

# Differences in pathogenesis of closely related environmental and clinical coxsackievirus B4 isolates

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Enteroviruses live in swarms displaying quasispecies dynamics, characterized by high rates of mutation and recombination. A high mutation rate is dangerous for a virus as it results in nonviable individuals, however, it has been hypothesized that high mutation rates create a “cloud” of potentially beneficial mutations at the population level, which allow the viral quasispecies a greater probability to evolve and adapt to new environments and different challenges during infections.

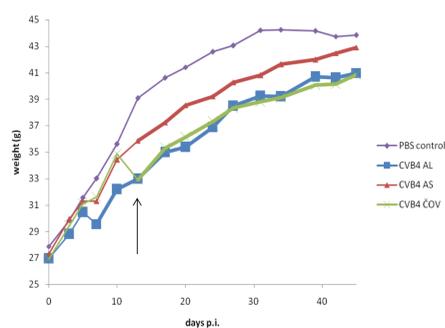
## Aim:

Our aim was to study the influence of intratypic virus strain variability on the course of infection in terms of pathogenesis, tissue and organ tropism, and possible persistent infection in relation to genetic variability of coxsackievirus strains isolated from different origins: environmental and clinical.

Outbred CD1 mice were infected with three isolates of coxsackievirus, serotype B4: (i) clinical isolate from the cerebrospinal fluid of a patient with diagnosed enteroviral meningitis – CVB4 AL, (ii) isolate from the stool of the same patient – CVB4 AS, (iii) environmental isolate from treated sewage waste – CVB4 COV. Mice were observed for mortality, morbidity and weights recorded for up to 45 days post infection (p.i.).

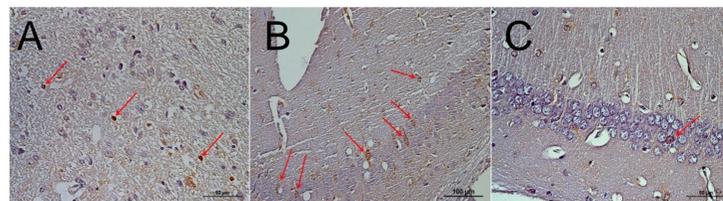
Organs (pancreas, heart, brain) and stool samples were collected from the mice at different days post infection. Presence of replicating virus, viral RNA and localization of the virus by immunohistochemical staining of VP1 protein in organ tissue sections was assessed. 5'NCR and VP1 regions were sequenced for comparing the original isolates and those isolated from different tissues of the infected mice. Morbidity, or signs of disease were absent, but differences in weight were observed during the course of experiment.

## Results

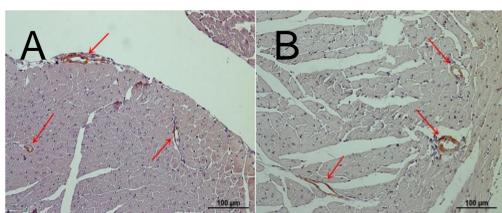


**Fig. 1: Weights of control and infected mice.**

Average weights of all mice groups are shown, statistically significant differences between infected and control groups are highlighted with bold lines. Arrow shows the statistically significant decrease in the weights of CVB4 COV-infected mice at day 13 p.i.

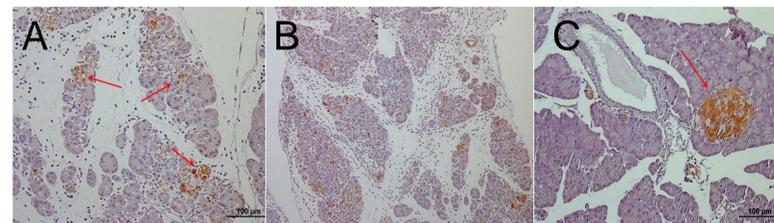


**Fig. 2: Immunohistochemical detection of VP1 protein in brains of mice.** Individual cells infected with CVB4 are highlighted with arrows. (A, B – CVB4 AL – infected mouse, day 10 p.i., C – CVB4 AL – infected mouse, day 5 p.i.)



**Fig. 3: Immunohistochemical detection of VP1 protein in hearts of mice.**

Blood vessels positive for the VP1 protein (arrows) can be observed. Cardiomyocytes are without any sign of infection. (A – CVB4 COV – infected mouse, day 5 p.i., CVB4 AL – infected mouse, day 10 p.i.)



**Fig. 4: Immunohistochemical detection of VP1 protein in pancreas of mice infected with CVB4 COV.**

Red arrows show virus in the exocrine and endocrine pancreas, mixed inflammatory infiltrate is visible. (A – day 5 p.i., B – day 10 p.i.) and detection of VP1 protein in pancreas of mice infected with CVB4 AS – C. Arrow shows the virus present in the islet of Langerhans. Weak positivity is visible in the blood vessels

		Percent Identity					
		1	2	3	4	5	
Divergence	1	100.0	99.2	88.4	86.5	1	CVB4 AL NCR
	2	0.0	100.0	99.2	88.4	86.5	2
	3	0.3	0.3	100.0	87.6	86.0	3
	4	12.8	12.8	13.2	100.0	92.9	4
	5	15.1	15.1	15.2	7.6	100.0	5
		1	2	3	4	5	

		Percent Identity					
		1	2	3	4	5	
Divergence	1	100.0	99.0	87.6	83.0	1	CVB4 AL VP1
	2	0.0	100.0	99.0	87.6	83.0	2
	3	1.0	1.0	100.0	87.9	83.5	3
	4	13.8	13.8	14.3	100.0	84.1	4
	5	19.5	19.5	20.0	19.5	100.0	5
		1	2	3	4	5	

**Fig. 5: Pair distances of 5'NCR (A) and VP1 (B) sequences of CVB4 AL, AS, COV isolates and E2 and JVB sequences.**

The distances were calculated using MegAlign software, ClustalW method.

## Results and conclusion

From day 7 p.i., the differences between weights in CVB4 AL-infected and control mice were statistically significant ( $P < 0,05$ ). The differences between CVB4 AS-, CVB4 COV- infected and control mice were statistically significant from the day 13 p.i. In addition, a small but significant decrease ( $P = 0,05$ ) in weights of mice infected with CVB4 COV isolate was observed at day 13 p.i. (fig. 1). The viral kinetics showed that CVB4 AL isolate had an affinity for the brain tissue and was detected in brain for up to 45 days. CVB4 AS isolate was detected in pancreases more than in brains and unlike other viruses; this was shedded in the stool for up to day 45 p.i. CVB4 COV was detected in pancreas of mice for up to day 45 p.i., this was the only isolate which induced acute pancreatitis in mice. Immunohistochemical analysis localised the environmental virus isolate in the acinar tissue as well as in islets of Langerhans, whereas clinical isolates were clearly detected only in islets of Langerhans (fig. 4). Sequence analysis of the isolates showed no differences between the CVB4 AL and CVB4 AS (clinical) isolates, neither in the 5'NCR, nor in the VP1 region. In the 5'NCR one difference was found between the CVB4 AL/AS and CVB4 COV isolates at position 573, three differences were found between the CVB4 AL/AS isolates and CVB4 COV isolate at positions 2660, 2711 and 2945 in the VP1 region.

In conclusion all three viruses showed differences among themselves and differed from the CVB4-E2 and prototype CVB4-JVB strains especially in the viral replication and histopathological changes in the organs after experimental infection (fig. 5).