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1 **Abstract**

2 *Usutu virus* has been causing avian mortality in Austria since its emergence in 2001.
3 Between 2003 and 2005 a total of 504 dead birds were examined by reverse-transcriptase
4 polymerase chain reaction and immunohistochemistry for the presence of *Usutu virus*
5 nucleic acid and antigen, respectively. In 2003, 92 birds (out of 177 birds) belonging to 5
6 different species were positive, while in 2004, only 11 (of 224) birds, and in 2005, 4 (of
7 103) birds proved positive, all of which were blackbirds (*Turdus merula*). Within the
8 surveillance period the virus had spread from its initial area of emergence and circulation,
9 the surroundings of Vienna, to large areas of the federal states of Lower Austria,
10 Burgenland and Styria. However, the absolute numbers of *Usutu virus* associated avian
11 deaths declined significantly during the course of the years. In addition, the proportion of
12 birds with low amounts of virus in their tissues increased continuously, which may indicate
13 developing herd immunity.

14

15 **Keywords:** *Usutu virus*; USUV; Austria; surveillance; avian mortality

16

1 **1. Introduction**

2

3 *Usutu virus* (USUV), a flavivirus of the Japanese encephalitis virus complex (Kuno
4 et al., 1998; Bakonyi et al., 2004) was for the first time identified and isolated outside
5 Africa in late summer of 2001 (Weissenböck et al., 2002). The virus was found to be
6 responsible for an episode of mortality among blackbirds (*Turdus merula*) and great grey
7 owls (*Strix nebulosa*) in and around Vienna, Austria. In the subsequent year, 2002, the
8 virus continued to kill birds in eastern Austria (Weissenböck et al., 2003a). The USUV-
9 associated bird mortality in two consecutive summers as well as the detection of the virus
10 in mosquitoes showed that USUV had managed to overwinter and had been able to
11 establish an efficient local bird-mosquito transmission cycle (Weissenböck et al., 2003b).
12 Based on pathological observations from the 2001 and 2002 USUV-affected birds, the
13 disease has been characterized by encephalitis, myocardial degeneration, and necrosis in
14 liver and spleen (Chvala et al., 2004). USUV has also spread to other European countries
15 (T. Bakonyi and G. Dorrestein, unpublished results) and recently, evidence for USUV-
16 associated bird mortality in Switzerland has been provided (ProMED, 2006).

17 Because the emergence of USUV in Austria provided a unique opportunity to study
18 the further development of the activity of a mosquito-borne virus in a previously
19 unexposed eco-system and a naïve host population, we established a surveillance system
20 which was maintained for a period of three consecutive years (2003-2005). Dead bird
21 surveillance has already been successfully applied for monitoring of *West Nile virus*
22 activity in North America (Eidson et al., 2001; Guphill et al., 2003; Mostashari et al., 2003;
23 Glaser, 2004; Public Health Agency of Canada, 2006). The present survey identifies the
24 geographical distribution of infected birds and the most frequently affected bird species,
25 reveals marked differences of USUV-associated bird mortality in the three seasons

1 investigated, and also demonstrates changes in the severity of pathological lesions and
2 viral loads of positive birds during the course of the years. In addition, sequencing results
3 of USUV amplification products of bird origin are presented in order to determine whether
4 one or more strains of USUV occur in Austria.

6 **2. Materials and methods**

8 *2.1. Organization of dead bird monitoring*

9 The goals of the surveillance program were 1) to gather information on local
10 episodes of avian mortality and to form an impression of the dimension of such
11 phenomena, 2) to have access to a representative number of dead birds from different areas
12 and different species for laboratory investigation, 3) to evaluate fluctuations in the
13 prevalence and the spatiotemporal distribution of cases of USUV-associated avian deaths
14 during several consecutive seasons, and 4) to evaluate, whether or not dead bird
15 surveillance is generally a useful tool for estimating USUV activity. According to the
16 experiences of 2001 and 2002, USUV-associated bird mortality can only be expected
17 between mid-July and mid-September (Weissenböck et al., 2002, 2003a). Thus, the active
18 surveillance period was restricted to the summer months July to September. The focus of
19 the surveillance program was the eastern part of Austria, because previously USUV-
20 associated bird deaths had only been noticed in this area. However, the surveillance also
21 included certain "outposts" in order to identify a more rapid spread than anticipated.

22 The surveillance program relied strongly on the participation of the general public
23 which was requested by mass media advises to collect and submit dead birds to local
24 collection centers usually installed in the headquarters of the district veterinarians. The
25 political districts included in the surveillance program were updated each year according to

1 the previous year of USUV activity and are shown in Fig. 1. The collected bird carcasses
2 were periodically shipped to the University of Veterinary Medicine Vienna for
3 examination. The locations of discovery of dead birds were geo-referenced on a scale
4 represented by cities using the Austrian national database of cities and inserted into maps
5 applying the generic mapping tools (GMT), an open source collection of tools for
6 processing geographic data sets (Wessel and Smith, 2006).

8 *2.2. Laboratory procedures*

9 The submitted dead birds were sorted by species, necropsied, and tissue samples
10 were fixed in 7% neutral buffered formalin for subsequent histological and
11 immunohistochemical examinations. Following embedding in paraffin wax, tissue sections
12 of brain, heart and spleen were subjected to immunohistochemistry (IHC) with the avidin-
13 biotin complex (ABC) technique using a rabbit USUV antiserum as primary antibody
14 (Weissenböck et al., 2004).

15 A portion of brain tissue was stored frozen at -20°C and was subsequently used for
16 reverse-transcriptase polymerase chain reactions (RT-PCR) to detect flavivirus-specific
17 nucleic acid. Brain samples were homogenized and viral RNA was isolated as described
18 previously (Weissenböck et al., 2002). Each sample was subjected to two independent RT-
19 PCR assays, employing Japanese encephalitis virus complex specific primers
20 (WNV10090f and WNV10807r) (Bakonyi et al., 2004), and USUV specific primers
21 (according to Weissenböck et al., 2004).

22 Selected positive samples were further investigated to compare the sequence
23 variance of viruses detected in different years and different geographic locations. Because
24 the E protein is the main surface protein of USUV and is most importantly and commonly
25 neutralized, it can be subjected to selective pressure of antibodies, and therefore its gene is

1 suspected to be more variable. Two USUV-specific primer pairs (Usu1155f and Usu1600r,
2 as well as Usu1537f and Usu2505r) (Bakonyi et al., 2004) producing overlapping
3 amplification products in the E protein coding region, were employed in RT-PCR
4 reactions. The amplicons were directly sequenced in both directions, compiled, and aligned
5 to each other as well as to the USUV Vienna strain isolated in 2001, and to the reference
6 SAAR-1776 strain isolated in South Africa in 1958.

7

8 **3. Results**

9

10 *3.1. Dead bird testing*

11 In the three-year surveillance period, a total of 107 birds (of 504) were found
12 USUV-positive by RT-PCR and/or by IHC. In 4 of these birds the diagnosis was based on
13 IHC alone and in 3 cases only RT-PCR was performed. In 100 positive birds both tests
14 were simultaneously applied. In 99 of these birds, both RT-PCR and IHC gave a
15 correspondingly positive result; only one bird was RT-PCR-positive and negative by IHC.
16 The vast majority of the investigated birds originated from the defined surveillance areas
17 (Figs. 2 and 3, Table 1). However, several birds were also submitted from other areas,
18 leading to detection of single cases outside the surveillance areas (2003: one bird each
19 from the districts Mistelbach, St. Pölten, Bruck a. d. Leitha, Eisenstadt and Oberpullendorf;
20 2004: one bird from the district Amstetten).

21 In 2003, a total of 177 birds of 27 classifiable different species (Table 1) found in
22 the federal states of Vienna, Lower Austria, Burgenland, Styria, Carinthia and Vorarlberg
23 were tested for USUV. 52% (92) tested positive, consisting of 87 blackbirds (*Turdus*
24 *merula*), 2 great tits (*Parus major*), 1 nuthatch (*Sitta europaea*), 1 song thrush (*Turdus*
25 *philomelos*) and one robin (*Erithacus rubecula*). The first USUV-positive bird was found

1 on July 13 and the last one on September 23. The count of USUV-positive dead birds
2 reached its peak in mid August (August 18-24, week 34) with 22% of the total (Fig. 4).

3 In 2004, 224 dead birds belonging to 30 species, found in the federal states of
4 Vienna, Lower Austria, Upper Austria, Burgenland, Styria and Tyrol were investigated.
5 Only 11 blackbirds (5%) tested positive (Table 1). The first USUV-positive bird was found
6 on July 31 and the last one on September 21. The majority of positive birds (37%) were
7 found in week 34 (August 16-22) (Fig. 4).

8 In 2005, 103 dead birds of 19 species from Vienna, Lower Austria, Burgenland,
9 Styria, Salzburg, Upper Austria and Carinthia were investigated. No more than 4
10 blackbirds found dead in Vienna (within two areas approximately 10 km apart) were found
11 to be USUV-positive (Table 1). Three of 4 blackbirds (75%) were positive in week 30
12 (July 25-31) (Fig. 4).

13

14 3.2. *Viral distribution in USUV-positive birds*

15 Viral distribution was assessed by IHC in the tissues most frequently affected by
16 USUV infection (Chvala et al., 2004).

17 Of the 107 USUV-positive birds, IHC data of brain from 101 birds, of spleen from
18 75, and of heart from 71 birds were evaluated. 92% of the tested brains, 83% of the tested
19 spleens, and 76% of the tested hearts were positive. In the majority of the tested organs, the
20 amount of positive signals ranged from low to moderate. Large amounts of viral antigen
21 were present in only a comparably low number of birds. In the blackbirds, positive signals
22 were found in 93 % of the examined brains, 86 % of the examined spleens and 78 % of the
23 examined hearts. Both positive great tits exhibited few positive neurons in the brain, no
24 positive signal in the heart, and one bird showed single positive cells in the spleen capsule.
25 The song thrush showed numerous positive cells in the brain, the spleen showed no

1 positive signal. Heart was not examined. The nuthatch showed few positive cells focally in
2 the brain, in the heart and in the spleen capsule. In the robin single positive cells were seen
3 only in the heart.

4 5 *3.3. Nucleotide sequence comparisons of selected USUV strains*

6 To reveal the genetic relatedness between the USUVs detected in three consecutive
7 years, 12 positive bird samples were selected for sequence comparisons. Four samples had
8 been collected in 2003 (from Vienna [W], Strasshof [GF], Neunkirchen [NK], and
9 Stegersbach [GS]); three in 2004 (from Graz [G], Biberbach [AM], and Klosterneuburg
10 [WU]), and four in 2005 (from Vienna [W]). The partial nucleotide sequence of the 12
11 USUV samples was determined between nucleotide (nt) positions 1159 and 2505, covering
12 88% of the E protein coding region (nt 976 to 2475) (Bakonyi et al., 2004). The sequences
13 exhibited 99.7 to 100% identity to each other and to the Vienna 2001 strain, and 95.9 to
14 96.2% identity to the South African reference strain SAAR-1776. Mutations were separate,
15 single nucleotide substitutions (Table 2). The nucleotide sequences were translated to
16 putative amino acid sequences. The deduced amino acid sequences were 100% identical to
17 each other and to the Vienna 2001 strain except for three identical 2005 Vienna samples, in
18 which one amino acid substitution (Met₄₁₉ to Val) was observed. The Austrian USUV
19 samples showed 98.9 to 99.1% amino acid identity to the SAAR-1776 strain.

20 21 **4. Discussion**

22
23 The presented data show that USUV has become a resident pathogen in central
24 Europe, and here predominantly in eastern Austria, which was the surveillance focus of
25 this study. The most remarkable trends were a peak of viral activity in late summer 2003,

1 resulting in considerable blackbird mortality, and a subsequent decline of USUV-
2 associated bird losses in the following summers of 2004 and 2005. We are fully aware that
3 only a fraction of birds dying in the wild are noticed by the public and still only a small
4 proportion of those are collected and made available for laboratory diagnosis. It is not
5 possible to give an estimation of regional USUV-associated bird losses based on such data.
6 However, the fact that a comparable number of dead birds in two consecutive years (2003
7 and 2004) from a largely similar area were analyzed clearly demonstrates that the
8 significant drop of the proportion of USUV-positives is factual and not biased. The design
9 of the surveillance program focusing on eastern Austria, the area of first emergence of
10 USUV in Europe, leaves the possibility of missing cases outside the area. Single additional
11 positive cases had been found in areas outside the surveillance zones, both, in 2003 and
12 2004. On the other hand we do not consider it very likely that significant virus activity was
13 missed, especially when looking at data from the "outpost" Graz in 2003, where despite a
14 considerable number of submissions no positives were found. Nonetheless, a more
15 widespread distribution of USUV cannot be categorically excluded, an assumption which
16 has recently received final proof by diagnosis of USUV-associated bird mortality in
17 Switzerland (ProMED, 2006). In the UK circulation of USUV in the wild bird population
18 has been claimed based on serological data (Buckley et al., 2003, 2006). However, except
19 for limited serosurveys in the UK, nowhere else in Europe as in Austria are, according to
20 our knowledge, surveillance programs in place. In the surveillance seasons 2004 and 2005
21 USUV-activity presenting as avian mortality significantly declined. Without active
22 surveillance it would most likely have been overlooked.

23 Diagnosis of USUV infections was achieved with two different reliable laboratory
24 procedures, RT-PCR and IHC. In 99% of the cases the results generated with both methods
25 were concordant, indicating an excellent correlation and a high specificity and sensitivity

1 of both techniques. One has to keep in mind that RT-PCR is very sensitive allowing
2 detection of traces of nucleic acid, but does not provide information whether the bird
3 necessarily died due to USUV infection. The additionally used IHC, however, gives
4 information on the amount and the cellular localization of viral antigen as well as its
5 association with tissue lesions, and may thus be used for estimation whether the USUV
6 infection was the cause of death or only accidentally present in a bird which died due to
7 another reason. While in the first 2 years of the epizootic in Austria the majority of the
8 investigated blackbirds had high amounts of viral signals and associated severe lesions in
9 their tissues (Chvala et al., 2004), we found an increasing proportion of birds with very
10 limited viral signals in certain tissues in this study. This trend could be explained by the
11 fact that viral replication was counteracted by a pre-existing immunity or that these birds
12 were in a more advanced stage of infection with immune-mediated clearance mechanisms
13 in action. Maybe both the reduction of USUV-positives within the total of dead birds as
14 well as the reduction of viral load among the positives reflect the situation of developing
15 herd immunity. This phenomenon could lead to a cyclic pattern of disease emergence in
16 the future, as noticed with other vector-borne viruses, e.g. *Rift Valley fever virus* (Chevalier
17 et al., 2004) or *Sindbis virus* (Brummer-Korvenkontio et al., 2002).

18 An alternative explanation for the decline of virus-positive birds might be climate
19 differences between the investigated years (Zentralanstalt für Meteorologie und
20 Geodynamik, 2006). The summer of 2003 had been extraordinarily hot and dry in Austria
21 while the two consecutive summers were more average with much more precipitation and
22 no longer periods of continuous high temperatures. It has been experimentally proven that
23 the extrinsic incubation period of flaviviruses in *Culex* mosquitoes is temperature
24 dependent (Turell et al., 2002). Thus it is not unlikely that the climate in the years 2004
25 and 2005 with lower average temperatures (Zentralanstalt für Meteorologie und

1 Geodynamik, 2006) significantly affected the transmission rate of USUV from mosquitoes
2 to birds by increasing the extrinsic incubation period beyond the average activity period of
3 the susceptible mosquitoes. However, preliminary serological data from wild birds point to
4 an increasing number of seropositives in these years (Weissenböck et al., 2006), which is
5 indicative of continuing transmission of the virus between birds and mosquitoes. This
6 observation makes herd immunity the more likely explanation for the conspicuous decline
7 of USUV-associated bird mortality.

8 As already noted, there is a theoretical possibility that USUV has been present for a
9 longer time and in a wider area in Europe and has gone unnoticed because of lack of any
10 flaviviral surveillance (Buckley et al., 2003). Also, USUV strains with differences in
11 pathogenicity could have been repeatedly introduced into Europe by migratory or imported
12 exotic birds. Neither hypothesis can be supported by the present sequencing data. Repeated
13 introductions of different strains, as well as the presence of a single strain for a longer time
14 period would result in a much higher nucleotide sequence diversity of the sequenced
15 viruses. Similarly, WNV in the Americas has developed low level of sequence diversity,
16 within four years after its emergence, compared to the diversity of WNV observed in the
17 Old World (Lanciotti et al., 2002; Ebel et al., 2004).

18 USUV surveillance based on dead bird investigations has been a useful method in
19 the years of the beginning epidemic, i.e. 2001 – 2003. In these years a rather high
20 proportion of the submitted birds were USUV-positive, showing the importance of this
21 infection as cause of death and allowing making assumptions of the presence or absence of
22 the virus in a certain region based on a rather low sample size. In the following years the
23 power of dead bird surveillance as indicator of USUV activity in certain areas was severely
24 weakened due to the substantially decreased percentage of USUV-positive dead birds. In
25 order to make any statistically significant statement, the number of submissions would

1 need to be strongly increased in the future. This is not a feasible approach. Thus, if USUV
2 surveillance is desired in the future, the dead bird surveillance method, which proved very
3 useful at the beginning of the epidemic, needs to be replaced by alternative surveillance
4 tools, such as serological screenings of wild birds or mosquito monitoring. Serological
5 approaches with the currently available techniques (plaque reduction neutralization test and
6 hemagglutination inhibition test) only reveal previous exposure to the virus without usually
7 giving information of the time point when it took place. However, certain strategies, e.g.
8 only including birds hatched within the season of interest (Nasci et al., 2002), should be
9 able to provide information on current USUV activity within a certain region.

10

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8 The nucleotide sequences determined in this study have been deposited in GenBank
9 database under the accession numbers EF078294 - EF078301 and EF393679 - EF393681.
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1 **Figure legends**

2

3 **Fig. 1.** Maps of Austria showing the location of all federal states and the location of the
4 political districts which were included into the USUV surveillance program from 2003 to
5 2005. Districts where USUV activity had been noticed in one of the previous seasons are
6 filled in red, districts without previous USUV activity are filled in green. Abbreviations of
7 federal states: Vb: Vorarlberg; T: Tyrol; S: Salzburg; C: Carinthia; U: Upper Austria; L:
8 Lower Austria; V: Vienna; St: Styria; B: Burgenland.

9

10 **Fig. 2.** Map of Austria including labelling of the political districts in which USUV-positive
11 birds were found during the 3-year surveillance period. The rectangle marks the area which
12 is shown in Figure 3.

13

14 **Fig. 3.** Map showing the north-eastern part of Austria (as indicated in Figure 2) including
15 all areas where at least one USUV-positive bird had been found. The circles show the
16 geocoded finding places of dead birds during 3 consecutive surveillance seasons. White
17 circles stand for USUV-negative birds of all species, red circles for positive blackbirds,
18 green circles for positive great tits, the yellow circle for the positive nuthatch, and the blue
19 circle for the positive robin.

20

21 **Fig. 4.** Comparison of the number of tested vs. USUV-positive birds, weekly breakdown,
22 during 3 consecutive surveillance seasons.

Table 1

List of birds examined for USUV-infection in Austria 2003-2005. Of species, which had at least one positive bird the districts of origin and the proportion of positives per season are given. Of species of which no bird tested positive the numbers of examined individual birds are given at the end of the table

Bird species	Federal state	District	District abbreviation	Year			Total	
				2003	2004	2005		
Blackbird	Vienna	Vienna	W	20 ^a /16 ^b	14/1	17/4	51/21	
		Lower Austria	Bruck a.d. Leitha	BL	1/1	0/0	1/0	2/1
			Mistelbach	MI	1/1	10/0	25/0	36/1
			Wr. Neustadt	WB	9/8	7/0	2/0	18/8
			Baden	BN	16/14	10/1	2/0	28/15
			Mödling	MD	4/2	1/0	0/0	5/2
			St. Pölten	PL	1/1	8/0	0/0	9/1
			Gänserndorf	GF	22/18	19/2	6/0	47/20
			Neunkirchen	NK	8/7	11/2	1/0	20/9
			Korneuburg	KO	7/2	0/0	1/0	8/2
			Tulln	TU	7/6	2/0	0/0	9/6
			Wien Umgebung	WU	7/5	7/2	4/0	18/7
			Schwechat	SW	1/1	0/0	0/0	1/1
			Amstetten	AM	0/0	1/1	0/0	1/1
		Burgenland	Güssing	GS	3/1	3/1	0/0	6/2
			Eisenstadt	EU	2/2	1/0	0/0	3/2
			Oberpullendorf	OP	2/2	2/0	0/0	4/2
	Styria	Graz	G	16/0	16/1	2/0	34/1	
	other ^c			6/0	37/0	8/0	51/0	
Great tit	Vienna	Vienna	W	2/1	5/0	0/0	7/1	
	Lower Austria	Gänserndorf	GF	1/1	0/0	0/0	1/1	
	other ^c			0/0	2/0	1/0	3/0	
Nuthatch	Lower Austria	Neunkirchen	NK	1/1	0/0	0/0	1/1	
Robin	Lower Austria	Baden	BN	1/1	0/0	0/0	1/1	
	other ^c			1/0	1/0	0/0	1/0	
Song thrush	unknown	unknown		1/1	0/0	0/0	1/1	
	other ^c			9/0	12/0	8/0	29/0	
Different ^d	other ^c			29/0	55/0	25/0	109/0	
				177/92	224/11	103/4	504/107	

^a Total birds examined

^b USUV positive birds

^c Birds from various districts, all negative for USUV

^d Different species (in brackets: number) of which all examined birds tested negative for USUV: House sparrow (*Passer domesticus*) (13); Blue tit (*Parus caeruleus*) (11); Dove (*Columba sp.*) (7); Pheasant (*Phasianus colchicus*) (4); Avocet (*Recurvirostra avosetta*) (4); Common swift (*Apus apus*) (4); Great spotted woodpecker (*Picoides major*) (4); Sand martin (*Riparia riparia*) (3); Warbler sp. (*Acrocephalus sp.*) (3); Black redstart (*Phoenicurus ochruros*) (3); White-faced whistling duck (*Dendrocygna viduata*) (2); Quail (*Coturnix coturnix*) (2); Lovebird sp. (*Agapornis sp.*) (2); King fisher (*Alcedo atthis*) (2); Tree sparrow (*Passer montanus*) (2); Swallow (*Hirundo rustica*) (2); Pied flycatcher (*Ficedula hypoleuca*) (2); Black-fronted white-eye (*Zosterops*

xanthochrous) (2); Yellowhammer (*Emberiza citrinella*) (2); Greenfinch (*Carduelis chloris*) (2); Starling (*Sturnus vulgaris*) (2); Jay (*Garrulus glandarius*) (2); Penguin (*Spheniscus sp.*) (1); White stork (*Ciconia ciconia*) (1); Flamingo (*Phoenicopterus ruber*) (1); Kestrel (*Falco tinnunculus*) (1); Capercaillie (*Tetrao urogallus*) (1); Caspian gull (*Larus cachinnans*) (1); Zebra dove (*Geopelia striata*) (1); Tawny owl (*Strix aluco*) (1); Long-eared owl (*Asio otus*) (1); House martin (*Delichon urbica*) (1); White wagtail (*Motacilla alba*) (1); Citrine wagtail (*Motacilla citreola*) (1); Red-backed shrike (*Lanius collurio*) (1); Hedge sparrow (*Prunella modularis*) (1); Blackcap (*Sylvia atricapilla*) (1); Coal tit (*Parus ater*) (1); Corn bunting (*Emberiza calandra*) (1); Violetaceous euphonia (*Euphonia violacea*) (1); Goldfinch (*Carduelis carduelis*) (1); Hawfinch (*Coccothraustes coccothraustes*) (1); Green-backed twin-spot (*Mandingoa nitidula*) (1); Golden oriole (*Oriolus oriolus*) (1); Rook (*Corvus frugilegus*) (1); Hooded crow (*Corvus corone cornix*) (1); Species not determined (5).

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Table 2. Nucleotide substitutions in the partial E protein coding region of Austrian Usutu viruses collected between 2001 and 2005

USUV Strain	Nucleotide position*													
	1209	1242	1251	1335	1351	1458	1470	1554	1731	1771	1785	2073	2121	2466
Vienna 2001	g	g	c	c	a	a	g	a	t	t	t	c	c	c
Vienna 2003	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gänsersdorf 2003	-	a	-	-	-	-	-	-	-	-	-	-	-	-
Neunkirchen 2003	-	-	-	-	-	-	-	-	-	-	-	t	-	-
Stegersbach 2003	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Klosterneuburg 2004	-	-	-	-	-	-	-	-	-	c	c	-	t	t
Graz 2004	-	-	-	-	-	-	-	g	-	-	-	-	-	-
Biberbach 2004	-	-	-	t	-	-	-	-	-	-	-	-	-	-
Vienna 2005a	a	-	t	-	gc	-	-	-	c	-	-	-	-	-
Vienna 2005b	a	-	t	-	gc	-	-	-	c	-	-	-	-	-
Vienna 2005c	a	-	t	-	gc	-	-	-	c	-	-	-	-	-
Vienna 2005d	-	-	-	-	-	g	a	-	-	-	-	-	-	-

* Nucleotide positions refer to the complete genome sequence of USUV Vienna 2001 (GenBank accession number: AY453411). Nucleotide substitution resulting in substitution of the deduced amino acid sequence is indicated in **bold**.



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