

Phylogenetic analysis of an Echovirus 30 Outbreak in Austria, 2008



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Introduction and Purpose

- Tasks of the National Reference Centre for Poliovirus (NRCP) in Vienna is the nationwide AFP (acute flaccid paralysis) surveillance among children aged less than 15 years and the laboratory based surveillance of enterovirus (EV) infections in Austria.
- In 2008 there was an Echovirus 30 (E30) outbreak in children (mean age 8 years) with symptoms of aseptic meningitis.
- The aim of our investigation was to compare virus isolates gained from outbreak cases by phylogenetic analyses with other E30 strains found in Austria in the years 2000, 2001, 2002 and 2010 (arbitrarily selected).

Methods

- Real time RT-PCR (AnDiaTec®) for Screening: stool specimens from 9 cases and case related contact persons (n=731)
- Virus isolation in RD (A) cells: EV-PCR positive tested specimens (n=68), specimens revealed an unreliable EV-PCR result and arbitrarily selected EV-PCR negative specimens (n=66)
- Molecular analysis of the VP1 genome region (Nix et al.)
- Further genotyping using a 3D-polymerase-(3DPol) gene fragment (Leitch et al.)
- Viral RNA Extraction with QIAmp Viral RNA Mini kit (Qiagen)
- Sequencing analyses with ABI Prism 3130 (Applied Biosystems)
- Data interpretation using CLC workbench 5

Outbreak description

- 10.Sept.2008: Case 1 (male, 7a), son of football trainer, aseptic meningitis
- 12.Oct.2008: Football tournament
- 16.Oct.2008: Two cases of EV-aseptic meningitis (case 2 and 3), both exposure to football club
- 20.Oct 2008: one case with aseptic meningitis (case 4)
- 21.Oct 2008: one case with aseptic meningitis (case 5)
- 22.Oct 2008: one case with aseptic meningitis (case 6), sister of a football club member
- 23.Oct.2008: two cases with aseptic meningitis (case 7 and 8), start with surface cleaning and disinfection
- 27.Oct.2008: one case with aseptic meningitis (case 9)

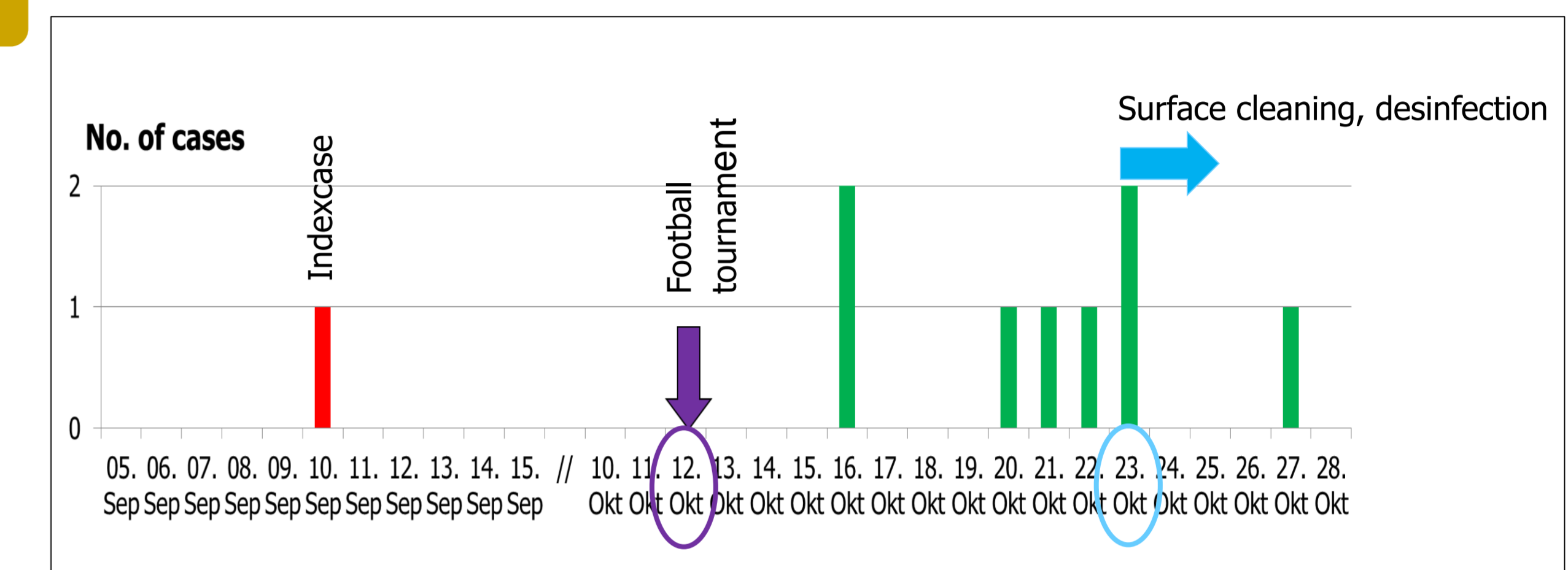


Figure 1: Cases by date of onset, outbreak of aseptic meningitis, Burgenland, 2008

Results

- 51 EV isolates (50 recovered from the EV-PCR positive specimens and one from a specimen with inhibited EV-PCR) were related to the E30-outbreak.
- Out of these isolates, 44 were identified by VP1 sequencing as E30 (86.3%), 3 as E25 (5,9%), 3 as Coxsackie A4 (5,9%) and one isolate as Non Polio EV (1,9%).
- The phylogenetic analyses of the E30 case isolates revealed a higher sequence similarity in the 3DPol region to E30 outbreak strains isolated in Finland in 2006 and 2007 (FJ538707.1, FJ538710.1) than to 6 E30 isolates found in 2000 (n=2), 2001 (n=1), 2002 (n=1) and 2010 (n=2) in Austria.

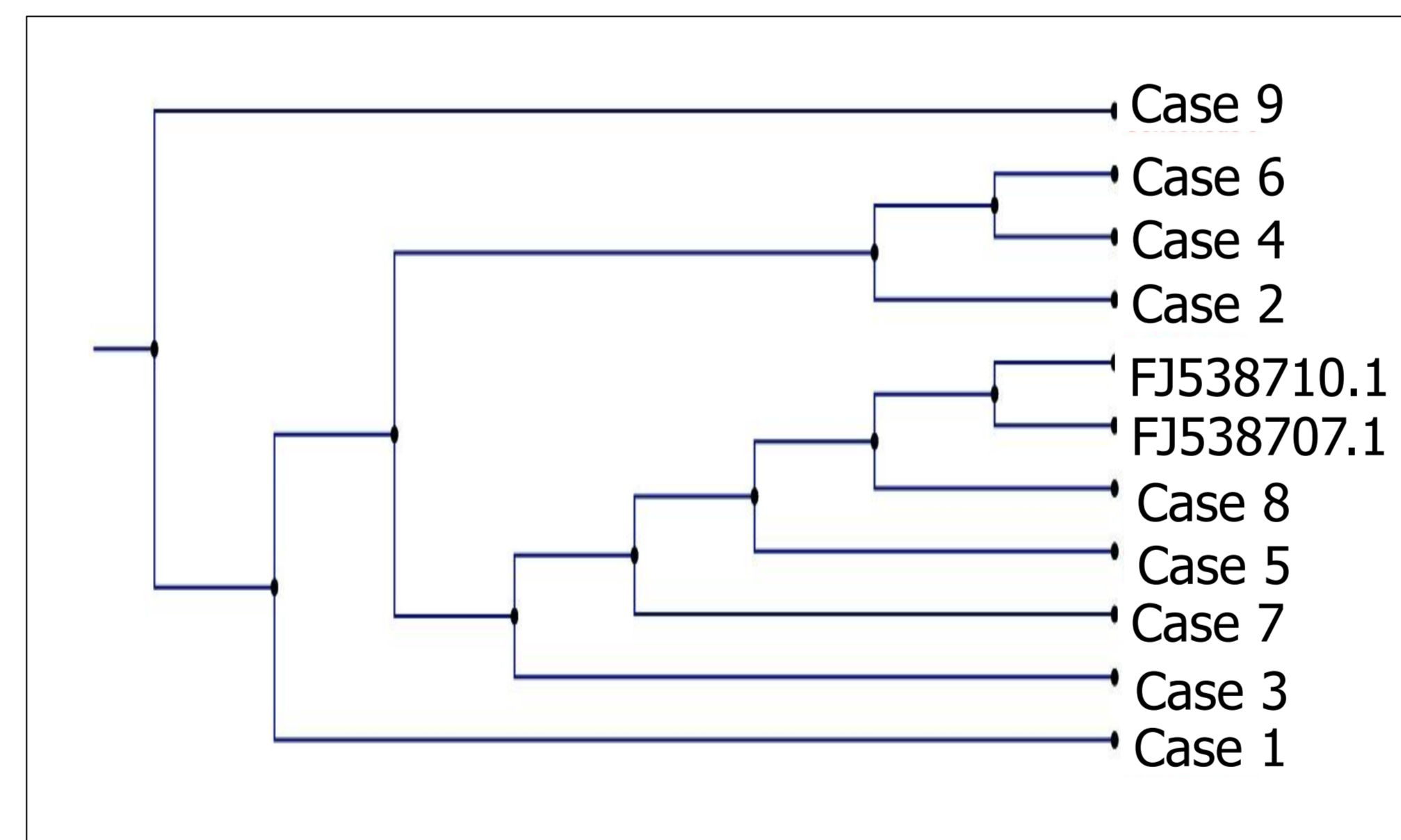


Figure 3: Maximum Likelihood phylogenetic tree, 3DPol region alignment, cases 1-9 and two E30 outbreak strains isolated in Finland 2006 and 2007 (FJ538710.1, FJ538707.1)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Case1	1	100,00	100,00	99,82	100,00	99,64	100,00	100,00	99,82	98,18	98,36	78,91	78,73	79,27	79,64	95,82	95,64
Case2	2	0	100,00	99,82	100,00	99,64	100,00	100,00	99,82	98,18	98,36	78,91	78,73	79,27	79,64	95,82	95,64
Case3	3	0	0	99,82	100,00	99,64	100,00	100,00	99,82	98,18	98,36	78,91	78,73	79,27	79,64	95,82	95,64
Case4	4	1	1	1	99,82	99,82	99,82	99,82	99,64	98,00	98,18	78,73	78,55	79,09	79,45	95,64	95,45
Case5	5	0	0	0	1	99,64	100,00	100,00	99,82	98,18	98,36	78,91	78,73	79,27	79,64	95,82	95,64
Case6	6	2	2	2	1	2	99,64	99,64	99,46	98,00	98,18	78,73	78,55	79,09	79,45	95,64	95,45
Case7	7	0	0	0	1	0	2	100,00	99,82	98,18	98,36	78,91	78,73	79,27	79,64	95,82	95,64
Case8	8	0	0	0	1	0	2	0	99,82	98,18	98,36	78,91	78,73	79,27	79,64	95,82	95,64
Case9	9	1	1	1	2	1	3	1	1	98,00	98,18	79,09	78,91	79,27	79,82	95,64	95,45
FJ538707.1	10	10	10	10	11	10	11	10	11	99,82	78,55	78,36	78,91	79,27	96,55	96,36	
FJ538710.1	11	9	9	9	10	9	10	9	10	1	78,73	78,55	79,09	79,45	96,73	96,55	
E30/1/2000	12	116	116	116	117	116	117	116	115	118	117	99,64	98,36	97,64	78,36	78,73	
E30/2/2000	13	117	117	117	118	117	118	117	116	119	118	2	98,00	97,64	78,18	78,55	
E30/2001	14	114	114	114	115	114	115	114	114	116	115	9	11	96,73	78,73	79,09	
E30/2002	15	112	112	112	113	112	113	112	111	114	113	13	13	18	79,09	79,49	
E30/1/2010	16	23	23	23	24	23	24	23	23	24	19	18	119	120	117	115	97,27
E30/2/2010	17	24	24	24	25	24	25	24	24	25	20	19	117	118	115	113	15

Figure 4: Pairwise comparison (3DPol region) of cases 1 -9, two E30 outbreak strains isolated in Finland 2006, 2007 and six E30 isolates found in 2000 (n=2), 2001 (n=1), 2002 (n=1) and 2010 (n=2) in Austria. Upper comparison: percent identity, lower comparison: differences in sequence

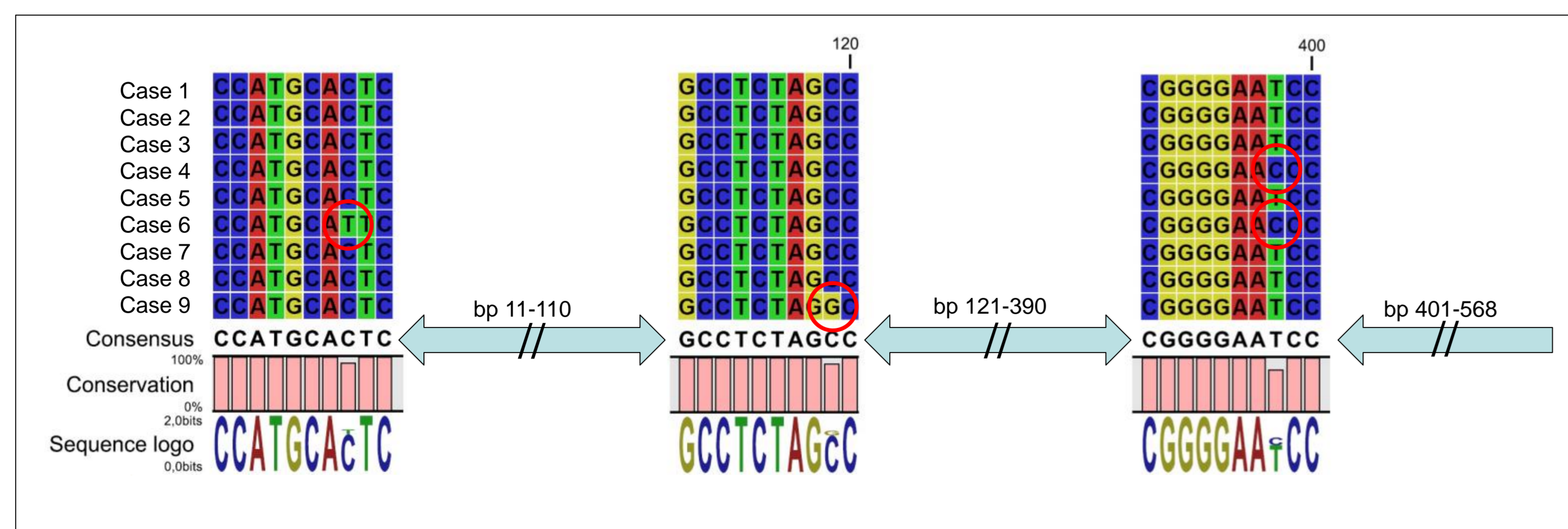


Figure 2: 3DPol region alignment, cases 1 -9, only distinctions in sequence comparison shown

Conclusions

- Our investigation in connection with the E30-outbreak in 2008 showed that the 3DPol region of the genome of EV strains circulating in Austria remained highly conserved.
- There seems to be a higher 3DPol region sequence similarity in outbreak related isolates than in free circulating strains, which are related neither in time nor in space.
- Further investigations may be helpful to get more information about virulence and relationship between the virus strains of outbreaks and epidemiologically unrelated strains. The planned whole genome sequencing may give us more insights.

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