

Concentration of carp edema virus (CEV) DNA in koi tissues affected by koi sleepy disease (KSD)

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Fig. S1. DNA sequence alignment presenting the location of the primers and probe in relation to the CEV sequences obtained from common carp (Q030) and koi (Japan, CEV_504, M141).

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Q030      GCTTTTAGGAGGACAAGTAAAATTGCCATCAGTTCCAACAAGGAAAGCAATTGATTTTAC
Japan     ACTTTTAGGAGGACAAGTAAAGTTACCACCAGCTCCTACAAGGAAAGCAATTGATTTTAT
CEV_504   ACTTTTAGGAGGACAAGTAAAGTTACCACCAGCTCCTACAAGGAAAGCAATTGATTTTAT
M141     ACTTTTAGGAGGACAAGTAAAGTTACCACCAGCTCCTACAAGGAAAGCAATTGATTTTAT
Primer/probe TTTAGGAGGACAAGTAAAGTTACCACCAGCTCCTACAAGGAAAGCAATTGA
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Q030      ACTTGAAGAACATTCTAGAAGAGAAGAGAAATTCTCAAGAATTAGAATTGCAACTTCTAG
Japan     ACTTGAAGAACAATCTAGAAGATTGGAGAAATTCTCAAGAATTAGAATTGCAACTTCTAG
CEV_504   ACTTGAAGAACATTCAAGAAGATTAGAGAAATTTCAAGAATTAGAATTGCAACTTCTAG
M141     ACTTGAAGAACATTCAAGAAGATTAGAGAAATTCTCAAGAATTAGAATTGCAACTTCTAG
Primer/probe

Q030      TCTTTCCAATCTCTCTAGATTTAGATTTAGATTGGCATGTAAATAACTTGCAA
Japan     TCTCTCTAGTTTTTCTAGATTTAGATTTAGGTTGGCATCGAAATAACTTGCAAT
CEV_504   TCTCTCTAGTTTTTCTAGATTTAGATTTAGGTTGGCATCGAAATAACTTGCAAT
M141     TCTCTCTAGTTTTTCTAGATTTAGATTTAGGTTGGCATCGAAATAACTTGCAAT
Primer/probe GGTTGGCATCGAAATAACTTG
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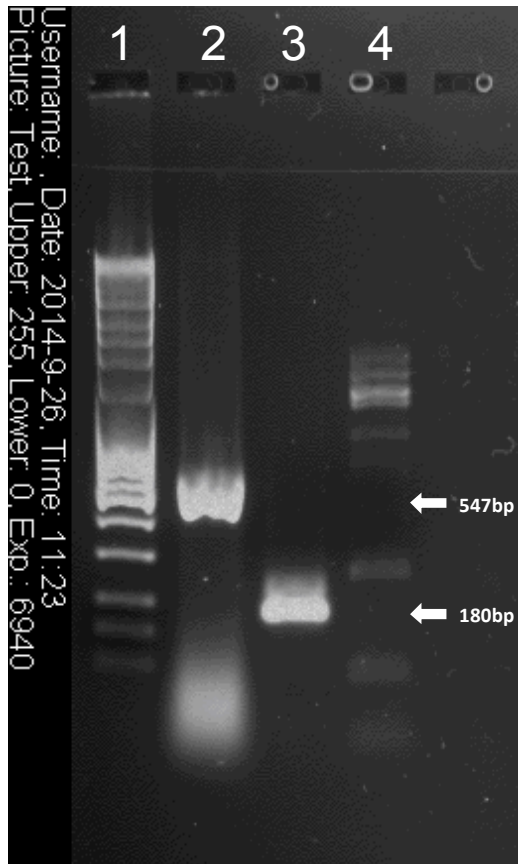


Fig. S2. Electrophoretic separation of PCR products amplified by using primers and PCR protocol described by Oyamatsu et. al. (1997). Lane 1 – DNA size marker, lane 2 – CEV_F1/R1 positive sample with a 547bp long product, lane 3 – nested CEV_F2/R2 positive sample with a 180bp long product, lane 4 - nested CEV_F2/R2 nonspecific amplification on CEV negative sample

Table S1. Repeated examination of samples by the newly developed qPCR. Shown are Ct values from selected positive samples measured with the qPCR in three independent reactions. The Ct, mean Ct and standard deviation (SD) of Ct values are presented per 250 ng of isolated DNA.

Fish	Run 1	Run 2	Run 3	Mean Ct	SD
2	22.84	22.22	22.19	22.42	0.37
8	37.16	37.58	36.66	37.13	0.46
10	32.39	32.02	32.22	32.21	0.19
11	32.48	32.73	33.72	32.98	0.66
14	37.83	37.74	37.93	37.83	0.10
15	20.21	20.23	20.62	20.35	0.23
18	20.54	19.96	19.13	19.88	0.71
25	32.24	31.81	31.29	31.78	0.48
26	26.98	26.53	26.43	26.65	0.29
27	37.11	36.45	36.14	36.57	0.50
28	29.38	29.36	28.61	29.12	0.44
29	35.64	35.2	36.75	35.86	0.80
30	36.22	38.83	36.23	37.09	1.50
32	23.09	23.54	22.09	22.91	0.74
33	25.04	24.55	24.98	24.86	0.27

Table S2. The Ct values and quantitative determination of the CEV load in tenfold diluted samples from Fish 1. Results are presented per 250 ng of isolated DNA.

Dilution	Ct	Copy number
10^{-1}	19.99	2.32E+05
10^{-2}	23.83	1.64E+04
10^{-3}	27.09	1.74E+03
10^{-4}	30.39	1.78E+02
10^{-5}	33.94	1.54E+01

LITERATURE CITED

Oyamatsu T, Matoyama H, Yamamoto K, Fukuda H (1997) A trial for detection of carp edema virus by using polymerase chain reaction. *Aquacult Sci* 45:247–251