2010-2017.

Genotype	Host	Location	Year	Isolate name	GenBank accession number
CTV-1	Atlantic salmon	New Brunswick	2010	CA/NB/2010-214/AtS	MZ438685
	Rainbow trout	Quebec	2011	CA/QC/2011-197/Rbt	MZ243139
	Arctic char	Quebec	2013	CA/QC/2013-181/ArC	MZ243138
	Rainbow Trout	Prince Edward Island	2015	CA/PE/2015-143/Rbt	MZ243137
	Atlantic salmon	Nova Scotia	2010	CA/NS/2010-67/AtS	MZ243115
	Atlantic salmon	New Brunswick	2010	CA/NB/2010-130/AtS	MZ243114
	Atlantic salmon	New Brunswick	2010	CA/NB/2010-133/AtS	MZ243135
	Atlantic salmon	Prince Edward Island	2010	CA/PE/2010-174/AtS	MZ243094
	Atlantic salmon	New Brunswick	2010	CA/NB/2010-200/AtS	MZ243109
	Atlantic salmon	Prince Edward Island	2010	CA/PE/2010-204/AtS	MZ438686
	Atlantic salmon	Nova Scotia	2011	CA/NS/2011-01/AtS	MZ243136
	Atlantic salmon	New Brunswick	2011	CA/NB/2011-06/AtS	MZ438687
	Atlantic salmon	New Brunswick	2011	CA/NB/2011-22/AtS	MZ243134

Atlantic salmon	New Brunswick	2011	CA/NB/2011-25/AtS	MZ243113
Atlantic salmon	New Brunswick	2011	CA/NB/2011-26/AtS	MZ243132
Atlantic salmon	New Brunswick	2011	CA/NB/2011-27/AtS	MZ243125
Atlantic salmon	Nova Scotia	2011	CA/NS/2011-104/AtS	MZ243103
Atlantic salmon	Nova Scotia	2011	CA/NS/2011-138/AtS	MZ243108
Atlantic salmon	Prince Edward Island	2011	CA/PE/2011-156/AtS	MZ243128
Atlantic salmon	Nova Scotia	2011	CA/NS/2011-172/AtS	MZ438688
Atlantic salmon	Prince Edward Island	2011	CA/PE/2011-218/AtS	MZ243093
Atlantic salmon	Prince Edward Island	2011	CA/PE/2011-241/AtS	MZ243112
Atlantic salmon	Prince Edward Island	2011	CA/PE/2011-242/AtS	MZ243107
Atlantic salmon	New Brunswick	2011	CA/NB/2011-251/AtS	MZ243111
Atlantic salmon	New Brunswick	2011	CA/NB/2011-252/AtS	MZ243117
Atlantic salmon	New Brunswick	2011	CA/NB/2011-263/AtS	MZ243133
Atlantic salmon	New Brunswick	2012	CA/NB/2012-01/AtS	MZ243126
Atlantic salmon	New Brunswick	2012	CA/NB/2012-25/AtS	MZ243096
Atlantic salmon	Nova Scotia	2012	CA/NS/2012-84/AtS	MZ243120

CTV-2b	Atlantic salmon	Nova Scotia	2012	CA/NS/2012-91/AtS	MZ243102
	Atlantic salmon	New Brunswick	2012	CA/NB/2012-99/AtS	MZ438689
	Atlantic salmon	New Brunswick	2012	CA/NB/2012-244/AtS	MZ243116
	Atlantic salmon	Prince Edward Island	2013	CA/PE/2013-02/AtS	MZ243092
	Atlantic salmon	New Brunswick	2013	CA/NB/2013-16/AtS	MZ243095
	Atlantic salmon	Nova Scotia	2013	CA/NS/2013-20/AtS	MZ243106
	Atlantic salmon	Nova Scotia	2013	CA/NS/2013-21/AtS	MZ243105
	Atlantic salmon	Nova Scotia	2013	CA/NS/2013-22/AtS	MZ243104
	Atlantic salmon	Prince Edward Island	2013	CA/PE/2013-23/AtS	MZ243129
	Atlantic salmon	New Brunswick	2013	CA/NB/2013-31/AtS	MZ438690
	Atlantic salmon	New Brunswick	2013	CA/NB/2013-37/AtS	MZ243100
	Atlantic salmon	New Brunswick	2013	CA/NB/2013-40/AtS	MZ243110
	Atlantic salmon	Prince Edward Island	2013	CA/PE/2013-202/AtS	MZ243124
	Atlantic salmon	Prince Edward Island	2013	CA/PE/2013-205/AtS	MZ243099
	Atlantic salmon	Nova Scotia	2014	CA/NS/2014-12/AtS	MZ243127
	Atlantic salmon	New Brunswick	2014	CA/NB/2014-18/AtS	MZ243091

Atlantic salmon	New Brunswick	2014	CA/NB/2014-102/AtS	MZ243123
Atlantic salmon	Nova Scotia	2014	CA/NS/2014-139/AtS	MZ243131
Atlantic salmon	Prince Edward Island	2014	CA/PE/2014-143/AtS	MZ243090
Atlantic salmon	Prince Edward Island	2014	CA/PE/2014-154/AtS	MZ243098
Atlantic salmon	New Brunswick	2014	CA/NB/2014-166/AtS	MZ243101
Atlantic salmon	New Brunswick	2015	CA/NB/2015-13/AtS	MZ243122
Atlantic salmon	New Brunswick	2015	CA/NB/2015-21/AtS	MZ438691
Atlantic salmon	Nova Scotia	2015	CA/NS/2015-46/AtS	MZ243130
Atlantic salmon	Prince Edward Island	2015	CA/PE/2015-145/AtS	MZ243097
Atlantic salmon	Prince Edward Island	2015	CA/PE/2015-149/AtS	MZ243118
Atlantic salmon	New Brunswick	2015	CA/NB/2015-158/AtS	MZ243121
Atlantic salmon	Nova Scotia	2015	CA/NS/2015-165/AtS	MZ243119
Rainbow Trout	Prince Edward Island	2015	CA/PE/2015-143/Rbt	MZ243137
Atlantic salmon	New Brunswick	2016	CA/NB/2016-05/AtS	MZ243089
Atlantic salmon	British Columbia	2017	CA/BC/2017-111/AtS	MZ438692

Table S2. Information of selected hepevirus complete genome sequences used in phylogenetic analyses presented in this study

Genus	Species	Genotype	Origin of reference isolate (isolate name)	Predominant host	GenBank accession
From GenBank					
<i>Orthohepevirus</i>					
	<i>Orthohepevirus A</i>	HEV-A1	Burma	Human	M73218
		HEV-A2	Mexico	Human	M74506
		HEV-A3	United States	Human, pig, rabbit, deer, mongoose	AF082843
		HEV-A4	China	Human, pig	AJ272108
		HEV-A5	Japan	Wild boar	AB573435
		HEV-A6	Japan	Wild boar	AB602441
		HEV-A7	United Arab Emirates	Camel	KJ496143
	<i>Orthohepevirus B</i>		United States	Chicken	AY535004
	<i>Orthohepevirus C</i>	HEV-C1	Germany	Rat	GU345042

		HEV-C2	Netherlands	Ferret	JN998606
	<i>Orthohepevirus D</i>		Germany	Bat	JQ001749
<i>Piscihepevirus</i>					
	<i>Piscihepevirus A</i>	CTV-1	United States	Trout	HQ731075
		CTV-2a	Canada	Salmon	MN995808
In this study					
<i>Piscihepevirus</i>					
	<i>Piscihepevirus A</i>	CTV-1	Canada	Salmon	MZ438685
		CTV-2b	Canada	Salmon	MZ438686
		CTV-2b	Canada	Salmon	MZ438687
		CTV-2b	Canada	Salmon	MZ438688
		CTV-2b	Canada	Salmon	MZ438689
		CTV-2b	Canada	Salmon	MZ438690
		CTV-2b	Canada	Salmon	MZ438691
		CTV-2a	Canada	Salmon	MZ438692

Table S3. Primer and probe sequences for RT-qPCR assays used in this study

Name^a	Sequence (5'-3')
ASHV_3848F	CACCAGCTGCCGACTCAA
ASHV_3989R	TCGTCAGGAACCGCACAC
ASHV_3493P	FAM-CGCATCAACCAGGGATCCTT-MGBNFQ
CTV 4578F	GTCTCYCTYGGCAAYGAGTTTCT
CTV 4809R	TCCTTTGAASAGSGCRAGRGCAT
CTV 4730P	FAM-GGCACMATGCTTTTCAA-MGBNFQ

^a Primers and probe denoted with “ASHV” target CTV isolates from Western Canada while the “CTV” denoted primers and probe target CTV isolates from Eastern Canada.

Table S4. Experimental parameters of the conspecific cohabitation trial.

Species	No. of tanks		Tank volume (l)	No. of fish per tank		Mean weight (g)	Stock Density (kg/m³)
	Treatment	Control		Donor	Sentinel		
Sockeye	2	2	450	21	21	76	8.8
Pink	2	2	50	40	40	12	19.2
Chinook	2	2	350	50	50	43	12.3

Table S5. Prevalence (%) of CTV-2 in Sockeye, Pink, and Chinook Salmon mucus in conspecific cohabitation challenge. Six fish were sampled per treatment at each timepoint with the exception of 12 wpi when five Chinook donor fish were sampled. NA denotes samples were not screened.

Weeks Post- Injection	Sockeye		Pink		Chinook	
	Donor	Sentinel	Donor	Sentinel	Donor	Sentinel

1	83	50	83	17	100	17
2	100	0	17	0	100	50
4	83	67	67	0	67	33
6	50	NA	33	NA	67	17
12	17	NA	0	NA	20	17
14	33	NA	17	NA	50	83
16	83	NA	17	NA	83	67
18	17	NA	0	NA	33	17

Table S6. Identification of PXXP motifs in Piscihepeviruses (GenBank accession #'s HQ731075, MN995808, MZ438685 - MZ438692) and Orthohepeviruses (HEV-1, 2, 3, 4, 5, 6, 7, B, C1, C2, D) isolates, respectively.

	ORF1 Non structural polyprotein		ORF2 Capsid		ORF3 Hypothetical protein	
	Length (aa)	PXXP motif sites*	Length (aa)	PXXP motif sites*	Length (aa)	PXXP motif sites*
Piscihepeviruses	1708-1713	P Y G P P T/S A P# P T/A L P P V/I Q P P A N/K P P A V P P A H P P S D P	629-635	P P K P P D/N S P P P Q/H P P P G P P S T P	NA	NA
Orthohepeviruses	1532-1709	P Y V/T P P F/G S/A P P A A/S P P T/A L/S/Q/T P P S/A/T L P P P/A C/A/G P P A/Q/L L/Q/P P	607-675	P M/S L/Y/S P P A P P P T N P P F A P P A/I/P R P P L S P P L/V N/S/G P P L/P L/M P P L/V-V P S/A/V/K R P	88-138	P S/L P/L/H P P M S/F/P P P L/Q/P/A R/H/P/C P P S A P

*Only sites present in at least 2 isolates are listed

#a634-637

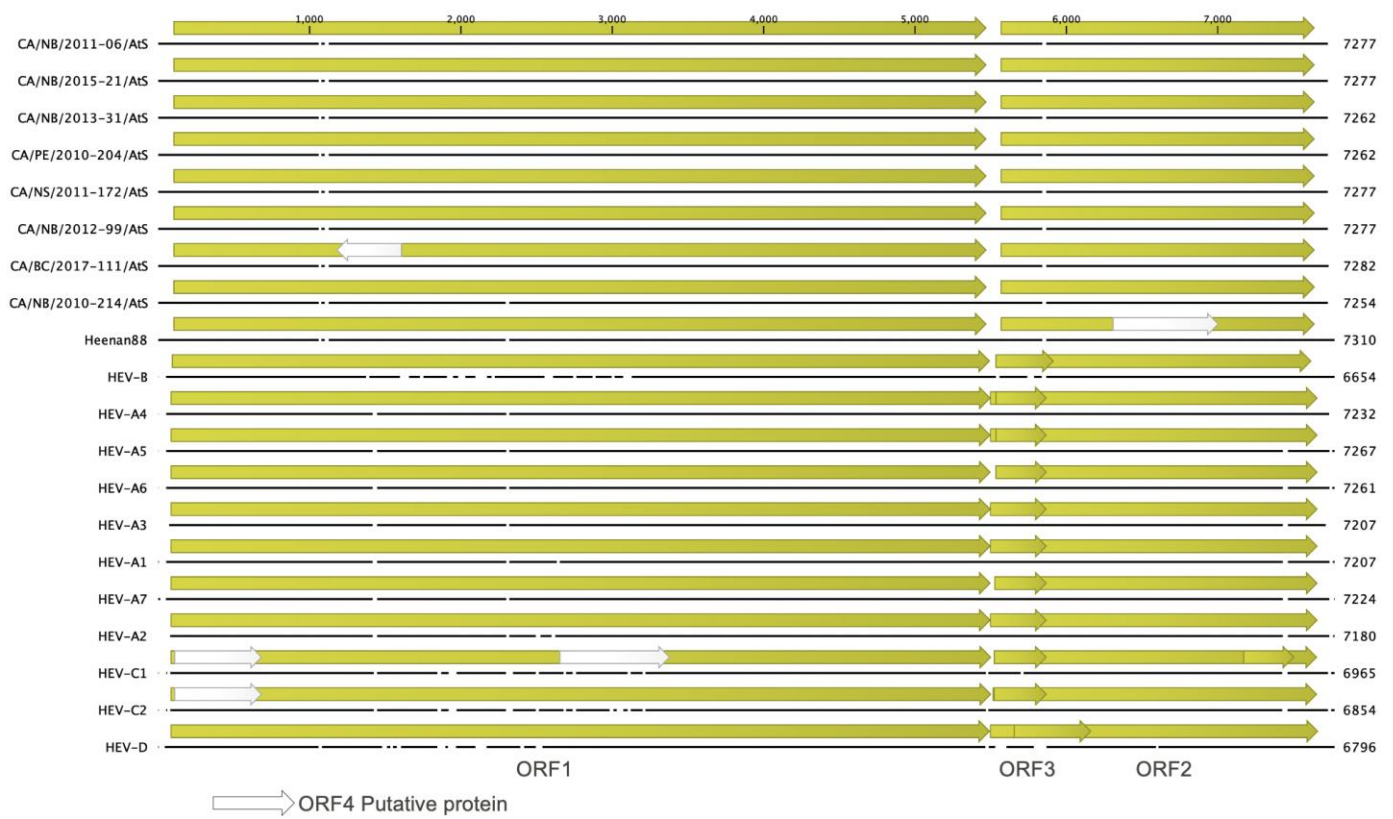


Figure S1. Alignment of the whole genome sequences including annotations of ORF1, ORF2 and ORF3 (when present). Putative protein ORF4s are indicated with white arrows.

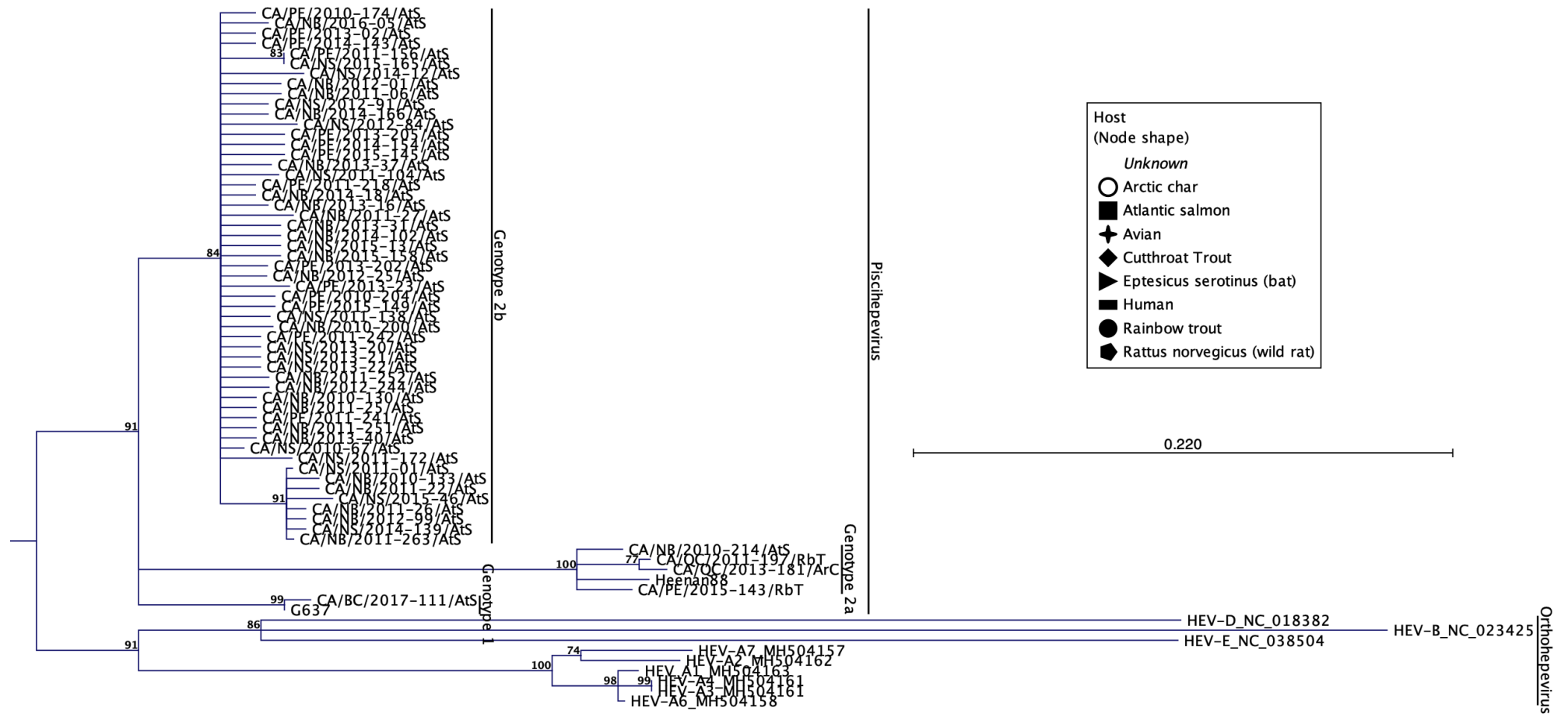


Figure S2. Phylogenetic tree showing the genetic relationship of 193 nt region of the RNA –dependent RNA polymerase of cutthroat trout virus isolates from Canada with other hepevirus isolates

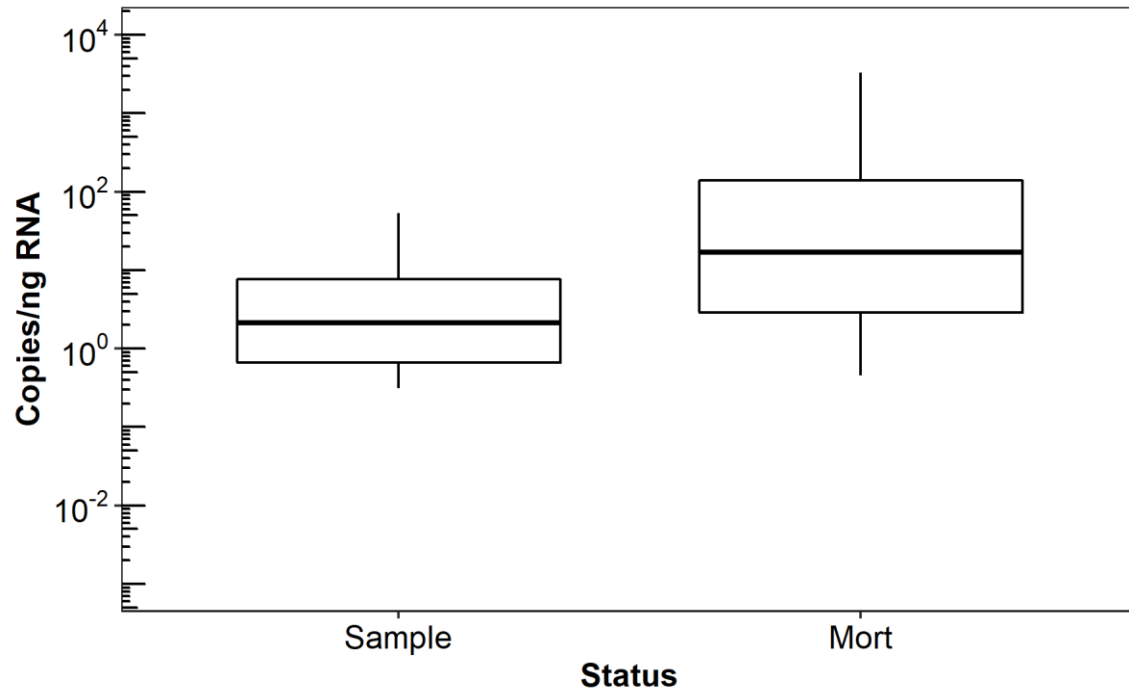


Figure S3. CTV-2 load in Atlantic salmon mortalities (n=9) and sampled fish (n=29) from the heterospecific cohabitation trial. Samples with RT-qPCR Ct values above the LOQ (54 c/rxn) were included in this analysis. Data are presented in box plots in which the inner horizontal line is the median, and the upper and lower boundaries of the box correspond to the first and third quartiles. The upper and lower whiskers denote the largest and smallest values no further than 1.5 times the inter-quartile range.

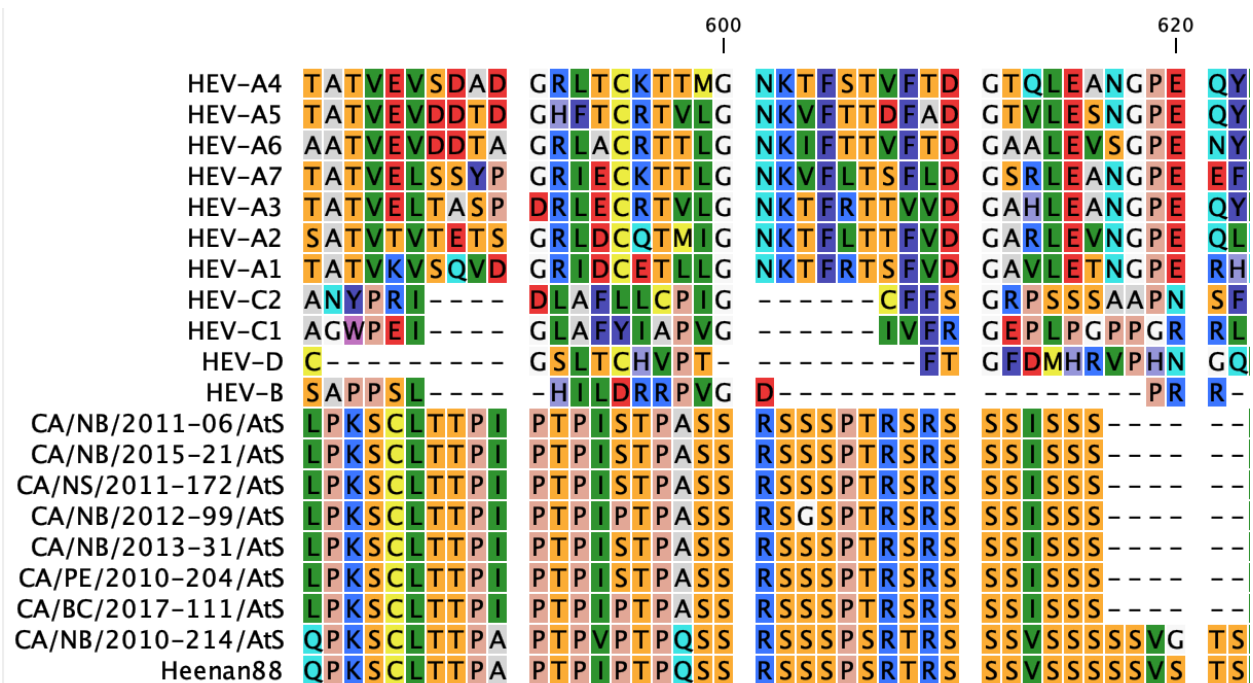


Figure S4. Amino acid alignment showing a serine rich region within the non-structural polyprotein of hepeviruses.