

West Nile and Usutu Virus Infections and Challenges to Blood Safety in the European Union

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West Nile virus (WNV) and Usutu virus (USUV) circulate in several European Union (EU) countries. The risk of transfusion-transmitted West Nile virus (TT-WNV) has been recognized, and preventive blood safety measures have been implemented. We summarized the applied interventions in the EU countries and assessed the safety of the blood supply by compiling data on WNV positivity among blood donors and on reported TT-WNV cases. The paucity of reported TT-WNV infections and the screening results suggest that blood safety interventions are effective. However, limited circulation of WNV in the EU and presumed underrecognition or underreporting of TT-WNV cases contribute to the present situation. Because of cross-reactivity between genetically related flaviviruses in the automated nucleic acid test systems, USUV-positive blood donations are found during routine WNV screening. The clinical relevance of USUV infection in humans and the risk of USUV to blood safety are unknown.

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West Nile virus (WNV) and Usutu virus (USUV) are arthropod-borne flaviviruses that belong to the Japanese encephalitis serocomplex. Their natural life cycle involves ornithophilic mosquitoes (predominantly *Culex* spp.) as vectors and birds as amplifying hosts. Mammals, including horses and humans, may act as accidental hosts. In temperate climate zones, WNV and USUV usually circulate from late spring to mid-autumn, when competent mosquitoes are active.

WNV was detected in Europe in 1958 (1). Among its 9 evolutionary lineages (2), WNV lineages 1 and 2 are the most notable. Most recent outbreaks of WNV infections in the European Union (EU) are caused by the central European lineage 2 WNV, which was introduced to southeastern Hungary in 2004 (3) and has spread to other countries in Europe since 2008 (4,5). Another (eastern European) lineage 2 WNV was independently introduced to the Volgograd area of Russia at approximately the same time (6) and spread from there to Romania (7) and Italy (8). In addition, lineage 1 WNV strains are still circulating in Europe, partly overlapping with lineage 2 strains (8,9). Around 80% of WNV infections in humans are asymptomatic (9,10). Most clinical WNV infections present with mild, influenza-like symptoms, and ≈1% of infected persons, most often the elderly or immunosuppressed, develop West Nile neuroinvasive disease (WNND), which causes variable rates of illness and death (9,10).

In humans, transmission of WNV through blood transfusion, organ transplantation, breast-feeding, and intrauterine means has been reported (11–14). WNV poses a risk to blood safety because an asymptomatic donor may donate infectious blood, which, if transfused, may cause a serious clinical illness in the recipient. To mitigate such risk, EU countries apply measures described in the EU Directives (15), professional guidelines (16), and the European Centre for Disease Prevention and Control (ECDC) coordinated blood safety preparedness plan (17). As part of these actions, blood banks should apply blood safety measures

directed to donors residing in or visiting areas affected by WNV (17). An area with ≥ 1 confirmed case of autochthonous WNV transmission to humans is considered affected (18). To support EU countries in applying legislation related to travelers visiting affected areas, the ECDC has developed web-based maps indicating areas at Nomenclature of Territorial Units for Statistics (NUTS) level 3 in the EU where confirmed cases of WNV infections in humans have been reported (19).

USUV is a mosquito-borne flavivirus closely related to WNV that was reported in Europe as a cause of death in birds (mainly blackbirds) in and around Vienna, Austria, in 2001 (20). It was also retrospectively identified as the etiologic agent of blackbird die-off in the Tuscany region of Italy in 1996 (21). Since then, the virus has spread across Europe (22,23). Clinically manifested cases of USUV infection in humans are rarely detected. In 2003, USUV-specific nucleic acid was identified in the blood of a young man with a rash in a USUV-endemic area around Vienna (24). In 2009, two human cases of USUV-related neuroinvasive illness were reported in Italy (25,26), and in 2013, three other human cases were reported in Croatia (27). USUV has also been recently associated with a clinical diagnosis of idiopathic facial paralysis in France (28). Transfusion-transmitted USUV infection has not been reported.

We evaluated the safety of the blood supply during WNV outbreaks in the EU by summarizing the preventive strategies applied, the functional use of WNV infection

distribution maps by blood banks and responsible blood safety authorities, the occurrence of WNV infections among blood donors, and cases of transfusion-transmitted WNV (TT-WNV) infection. Because USUV circulates or cocirculates with WNV in certain EU countries (29) and current virus RNA detection systems show cross-reactivity between these viruses, we also discuss the threat posed by USUV to blood safety.

Materials and Methods

ECDC organized an expert meeting in Vienna in March 2018. Experts and representatives of the National Competent Authorities for blood and blood components from 11 countries in Europe presented relevant data, which we used in this evaluation. The European Blood Alliance provided data on preventive measures from the other EU member states. We also reviewed the scientific literature on blood donation and transfusion-related WNV and USUV infections, and retrieved data on reported cases of WNV infection in the EU population from The European Surveillance System (TESSy) of ECDC.

Results

According to data reported to TESSy during 2009–2017, a total of 1,757 cases of WNV infection, with an annual range from 30 to 356 cases, were reported in the EU countries (Figure). Most cases (1,695) were reported to be locally acquired; only 62 cases were imported (30). We summarized data on WNV infection among blood donors, which were

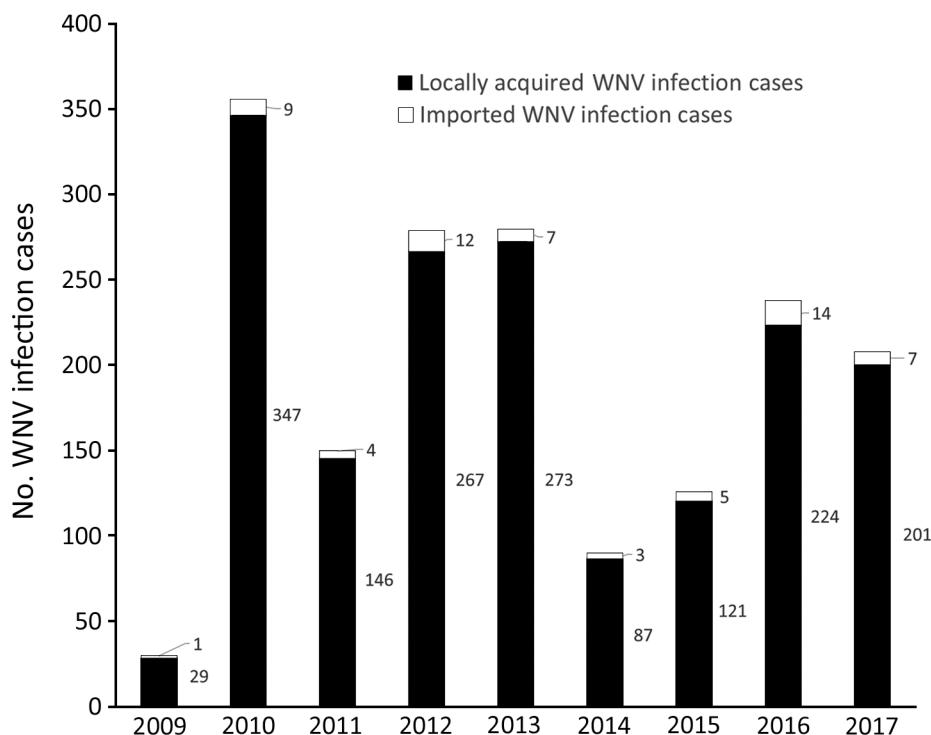


Figure. Annual number of locally acquired and imported WNV infection cases in the European Union reported to the European Surveillance System during 2009–2017. WNV, West Nile virus.

not consistently reported. Detailed descriptions of blood safety measures and frequencies of WNV-and USUV-positive blood donations in the EU are provided in the Appendix (<https://wwwnc.cdc.gov/EID/article/25/6/18-1755-App1.pdf>).

Preventive Strategies

As of August 31, 2018, most EU countries (15/17; 88%) without local WNV transmission to humans apply a 28-day deferral of asymptomatic blood donors after they leave an area with ongoing transmission of WNV to humans. These countries defer donors with a travel history to WNV-affected areas within EU at NUTS 3 level or at a country level in affected non-EU countries such as the United States, Canada, and countries in the tropical zone (delimited by latitudes 23°26'12.5" in the Northern and Southern Hemispheres). The minimum time spent in an affected area that countries consider as a deferral criterion varies from 1 night to 2 consecutive days. As alternatives to the 28-day deferral, the United Kingdom uses a minipool (MP) of 6 samples and Ireland performs individual donation (ID) WNV screening using nucleic acid testing (NAT) of blood donations from traveling donors who have visited a WNV-affected country in the previous 28 days during the mosquito season (May–November). These methods are used to avoid donor shortages resulting from deferral and the cost incurred to maintain an adequate donor base by recruiting new donors. Some blood banks in Germany perform either MP or ID-NAT screening of donations from travelers to affected areas.

As of August 31, 2018, a total of 11 of 28 (39%) EU countries have reported local autochthonous transmission of WNV; 9 (81%) of those countries implemented NAT screening of blood donations (Table 1). For purposes of implementation of local blood safety measures, geographic determination of an area in which a vectorborne disease is present is based on epidemiologic analysis and risk assessment (18).

ECDC's WNV Maps

All blood banks in the EU use WNV maps to assess the travel-related risk of WNV infection among blood donors. Although the weekly updated maps are publicly available, some National Competent Authorities for Blood use ECDC WNV maps and supporting tables to produce national instructive documents for blood banks. Upon detection of a WNV NAT-reactive donor, blood banks retrieve and quarantine blood components derived from whole blood donated by the involved donor 120 days before the date of collection of the reactive donation and initiate a retrospective (lookback) analysis of recipients of potentially implicated blood components. There have been instances of observed differences between data on

confirmed and probable human cases shown in the maps and reported by other sources (e.g., Rapid Alert System for Blood and Blood Components [RAB]), which is partially the result of occasional delays of longer than 10 days in reporting of cases to ECDC by some member states. These discrepancies could cause uncertainties for the map users and delays in the implementation of blood safety measures.

WNV Infections among Blood Donors

During 2010–2017, blood banks in the affected areas of 7 EU countries (Austria, France, Greece, Italy, Portugal, Romania, and Spain) detected 152 WNV RNA-reactive donations among 2,636,653 donated blood units, corresponding to a mean frequency of 0.60 (range 0–2.95) positive donations/10,000 donations tested (Table 2). This estimation of WNV-positive donations in the EU is biased by the small amount of data from countries with sporadic outbreaks. In countries with established continuous WNV circulation

Table 1. Occurrence of locally transmitted or imported cases of West Nile virus infection and blood safety measures by country in the European Union as of August 31, 2018*

Country	Affected areas with local transmission cases (measure)	Nonaffected areas with imported cases (measure)
Austria	+ (MP-NAT)	0 (D/MP-NAT†)
Belgium	0	0 (D)
Bulgaria	+ (D)	0 (D)
Croatia	+ (ID-NAT‡)	0 (D)
Cyprus	0	0 (D)
Czech Republic	0	0 (D)
Denmark	0	0 (D)
Estonia	0	0 (D)
Finland	0	0 (D)
France	+ (ID-NAT)	0 (D)
Germany	0	0 (D/MP/ID-NAT†)
Greece	+ (ID-NAT/D/TSD)	+ (D)
Hungary	+ (D)	0 (D)
Ireland	0	+ (ID-NAT)
Italy	+ (ID-NAT)	+ (D/ID-NAT†)
Latvia	0	0 (D)
Lithuania	0	0 (D)
Luxembourg	0	0 (D)
Malta	0	0 (D)
Netherlands	0	+ (D)
Poland	0	0 (D)
Portugal	+ (ID-NAT)	0 (D)
Romania	+ (2D-NAT†/D/TSD)	+ (D)
Slovakia	0	0 (D)
Slovenia	+ (ID-NAT‡)	0 (D)
Spain	+ (ID-NAT)	0 (D)
Sweden	0	+ (D)
United Kingdom	0	+ (MP-NAT)

*D, 28-d deferral of donors coming from affected areas; ID-NAT, individual donation NAT; 2D-NAT, 2 donations NAT; MP-NAT, minipool NAT; NAT, nucleic acid testing; TSD, temporary stop of donations in the area; +, presence of cases; 0, absence of cases. More information about data collection in the different European Union countries is provided in the Appendix (<https://wwwnc.cdc.gov/EID/article/25/6/18-1755-App1.pdf>).
 †NAT screening in some blood banks.
 ‡NAT screening began in the end of summer 2018.

in humans, such as Greece and Italy, the number of WNV-positive blood donations is proportional to the number of reported clinical cases in the general population, showing correlation coefficients of, for instance, $R = 0.92$ for Greece and $R = 0.69$ for Italy. Consequently, during the peak years, the positivity rate per 10,000 donations was 2.95 in Greece and 1.20 in Italy. In Austria, France, Spain, and Portugal, no positive blood donation was observed when only 1–3 autochthonous human WNV infections were detected in the population.

Transfusion-Transmitted WNV Infection

One report of WNV infectious blood donation noted that this donation resulted in TT-WNV infections in 2 patients (31). In 2012, the Greek Haemovigilance Centre reported these 2 TT-WNV infections: 1 patient received a platelet transfusion and developed WNND, and another patient received fresh frozen plasma and became positive for WNV but remained asymptomatic. Both blood components, including nontransfused erythrocytes, were prepared from a single whole blood donation and tested positive for WNV RNA

after notification. These erythrocytes were discarded. The implicated donor, who retrospectively received a diagnosis of WNV infection, donated blood 8 days before a case of WNV infection in Greece was reported and preventive measures initiated (31).

Threat to Blood Safety Posed by Emerging USUV

Because of NAT cross-reactivity, USUV-infected donations in the EU blood supply have been detected during routine screening of blood donations for WNV RNA. A USUV RNA-positive blood donor in the EU was detected in Germany in 2016 (32). In 2017, the follow-up investigation of 7 donors among 12,047 donations from eastern Austria whose blood tested positive by NAT (Cobas WNV assay; Roche Diagnostics, <https://diagnostics.roche.com>), showed by virus-specific NATs and sequencing that 6 of them had USUV infection, not WNV (33). Retrospective analyses of 4 blood donations among 70,864 donations from eastern Austria diagnosed as WNV-positive in 2016 showed 1 USUV-positive sample (33). In the 2018 transmission season, the highest-ever number of WNV

Table 2. Autochthonous and imported cases of WNV infection and WNV-positive blood donations by country in the European Union, 2009–2017*

Country and year	No. cases of WNV infection, autochthonous (imported)	No. WNV-positive blood donations	No. blood donations screened	WNV-positive blood donations/10,000 donations
Austria†				
2014	2	0	67,800	0.00
2015	6	5	74,677	0.67
2016	5	3	70,864	0.42
2017	6	2	67,544	0.30
France†				
2015	1	0	30,900	0.00
2017	2	0	4,044	0.00
Greece				
2010	262	8	27,108	2.95
2011	99 (1)	5	105,610	0.47
2012	160 (2)	4	36,911	1.08
2013	86	1	26,910	0.37
2014	15	0	6,662	0.00
2017	48	0	3,779	0.00
Italy				
2009	18	2	59,815	0.33
2010	11	6	118,295	0.51
2011	32	4	148,255	0.27
2012	73	14	116,255	1.20
2013	126 (3)	19	284,564	0.67
2014	24	4	334,356	0.12
2015	61 (1)	16	322,196	0.50
2016	76 (5)	31	455,930	0.68
2017	57 (1)	25	338,900	0.74
Portugal				
2015	1	0	4,247	0.00
Romania‡				
2016	17	1	10,694	0.94
2017	16	1	11,390	0.88
Spain				
2010	2	0	10,768	0.00
2016	3	0	9,457	0.00

*USUV, Usutu virus; WNV, West Nile virus.

†Austria corrected numbers of WNV-positive cases and donations by excluding USUV-positive donations in 2016 and 2017. Other affected countries did not make corrections related to possible USUV positivity.

‡Data from affected areas where blood donations were screened.

and USUV NAT-positive blood donations were identified in eastern Austria (5 WNV NAT-positive, 17 USUV NAT-positive, and 1 WNV/USUV double infection among 31,598 blood donations) (34). Similarly, in the Lazio region of Italy, all 5 WNV NAT-reactive blood donations in 2017 and 2018 turned out to contain USUV, and not WNV, RNA (35). USUV antibodies in blood donors had been detected earlier. In 2009, four of 359 healthy blood donors were positive for USUV IgG in Italy (36), and in 2012, one of 4,200 screened blood donations tested positive for USUV IgG and IgM in southwest Germany (37).

Discussion

The differences in the applied WNV blood safety measures in the EU countries with autochthonous human WNV transmission probably reflect the fact that the EU Blood Directives define measures only for donors returning from WNV-affected areas but not for donors residing in affected areas (15,38). Therefore, in the affected areas, each EU country applies blood safety interventions consistent with its national epidemiologic situation, economic capabilities, and experiences, as well as the EU preparedness plan for the prevention of TT-WNV (17), the European blood guide (16), recommendations from the US Food and Drug Administration (39), the American Association of Blood Banks standards (40), and other sources. Because of the smaller magnitude of outbreaks in affected EU countries, various blood safety measures are applied only locally. Conversely, driven by a continuous high number of WNV infection cases, an all-year universal donor screening has been implemented nationwide in the United States, but in Canada, donor screening is done only during the summer. Both countries switch from MP to ID-NAT depending on WNV activity levels (39,41). As predicted by models of environmental and climatic drivers (42), and after a substantial increase in human cases in 2018 (43), it is likely that WNV activity and the prevalence of infection in the donor population will increase across the EU in the future, which will inevitably call for greater screening of blood donations.

Changes in the epidemiology of existing infections and increasingly frequent emerging and reemerging infections may challenge the relevance of the current EU legislation on blood, tissues, and cells. Because the affected area at NUTS 3 level, which is currently used by EU countries in the donor selection procedure for travelers, could be considerably broader than the geographic area with ongoing transmission to humans, a new term should be developed for such affected areas to avoid misinterpretations. The role of ECDC in coordination of the development of common preparedness plans at the EU level (e.g., EU preparedness plans for the prevention of transfusion-transmitted WNV and Zika virus infections and safety of substances of human

origin [17,44]) is to identify gaps in measures mandated through existing legislation and to achieve a high consistency and efficacy of implemented measures.

The mean frequency of 0.60 WNV RNA-positive donations/10,000 blood donations at the EU level is similar to data from the United States, where the American Red Cross detected 1,576 WNV-positive donations among 27 million donations during 2003–2012 (0.59 [range 0.18–1.49] positive/10,000 donations) (45). Observed data in the EU and United States show that the mean frequencies of WNV-positive blood donations in affected areas are low but not negligible. These data and experiences from the EU countries suggest that, in newly affected areas, initial cases of WNV transmission to humans tend to be sporadic, posing a low risk to potential blood donors, especially travelers. Such risk increases when more clinical cases are reported in the population, indicating that continuous transmission of WNV to humans has been established. However, in previously affected or endemic areas in Greece and Italy, WNV-positive donations had been reported before clinical cases were diagnosed in the general population (46–48). In Greece, lookback studies in 2010 of possible TT-WNV among patients with thalassemia revealed that some WNV-positive blood units might have been donated before the criteria used for initiating the implementation of blood screening with NAT were met (48). In Italy, circulation of the virus between birds and mosquitoes or a single case of transmission to a human or animal host are considered indicators of an increased risk of virus transmission to humans, and screening of blood donation starts even before the first case in humans is confirmed. Conversely, the absence of WNV-positive blood donated by travelers returning from affected areas calls for reevaluating triggers for implementation of traveler risk-related safety interventions because increased deferrals may have an effect on donor availability, especially during summer months.

Only 1 case of fully documented WNV transmission from a single donation to 2 recipients has been reported in the EU (31), which suggests that the current blood safety measures effectively prevent WNV infectious blood donations from entering the blood supply in the EU. However, besides blood safety interventions, other factors that constitute the risk of TT-WNV infection also contribute to the current situation. Considering that the projected incidence of WNV-positive donations generally correlates with WNN case frequencies (49), it is possible that the number of WNV-positive donations associated with the annual number of 30–356 cases of WNV infection in the EU (30) is too low for the occurrence of low-viremic donations that might escape safety interventions. In the United States, where substantially more WNV cases are reported annually, some breakthrough transfusion-transmitted cases

occurred because of low-level viremia that escaped detection by MP-NAT (50). It is also believed that, despite the implemented surveillance and hemovigilance protocols and improvements in diagnostic tests, many WNV-like symptoms were undiagnosed, or the routes of infection were not investigated. Thus, a certain level of underrecognition and underreporting certainly contributes to the lack of TT-WNV cases.

Recent molecular and serologic surveillance studies in Germany, Austria, and Italy identified USUV infections in blood donors (32,33,36,37). USUV is currently circulating more widely than WNV in the EU (22,23,33). Historically, USUV was introduced to Europe decades after WNV, although WNV activity was low in Europe before 2008. Because of the similar environmental requirements, mosquito vectors, and amplifying cycle in birds, USUV has easily been introduced in areas where WNV was present, resulting in a substantial geographic overlap in the circulation of these 2 viruses. USUV is currently spreading more intensively into new areas than WNV (22). Consequently, USUV but not WNV is currently circulating in the Netherlands, Belgium, and Switzerland (33). Furthermore, in Germany, only a few cases of WNV infections in birds were identified in 2018, whereas USUV is endemic throughout the country. No transfusion-associated USUV infection has been reported. However, the occurrence of USUV among blood donors is not fully determined because countries with USUV but without WNV circulation are not required to screen blood donations for flavivirus RNA. Assessing the risk of USUV transmission through blood transfusion and the clinical relevance of USUV infections in humans is therefore crucial. The currently used ID-NATs for blood screening are highly sensitive. The cross-reactivity of these test systems with USUV (33,34) and potentially with other members of the Japanese encephalitis virus complex can contribute to the detection of these flaviviruses in donated blood. WNV NAT-reactive donations should therefore undergo virus-specific confirmatory tests to determine the actual flavivirus present in donated blood.

In summary, the paucity of reported TT-WNV cases provides reassurance about the efficacy of WNV blood safety interventions in the EU. However, the cocirculation of WNV and USUV in several EU countries, together with the yet unknown transfusion risk and clinical relevance of human USUV infections, needs further attention.

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Dr. Domanović is a senior expert in the vigilance and traceability of substances of human origin at the European Centre for Disease Prevention and Control, Stockholm, Sweden. His research interests are donor-derived infections, emerging infections, and risk of infectious diseases transmission through substances of human origin.

References

- Hubálek Z, Halouzka J. West Nile fever—a reemerging mosquito-borne viral disease in Europe. *Emerg Infect Dis*. 1999;5:643–50. <http://dx.doi.org/10.3201/eid0505.990505>
- Pachler K, Lebl K, Berer D, Rudolf I, Hubalek Z, Nowotny N. Putative new West Nile virus lineage in *Uranotaenia unguiculata* mosquitoes, Austria, 2013. *Emerg Infect Dis*. 2014;20:2119–22. <http://dx.doi.org/10.3201/eid2012.140921>
- Bakonyi T, Ivanics E, Erdélyi K, Ursu K, Ferenczi E, Weissenböck H, et al. Lineage 1 and 2 strains of encephalitic West Nile virus, central Europe. *Emerg Infect Dis*. 2006;12:618–23. <http://dx.doi.org/10.3201/eid1204.051379>
- Bakonyi T, Ferenczi E, Erdélyi K, Kutasi O, Csörgő T, Seidel B, et al. Explosive spread of a neuroinvasive lineage 2 West Nile virus in Central Europe, 2008/2009. *Vet Microbiol*. 2013;165:61–70. <http://dx.doi.org/10.1016/j.vetmic.2013.03.005>
- Papa A, Bakonyi T, Xanthopoulou K, Vazquez A, Tenorio A, Nowotny N. Genetic characterization of West Nile virus lineage 2, Greece, 2010. *Emerg Infect Dis*. 2011;17:920–2. <http://dx.doi.org/10.3201/eid1705.101759>
- Platonov AE, Karan' LS, Shopenskaia TA, Fedorova MV, Koliashnikova NM, Rusakova NM, et al. Genotyping of West Nile fever virus strains circulating in southern Russia as an epidemiological investigation method: principles and results [in Russian]. *Zh Mikrobiol Epidemiol Immunobiol*. 2011;2:29–37.
- Sirbu A, Ceianu CS, Panculescu-Gatej RI, Vazquez A, Tenorio A, Rebreanu R, et al. Outbreak of West Nile virus infection in humans, Romania, July to October 2010. *Euro Surveill*. 2011;16:19762.
- Ravagnan S, Montarsi F, Cazzin S, Porcellato E, Russo F, Palei M, et al. First report outside eastern Europe of West Nile virus lineage 2 related to the Volgograd 2007 strain, northeastern Italy, 2014. *Parasit Vectors*. 2015;8:418. <http://dx.doi.org/10.1186/s13071-015-1031-y>
- Sambri V, Capobianchi M, Charrel R, Fyodorova M, Gaibani P, Gould E, et al. West Nile virus in Europe: emergence, epidemiology, diagnosis, treatment, and prevention. *Clin Microbiol Infect*. 2013;19:699–704. <http://dx.doi.org/10.1111/1469-0691.12211>
- Barzon L, Papa A, Lavezzo E, Franchin E, Pacenti M, Sinigaglia A, et al. Phylogenetic characterization of central/southern European lineage 2 West Nile virus: analysis of human outbreaks in Italy and Greece, 2013–2014. *Clin Microbiol Infect*. 2015;21:1122.e1–10. <http://dx.doi.org/10.1016/j.cmi.2015.07.018>
- Harrington T, Kuehnert MJ, Kamel H, Lanciotti RS, Hand S, Currier M, et al. West Nile virus infection transmitted by blood transfusion. *Transfusion*. 2003;43:1018–22. <http://dx.doi.org/10.1046/j.1537-2995.2003.00481.x>
- Hinckley AF, O'Leary DR, Hayes EB. Transmission of West Nile virus through human breast milk seems to be rare. *Pediatrics*. 2007;119:e666–71. <http://dx.doi.org/10.1542/peds.2006-2107>
- Centers for Disease Control and Prevention (CDC). Intrauterine West Nile virus infection—New York, 2002. *MMWR Morb Mortal Wkly Rep*. 2002;51:1135–6.
- Pealer LN, Marfin AA, Petersen LR, Lanciotti RS, Page PL, Stramer SL, et al.; West Nile Virus Transmission Investigation Team. Transmission of West Nile virus through blood transfusion in the United States in 2002. *N Engl J Med*. 2003;349:1236–45. <http://dx.doi.org/10.1056/NEJMoa030969>
- European Commission. Commission Directive 2014/110/EU of 17 December 2014 amending Directive 2004/33/EC as regards temporary deferral criteria for donors of allogeneic blood donations 2014 [cited 2018 Mar 11]. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ%3AJOL_2014_366_R_0011
- European Directorate for the Quality of Medicines and Healthcare. Guide to the preparation, use and quality assurance of blood components, 19th ed. Strasbourg (France): Council of Europe; 2016.

17. European Commission. West Nile virus and blood safety—introduction to a preparedness plan in Europe 2012 [cited 2018 Mar 11]. https://ec.europa.eu/health/sites/health/files/blood_tissues_organs/docs/wnv_preparedness_plan_2012.pdf
18. Domanovic D, Giesecke J. How to define an area where transmission of arthropod-borne disease is occurring? *Euro Surveill.* 2012;17:20171.
19. European Center for Disease Prevention and Control (ECDC). West Nile fever maps 2012 [cited 2012 Feb 22]. http://ecdc.europa.eu/en/activities/diseaseprogrammes/emerging_and_vector_borne_diseases/pages/west_niles_fever_risk_maps.aspx
20. Weissenböck H, Kolodziejek J, Ürl A, Lussy H, Rebel-Bauder B, Nowotny N. Emergence of Usutu virus, an African mosquito-borne flavivirus of the Japanese encephalitis virus group, central Europe. *Emerg Infect Dis.* 2002;8:652–6. <http://dx.doi.org/10.3201/eid0807.020094>
21. Weissenböck H, Bakonyi T, Rossi G, Mani P, Nowotny N. Usutu virus, Italy, 1996. *Emerg Infect Dis.* 2013;19:274–7. <http://dx.doi.org/10.3201/eid1902.121191>
22. Cadar D, Lühken R, van der Jeugd H, Garigliany M, Ziegler U, Keller M, et al. Widespread activity of multiple lineages of Usutu virus, western Europe, 2016. *Euro Surveill.* 2017;22:30452. <http://dx.doi.org/10.2807/1560-7917.ES.2017.22.4.30452>
23. Bakonyi T, Erdélyi K, Brunthaler R, Dán Á, Weissenböck H, Nowotny N. Usutu virus, Austria and Hungary, 2010–2016. *Emerg Microbes Infect.* 2017;6:e85.
24. Weissenböck H, Chvala-Mannsberger S, Bakonyi T, Nowotny N. Emergence of Usutu virus in central Europe: diagnosis, surveillance and epizootiology. In: Takken W, Knols BGJ, editors. *Emerging pests and vector-borne diseases in Europe—ecology and control of vector-borne diseases.* Wageningen (the Netherlands): Wageningen Academic Publishers; 2007. p. 153–68.
25. Pecorari M, Longo G, Gennari W, Grottole A, Sabbatini A, Tagliacucchi S, et al. First human case of Usutu virus neuroinvasive infection, Italy, August–September 2009. *Euro Surveill.* 2009;14:19446.
26. Cavrini F, Gaibani P, Longo G, Pierro AM, Rossini G, Bonilauri P, et al. Usutu virus infection in a patient who underwent orthotopic liver transplantation, Italy, August–September 2009. *Euro Surveill.* 2009;14:19448.
27. Santini M, Vilibic-Cavlek T, Barsic B, Barbic L, Savic V, Stevanovic V, et al. First cases of human Usutu virus neuroinvasive infection in Croatia, August–September 2013: clinical and laboratory features. *J Neurovirol.* 2015;21:92–7. <http://dx.doi.org/10.1007/s13365-014-0300-4>
28. Simonin Y, Sillam O, Carles MJ, Gutierrez S, Gil P, Constant O, et al. Human Usutu virus infection with atypical neurologic presentation, Montpellier, France, 2016. *Emerg Infect Dis.* 2018;24:875–8. <http://dx.doi.org/10.3201/eid2405.171122>
29. Nikolay B. A review of West Nile and Usutu virus co-circulation in Europe: how much do transmission cycles overlap? *Trans R Soc Trop Med Hyg.* 2015;109:609–18. <http://dx.doi.org/10.1093/trstmh/trv066>
30. European Center for Disease Prevention and Control. The European surveillance system 2018 [cited 2018 Aug 31]. <https://ecdc.europa.eu/en/publications-data/european-surveillance-system-tessy>
31. Pervanidou D, Detsis M, Danis K, Mellou K, Papanikolaou E, Terzaki I, et al. West Nile virus outbreak in humans, Greece, 2012: third consecutive year of local transmission. *Euro Surveill.* 2014;19:20758. <http://dx.doi.org/10.2807/1560-7917.ES2014.19.13.20758>
32. Cadar D, Maier P, Müller S, Kress J, Chudy M, Bialonski A, et al. Blood donor screening for West Nile virus (WNV) revealed acute Usutu virus (USUV) infection, Germany, September 2016. *Euro Surveill.* 2017;22:30501. <http://dx.doi.org/10.2807/1560-7917.ES.2017.22.14.30501>
33. Bakonyi T, Jungbauer C, Aberle SW, Kolodziejek J, Dimmel K, Stiasny K, et al. Usutu virus infections among blood donors, Austria, July and August 2017—raising awareness for diagnostic challenges. *Euro Surveill.* 2017;22:17-00644. <http://dx.doi.org/10.2807/1560-7917.ES.2017.22.41.17-00644>
34. Aberle SW, Kolodziejek J, Jungbauer C, Stiasny K, Aberle JH, Zoufaly A, et al. Increase in human West Nile and Usutu virus infections, Austria, 2018. *Euro Surveill.* 2018;23:1800545. <http://dx.doi.org/10.2807/1560-7917.ES.2018.23.43.1800545>
35. Carletti F, Colavita F, Rovida F, Percivalle E, Baldanti F, Ricci I, et al. Expanding Usutu virus circulation in Italy: detection in the Lazio region, central Italy, 2017 to 2018. *Euro Surveill.* 2019;24:1800649. <http://dx.doi.org/10.2807/1560-7917.ES.2019.24.3.1800649>
36. Gaibani P, Pierro A, Alicino R, Rossini G, Cavrini F, Landini MP, et al. Detection of Usutu-virus-specific IgG in blood donors from northern Italy. *Vector Borne Zoonotic Dis.* 2012;12:431–3. <http://dx.doi.org/10.1089/vbz.2011.0813>
37. Allering L, Jöst H, Emmerich P, Günther S, Lattwein E, Schmidt M, et al. Detection of Usutu virus infection in a healthy blood donor from south-west Germany, 2012. *Euro Surveill.* 2012;17:20341.
38. European Commission. Commission Directive 2004/33/EC of 22 March 2004 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components, 2004 [cited 2018 Apr 25]. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:091:0025:0039:EN:PDF<eref>>
39. US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for industry, use of nucleic acid tests to reduce the risk of transmission of West Nile virus from donors of whole blood and blood components intended for transfusion. 2009 [cited 2018 Apr 25]. <https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM189464.pdf>
40. American Association of Blood Banks. Standards for blood banks and transfusion services. 31st edition. Bethesda (MD): The Association; 2018.
41. Vamvakas EC, Kleinman S, Hume H, Sher GD. The development of West Nile virus safety policies by Canadian blood services: guiding principles and a comparison between Canada and the United States. *Transfus Med Rev.* 2006;20:97–109. <http://dx.doi.org/10.1016/j.tmr.2005.11.001>
42. Semenza JC, Tran A, Espinosa L, Sudre B, Domanovic D, Paz S. Climate change projections of West Nile virus infections in Europe: implications for blood safety practices. *Environ Health.* 2016;15(Suppl 1):28. <http://dx.doi.org/10.1186/s12940-016-0105-4>
43. Haussig JM, Young JJ, Gossner CM, Mezei E, Bella A, Sirbu A, et al. Early start of the West Nile fever transmission season 2018 in Europe. *Euro Surveill.* 2018;23:1800428. <http://dx.doi.org/10.2807/1560-7917.ES.2018.23.32.1800428>
44. European Center for Disease Prevention and Control. Zika virus and safety of substances of human origin: a guide for preparedness activities in Europe 2017 [cited 2018 Aug 31]. <https://ecdc.europa.eu/en/publications-data/zika-virus-and-safety-substances-human-origin-guide-preparedness-activities-0>
45. Dodd RY, Foster GA, Stramer SL. Keeping blood transfusion safe from West Nile virus: American Red Cross experience, 2003 to 2012. *Transfus Med Rev.* 2015;29:153–61. <http://dx.doi.org/10.1016/j.tmr.2015.03.001>
46. Rizzo C, Napoli C, Venturi G, Pupella S, Lombardini L, Calistri P, et al.; Italian WNV surveillance working group. West Nile virus transmission: results from the integrated surveillance system in Italy, 2008 to 2015. *Euro Surveill.* 2016;21:30340. <http://dx.doi.org/10.2807/1560-7917.ES.2016.21.37.30340>

47. Politis C, Tsoukala A, Hatzitaki M, Pappa A, Englezou A, Zafeiriou C, et al. West Nile virus (WNV) outbreak in Greece and blood safety measures. *Vox Sang.* 2011;101(Suppl1):42.
48. Politis C, Pappa A, Hassapopoulou H, Pantelidou D, Perifanis V, Teli A, et al. Transfusion transmitted West Nile virus (WNV) infection in thalassaemic patients in Greece. *Vox Sang.* 2011;101(Suppl2):236.
49. Betsem E, Kaidarova Z, Stramer SL, Shaz B, Sayers M, LeParc G, et al. Correlation of West Nile virus incidence in donated blood with West Nile neuroinvasive disease rates, United States, 2010–2012. *Emerg Infect Dis.* 2017;23:212–9. <http://dx.doi.org/10.3201/eid2302.161058>
50. Stramer SL, Fang CT, Foster GA, Wagner AG, Brodsky JP, Dodd RY. West Nile virus among blood donors in the United States, 2003 and 2004. *N Engl J Med.* 2005;353:451–9. <http://dx.doi.org/10.1056/NEJMoa044333>

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West Nile and Usutu Virus Infections and Challenges to Blood Safety in the European Union

Appendix

West Nile Virus and Blood Donations in Selected European Countries

Austria

Autochthonous human WNV infections in Austria were documented in 3 patients (2 in 2009 [1 WNND and 1 West Nile fever (WNF) case] and 1 WNND case in 2010) whose serum and cerebrospinal fluid samples tested positive during a retrospective examination in 2012 (1). Following countrywide measures based on the deferral of potentially exposed donors, WNV-NAT screening of blood donations was introduced in eastern Austria (federal states of Vienna, Lower Austria, and Burgenland) in 2014, and in the first year of screening 1 WNV NAT-positive blood donation was identified (2,3). Seasonal screening of all donations between June 1 and November 30 was a measure to avoid deferrals of donors who had visited 1 of the 3 WNV-affected neighboring countries. Screening has been performed in pools of 19 samples, meeting the sensitivity limit enacted by Germany's Paul Ehrlich Institute of 250 copies WNV-lineage 2-RNA/mL (95% limit of detection) in the Roche Cobas 6800/8800 WNV assay. During 2014–2017, WNV nucleic acid was found in 10 out of 303,400 tested blood donations (2–4). In 2017, the follow-up of 7 donors from eastern Austria, whose blood reacted positive in the Cobas WNV assay among 12,047 donations, revealed that 6 of them had actually USUV and not WNV infection (5). In the other (not yet WNV affected) parts of Austria and offseason in eastern Austria, donors who have visited an affected country or region (based on the data of ECDC WNV maps at NUTS 3 level and other sources) are deferred for 28 days after having left the affected area. Although WNV positive donations have been detected, no transfusion-transmitted WNV infection was observed.

Croatia

Systematic WNV monitoring of animals started in 2012 and showed permanent seasonal circulation of the virus in Croatia. Monitoring is performed under the competence of the Ministry of Agriculture and in cooperation with the veterinary faculty in Zagreb (6). Clinical human cases of neuroinvasive WNV infection were detected in 2012 in eastern Slavonia. The seroprevalence of WNV infection in humans from that region in 2011 was 0.3% (7). During 2012–2017, the number of human WNV infections in Croatia varied by year (7 in 2012, 20 in 2013, 1 in 2014, 1 in 2015, 2 in 2016, 1 in 2017; data for 2017 not complete) and also occurred in the other parts of the country (8). From 2012 until March 16, 2018, there were no cases of WNV among blood donors in Croatia. Considering the possibility of blood transmission and potential hazards in patients after rapid alert sent by the Ministry of Health, the transfusion service in Croatia is undertaking the following measures: donors are deferred from donation for 28 days if they visited an affected area in the period from April 1 to November 30. ECDC maps showing human WNV cases are used to identify affected areas in the EU. Potential donors with confirmed or suspected WNV infections may donate blood 120 days after diagnosis. Postdonation information is reinforced. Cases of transfusion-transmitted WNV infection have not been reported.

France

The French Advisory Group for Substances of Human Origin is a multisector body responsible for providing recommendations on blood safety measures to be implemented in the case of arboviral epidemiologic alerts, which are managed according to French interministry guidelines. WNV blood safety measures were implemented for the first time during the equine outbreak of WNF in the Camargue in 2000. For local WNV transmission, the trigger for applying the measures is a confirmed case of WNV infection in humans. Once implemented, blood safety measures last until the end of the mosquito season. The measures in the affected area comprise the cancellation of blood collection sessions or temporary deferral of donors for 28 days unless WNV NAT screening and/or pathogen reduction method for platelets and plasma are implemented. Potential blood donors visiting affected areas in France or abroad are temporarily deferred for 28 days after return. When applied, blood donation screening uses ID-NAT testing (Procleix WNV assay on the Grifols Tigris System, <https://www.grifols.com>). In 2015, 1 human WNF case in the department Gard, and 41 equine WNF cases in departments 13, 30, and 34 were detected. During this outbreak, 30,900 blood donations were screened using ID-NAT during

September–November 2015; all were negative for WNV RNA. During October 13–November 27, 2017, 1 equine and 2 human WNF cases in the department of Alpes Maritimes were reported. Retrospective testing of cerebrospinal fluid and/or serum samples from 61 neurologic patients in August and September at the Hospital of Nice were all WNV negative. All donations tested in ID-NAT WNV (n = 4044) by the French National Blood Service during October 20–November 21 were negative. By the time of the expert meeting, no donors were found positive and no cases of transfusion-transmitted WNV infection were diagnosed in France.

Germany

Monitoring of migratory and resident birds for zoonotic arbovirus infections showed that WNV was not present in Germany before March 2018. Although WNV-specific RNA was not detected, USUV was already widespread in birds in Germany. In addition, neutralizing antibodies to WNV and USUV were identified (9,10). Human arbovirus infections, which were insufficiently reported in Germany under “other threatening diseases,” became an explicit reporting obligation from May 2016 onward. Overall, 10 imported cases of WNV infections were reported in Germany. WNV substances of human origin safety measures entail donor deferral for 4 weeks after residence in an area with ongoing transmission of WNV to humans for >2 consecutive days, starting after leaving this area. If the donor/donation WNV-NAT testing is applied, the minimum sensitivity of 250 copies of WNV-RNA/mL related to a single donation and concurrent detection of WNV lineages 1 and 2 are required. Affected areas are countries considered with ≥ 1 confirmed autochthonous human WNV cases in the current and the previous year. Areas for geographic deferral of visitors are defined as regions at NUTS 3 level (Eurostat definition). For the donor selection procedure, blood banks use the list of affected areas in the EU, which is based on the ECDC Surveillance Atlas and published on the homepage of the Paul Ehrlich Institute (www.pei.de/wnv-spenderrueckstellung). Updates are performed on the first working day of each month. Data from the ECDC Surveillance Atlas are exported and transformed to meet only confirmed cases and translated into German. Measures are applied from June 1 to November 30.

Greece

Surveys, undertaken in some parts of Greece before 2010, showed that $\approx 1\%$ of the population had WNV antibodies, indicating a circulation of WNV or related viruses in the observed areas, but no clinical disease or WNV NAT positivity in humans were detected (11,12).

In 2010, the first outbreak of WNV infection in Greece was recorded as the largest in Europe since 1996 (13). WNV lineage 2–Nea Santa was introduced into Greece in or before 2010 and has since been responsible for 672 diagnosed symptomatic clinical cases, of which 30% were WNF and 70% were WNND; 77 patients with WNND died (13,14). Raising awareness among physicians and susceptible populations (the elderly and persons with concurrent conditions) and enhanced surveillance during mosquito transmission season were implemented. The occurrence of human cases in 5 consecutive years (2010–2014) and in 2017 suggests that WNV lineage 2 has become established in Greece (13,15). A study of symptomatic WNV lineage 2 in Greece suggests blood group A RhD negativity as a new genetic risk factor associated with WNV infection and level of illness. The possibility that HLA C*08, DRB1*04:05, and DQB1*02 are protective alleles and DRB1*10:01 a “susceptible” allele to WNV infection and the role of secretor status of an individual (defined by the phenotype in the Lewis blood group) in relation to this infection has also been suggested (16). Blood safety and hemovigilance measures are in conformity with Directives 2004/33/EC, 2014/110/EU (17,18). A trigger for the implementation of measures would be the first laboratory confirmed human case of WNV infection. In addition to donation screening in affected areas defined at NUTS3 level, the quarantine of blood components collected 15 days before starting the measures, enhanced postdonation and posttransfusion information have also been in place since 2010 (15). ECDC WNV maps are in use for the selection of donors traveling abroad. Additional information on affected areas is obtained from the Rapid Alert System for Blood and Blood Components (RAB) platform. During 2010–2017 the annual number of affected NUTS 3 areas with human cases varied from 4 (2014) to 19 (2013), with only equine cases from 3–8 areas, and with both human and equine cases from 2–5 areas. In the same period, the rate of reactive donations for WNV-RNA was 0.87/10,000 (from 206,980 donations tested by ID-NAT, 18 were positive, for a frequency of 1:11,498 donations). Before the start of NAT screening of blood donations in 2012, WNV infection was transmitted to 2 patients who received transfusions of 2 different blood components (fresh frozen plasma and platelets) derived from the same blood unit of an asymptomatic blood donor. One of the 2 infected patients, who received a transfusion of whole blood derived platelets, had severe transfusion-transmitted WNND (13).

Hungary

Hungary was the first country in Europe in which the lineage 2 WNV emerged (19). During 2003–2007, a yearly average of 6 cases of WNND were diagnosed (19). In 2010, a lineage 2 WNV outbreak in humans, including several cases of encephalitis, was observed. Public health measures to prevent WNV transmission are in place from April to the end of November annually and include enhanced human and animal surveillance, as well as vector control measures. Blood safety measures are based on blood donor deferral for 28 days after being in an affected area for >24 hours. Measures in the country are valid until the end of the mosquito season. During 2010–2017, Hungary reported to ECDC 163 probable and confirmed cases of WNV infection in the population. Data on positive blood donations/donors are not available. Cases of transfusion-transmitted WNV infection have not been reported in Hungary.

Italy

Following the first human cases of WNV infection identified in the Emilia-Romagna region in 2008, Italy implemented a 28-day deferral policy of blood donors living in areas with ongoing WNV transmission. During June–November 2009–2014, the National Blood Centre used WNV NAT screening of blood donations in provinces corresponding to NUTS 3 units where the virus had been circulating among animals and mosquitoes or where a human case of WNND and/or a WNV NAT-positive donor had been confirmed. Besides NAT screening, the National Blood Centre enforced a nationwide 28-day deferral for blood donors who had spent ≥ 1 night in areas with active WNV circulation. In 2008, an integrated surveillance targeting mosquitoes, birds, and humans was put into effect starting in the Emilia-Romagna region; in the following years, this surveillance was extended to 4 other regions (Piedmont, Lombardy, Friuli Venetia Giulia, and Veneto) (20). The remaining regions adopted the national surveillance plan. Currently, the triggers for WNV ID-NAT screening of blood donations in the June–November period are notification of WNV circulation in mosquitoes and/or wild birds in the regions where the integrated surveillance is in place, notification of a WND equid case, and/or notification of WNV infection in humans. WNV NAT screening continues until the end of the surveillance season in November (20). Nationwide, all donors having spent ≥ 1 night in the Italian provinces where NAT testing has been introduced are deferred for 28 days unless tested negative by ID-NAT. The same protocol is adopted for donors coming from the United States, Canada, and any other EU or non-EU countries where WNV human cases were reported by the ECDC. Other

general measures include strengthening of the predonation questionnaire and donor physical examination, enforcing postdonation information, implementation of ad hoc hemovigilance procedures, and communication and collaboration among all institutional healthcare bodies involved. The number of screened donations was $\approx 150,000$ in 2011 and $\approx 340,000$ in 2017, with a peak in 2016 ($\approx 460,000$ tested donations). The number of WNV-positive donations also increased, from 4 in 2011 to 25 in 2017, with a peak of 31 positive donations in 2016. In 2017, 27 cases of WNND were confirmed in Italy. Specific measures for hematopoietic stem cell donations (WNV NAT testing and 28-day deferral criteria, if applicable, on the basis of transplant timing) were applied in Emilia-Romagna, Veneto, Lombardy, Piedmont, and Sardinia. There are no records of transfusion-transmitted WNV infection in Italy.

Portugal

The National Hemovigilance Office, which is responsible for blood safety monitoring in Portugal, obtains data from the weekly bulletin issued by the Directorate General of Health, the European Commission platform RAB, and ECDC's WNV maps. In Portugal, only 4 human autochthonous WNV cases have been reported: 3 cases in the Algarve and 1 in the Sado Valley of the Lisbon NUTS 3 region. Three of these cases were classified as probable cases: 2 cases detected in 2004 (21) and 1 case in 2010. The first confirmed case was in 2015 (Algarve). Following this case and in line with the Blood Directives, the blood safety measures have been implemented during August 31–November 4, 2015 (22). In the affected area, blood collection is halted until the implementation of WNV ID-NAT screening, which lasts until the end of the mosquito season; blood components in stock are quarantined and retrospectively screened by WNV ID-NAT testing, when possible. If available, pathogen inactivation of plasma and platelets is implemented. Potential blood donors who spent ≥ 1 night in an affected area are deferred for a period of 28 days unless tested negative by ID-NAT. Blood services defer confirmed cases of WNV infection for 120 days after recovery and enhance postdonation information and posttransfusion hemovigilance. During the outbreak in 2015, 4,274 blood donations were screened during September 4–November 4; no WNV RNA-positive blood donation was found. There were no reports of transfusion-transmitted WNV infection or decreases in the number of blood donations in the affected area. The number of potential blood donors deferred in nonaffected areas could not be assessed. During 2015–2017, 108 equine WND cases were identified in the Algarve, Alentejo, and Sado Valley.

Romania

Circulation of WNV in Romania has been reported since the 1950s. Following the largest outbreak of human WNV infection in Europe to date in 1996, when ≈ 400 clinical cases of WNND disease were reported in Bucharest (23), Romania implemented a WNV surveillance system. During 1997–2009, sporadic human cases were reported in the southern part of the country. In 2010, WNV reemerged, with 52 human cases in the central and northern part of the country. Blood safety measures have been implemented since 2010 by the National Center for Prevention and Control of Infectious Diseases, which also elaborates on and disseminates the methodology for the surveillance and control of WNV infection, assesses the risk of transfusion-transmitted WNV infection, and informs all stakeholders, including the veterinary agency, about human WNV cases. Blood safety measures are in line with the current EU directives and defined in the preparedness plan. Based on information provided by ECDC and RAB, blood banks apply a 28-day deferral for potential donors who have previously traveled to affected areas inside or outside the country. Specific measures in affected villages and small towns include temporary deferral for resident donors, suspension of mobile blood collection sessions during the season, and 28 days deferral for potential donors having traveled in affected areas. Based on the risk assessment in larger towns, ID-NAT or a pool of 2D-NAT is implemented or there is a temporary deferral of resident donors, 14-day quarantine of donated blood, and release after post-donation information. It is estimated that $\approx 1,000$ prospective donors are temporarily deferred nationwide each year. Hospitals in affected areas are strengthening the rational use of blood and perform the lookback procedure if blood from a patient who has received a transfusion is found positive for WNV. Cases of transfusion-transmitted WNV infection have not been reported. During 2010–2017, the estimated risk of viremic donations from asymptomatic donors among the released units varied from 1.3 to 13 per 100,000 blood units. WNV ID and pool 2D-NAT testing during August 17–October 31, 2016 showed 1 positive per 10,694 tested donations (0.93/10,000). In 2017 (2D pool NAT), there was 1 positive donation in 11,390 donations (0.88/10,000).

Spain

The circulation of WNV in birds in Spain was documented in 2004 when, according to retrospective analysis, the first human case of WNV infection occurred (24–26). Two confirmed human cases in Cádiz and 43 equine cases in Cádiz, Huelva, Malaga, and Sevilla were reported

in September 2010. Since 2010, the System for Epidemiologic Vigilance in Andalusia runs an active WNV surveillance in humans from April to the end of November each year. During 2011–2015, the virus was detected in horses and wild birds. Three WNV human cases were diagnosed in 2016. The trigger for the implementation of blood safety measures is a confirmed locally acquired WNV infection in Spain. The Spanish Society of Blood Transfusion provides a list of the Spanish group of diseases transmissible by transfusion to all blood collection centers with weekly updates and a web link to the ECDC maps. Preventive measures include the deferral for 28 days of donors who have spent ≥ 1 night in an affected area and deferral for 120 days for donors with a diagnosis of WNF or who present or have presented a clinical picture compatible with WNV infection. In areas of 10 km around the central focus of a locally transmitted case, blood banks cancel blood collections and quarantine or retest by ID-NAT the blood components in stock that are derived from blood donated 15 days before the first case of WNV infection was reported. The recovered and plasmapheresis plasma from affected areas can be used for fractionation. Donors are informed to report to the blood bank whether they developed symptoms of WNF within 14 days after the donation. There was no WNV RNA-positive result among 10,768 screened in 2010 and 9,457 blood donations screened in 2016.

United Kingdom

The primary strategy for minimizing the risk of WNV infection in the UK population is surveillance. Activities targeting humans, animals, and vector sources have been in place since 2002; these include passive surveillance of equids and wild birds and targeted surveillance of mosquitoes. This program has identified the *Culex modestus* mosquito, the bridge WNV vector in continental Europe, in the 2 counties in the river Thames estuary, where the vector has been endemic since 2010. According to the WNV risk assessment published by the Human Animal Infections and Risk Surveillance group in December 2017 (27), there continues to be no evidence of WNV presence in the UK, with only 3 travel-related cases having been reported: 2 in 2014 (from Egypt and the United States) and 1 in 2017 (from South Africa). The Human Animal Infections and Risk Surveillance group recommended continued monitoring of vector and host populations, and surveillance and promotion of awareness among clinicians to encourage appropriate testing of clinical cases in the UK. To protect safety and sufficiency of the blood supply, WNV ID-NAT screening was implemented in 2012, replacing the 28-day deferral of donors who have visited areas with ongoing WNV activity. The Donor Selection Guidelines

devised by the Joint United Kingdom Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee stipulates deferral of 6 months after proven WNV infection or development of compatible symptoms within 28 days after return from an affected area and 28 days after return where there has been no diagnosis nor symptoms. A negative result using a validated WNV NAT allows donor acceptance in the asymptomatic group. This discretionary screening is applied each year during the May–November mosquito season, using mini-pools of 6 donations. During 2012–2017, the National Health Services Blood and Transplant tested 185,258 donations, accounting for ≈2% of all blood donations. There have not been any confirmed positive WNV RNA results in this period. If countries that receive high numbers of visitors from the UK (e.g., Spain, France, Portugal), were to become affected WNV areas, testing in England would increase, thereby influencing the cost-effectiveness of screening versus deferral.

West Nile Virus and Blood Donations in Other EU Countries

In 2018, the European Blood Alliance (EBA) performed a questionnaire survey of the WNV blood safety measures applied by EBA members. Among 14 EBA members from the EU, WNV circulation and transmission was absent in 10 countries (Belgium, Denmark, Finland, Germany, Ireland, Malta, the Netherlands, Sweden, Switzerland, United Kingdom), whereas in Austria, France, Slovenia, and Spain, transmission occurred sporadically. EBA has a global partnership with the Alliance of Blood Operators (Canadian Blood Service, America's Blood Centers, American Red Cross, the Australian Red Cross Blood Service, Blood Systems Inc., and the NHS Blood and Transplant). In the survey, Australia reported sporadic WNV cases, whereas the United States was endemic. In the EU countries, surveillance systems are in place, although the monitoring is mostly passive and triggered by animal and/or human cases. ECDC maps displaying human WNV cases are helpful and appropriate for identifying WNV-affected areas for the selection or exclusion of donors. Discrepancies between the number of reported cases by ECDC and other sources have been observed. Confirmatory testing takes time, which prolongs reporting. For the purpose of applying blood safety measures, only confirmed cases are relevant; however, some EU countries have no capacity to perform confirmatory tests. Reporting of additional information about cases has been suggested and a tighter timeline for reporting emphasized.

Most EU countries apply a donor deferral of 28 days after leaving a WNV-affected area. In the UK and in Ireland, where traveling donors are screened by WNV NAT, and in France, where donors in affected areas have been tested, no WNV RNA-positive donations were found. In the WNV-endemic area of eastern Austria, several WNV RNA positive blood donors have been identified; however, USUV RNA has been found more frequently since 2017 (5). Estimation performed by the European Up-Front Risk Assessment Tool shows a very low risk for blood safety posed by WNV, especially for blood banks in nonaffected countries. The EBA emerging infectious diseases monitoring group concluded that the current measures to prevent transfusion-transmitted WNV infection in nonaffected countries tend to be disproportional if the risk remains very low. The group also emphasized that there is a need to discuss the appropriateness of triggers for implementing a geographic deferral or screening of travelers to affected areas.

References

1. Aberle S, Heinz FX. Virus epidemiologic information No. 12/12. West Nile virus infections in Austria. 2012 [cited 2018 Mar 11].
<http://www.virologie.meduniwien.ac.at/fileadmin/virologie/files/Epidemiologie/2012/1212s.pdf>
2. Jungbauer C, Hourfar MK, Stiasny K, Aberle SW, Cadar D, Schmidt-Chanasit J, et al. West Nile virus lineage 2 infection in a blood donor from Vienna, Austria, August 2014. *J Clin Virol*. 2015;64:16–9. <http://dx.doi.org/10.1016/j.jcv.2015.01.003>
3. Kolodziejek J, Seidel B, Jungbauer C, Dimmel K, Kolodziejek M, Rudolf I, et al. West Nile virus positive blood donation and subsequent entomological investigation, Austria, 2014. *PLoS One*. 2015;10:e0126381. <http://dx.doi.org/10.1371/journal.pone.0126381>
4. Kolodziejek J, Jungbauer C, Aberle SW, Allerberger F, Bagó Z, Camp JV, et al. Integrated analysis of human-animal-vector surveillance: West Nile virus infections in Austria, 2015–2016. *Emerg Microbes Infect*. 2018;7:1–15. <http://dx.doi.org/10.1038/s41426-018-0021-5>
5. Bakonyi T, Jungbauer C, Aberle SW, Kolodziejek J, Dimmel K, Stiasny K, et al. Usutu virus infections among blood donors, Austria, July and August 2017—raising awareness for diagnostic challenges. *Euro Surveill*. 2017;22. <http://dx.doi.org/10.2807/1560-7917.ES.2017.22.41.17-00644>

6. Barbić L, Vilibić-Čavlek T, Stevanović V, Savić V, Klobučar A, Pem-Novosel I, et al. [“One health”—detection and surveillance of emerging and re-emerging arboviruses in Croatia]. *Croat J Infect.* 2015;35:53–60.
7. Vilibić-Cavlek T, Barbić L, Ljubin-Sternak S, Pem-Novosel I, Stevanović V, Gjenero-Margan I, et al. [West Nile virus infection: re-emergent disease in Croatia]. *Lijec Vjesn.* 2013;135:156–61.
8. Health ClOIP. Communicable diseases in Croatia in 2016 [cited 2018 Feb 15].
<https://www.hzjz.hr/en/news/communicable-diseases-in-croatia-in-2016>
9. Michel F, Fischer D, Eiden M, Fast C, Reuschel M, Müller K, et al. West Nile virus and Usutu virus monitoring of wild birds in Germany. *Int J Environ Res Public Health.* 2018;15:171.
<http://dx.doi.org/10.3390/ijerph15010171>
10. Ziegler U, Seidowski D, Angenwoort J, Eiden M, Müller K, Nowotny N, et al. Monitoring of West Nile virus infections in Germany. *Zoonoses Public Health.* 2012;59(Suppl 2):95–101.
<http://dx.doi.org/10.1111/zph.12015>
11. Papa A, Perperidou P, Tzouli A, Castilletti C. West Nile virus—neutralizing antibodies in humans in Greece. *Vector Borne Zoonotic Dis.* 2010;10:655–8. <http://dx.doi.org/10.1089/vbz.2010.0042>
12. Kantzanou MN, Moschidis ZM, Kremastinou G, Levidiotou S, Karafoulidou A, Politis C, et al. Searching for West Nile virus (WNV) in Greece. *Transfus Med.* 2010;20:113–7.
<http://dx.doi.org/10.1111/j.1365-3148.2009.00964.x>
13. Pervanidou D, Detsis M, Danis K, Mellou K, Papanikolaou E, Terzaki I, et al. West Nile virus outbreak in humans, Greece, 2012: third consecutive year of local transmission. *Euro Surveill.* 2014;19:20758. <http://dx.doi.org/10.2807/1560-7917.ES2014.19.13.20758>
14. Papa A, Bakonyi T, Xanthopoulou K, Vazquez A, Tenorio A, Nowotny N. Genetic characterization of West Nile virus lineage 2, Greece, 2010. *Emerg Infect Dis.* 2011;17:920–2.
<http://dx.doi.org/10.3201/eid1705.101759>
15. Politis C, Tsoukala A, Hatzitaki M, Pappa A, Englezou A, Zafeiriou C, et al. West Nile virus (WNV) outbreak in Greece and blood safety measures. *Vox Sang.* 2011;101(Suppl1):42.
16. Politis C, Parara M, Kremastinou J, Hasapopoulou E, Iniotaki A, Siorenta A, et al. Associations of ABO, D, and Lewis blood groups and HLA class I and class II alleles with West Nile virus lineage 2 disease outcome in Greece, 2010 to 2013. *Transfusion.* 2016;56:2115–21.
<http://dx.doi.org/10.1111/trf.13667>

17. European Commission. Commission Directive 2004/33/EC of 22 March 2004 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components, 2004 [cited 2018 Apr 25]. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:091:0025:0039:EN:PDF>
18. European Commission. Commission Directive 2014/110/EU of 17 December 2014 amending Directive 2004/33/EC as regards temporary deferral criteria for donors of allogeneic blood donations 2014 [cited 2018 Apr 25]. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ%3AJOL_2014_366_R_0011
19. Krisztalovics K, Ferenczi E, Molnar Z, Csohan A, Ban E, Zoldi V, et al. West Nile virus infections in Hungary, August–September 2008. *Euro Surveill.* 2008;13:19030.
20. Velati C, Angelini P, Pupella S. State of the art: West Nile virus circulation surveillance in Italy and transfusion risk early prevention methods. *Transfus Clin Biol.* 2017;24:172–5. <http://dx.doi.org/10.1016/j.tracli.2017.06.019>
21. Connell J, Garvey P, Cotter S, Conway A, O’Flanagan D, O’Herlihy BP, et al. Two linked cases of West Nile virus (WNV) acquired by Irish tourists in the Algarve, Portugal. *Euro Surveill.* 2004;8:2517. <https://dx.doi.org/10.2807/esw.08.32.02517-en>
22. Lopez LA, Yang SJ, Hauser H, Exline CM, Haworth KG, Oldenburg J, et al. Ebola virus glycoprotein counteracts BST-2/Tetherin restriction in a sequence-independent manner that does not require tetherin surface removal. *J Virol.* 2010;84:7243–55. <http://dx.doi.org/10.1128/JVI.02636-09>
23. Tsai TF, Popovici F, Cernercu C, Campbell GL, Nedelcu NI. West Nile encephalitis epidemic in southeastern Romania. *Lancet.* 1998;352:767–71.
24. Figuerola J, Jiménez-Clavero MA, López G, Rubio C, Soriguer R, Gómez-Tejedor C, et al. Size matters: West Nile Virus neutralizing antibodies in resident and migratory birds in Spain. *Vet Microbiol.* 2008;132:39–46. <http://dx.doi.org/10.1016/j.vetmic.2008.04.023>
25. López G, Jiménez-Clavero MA, Tejedor CG, Soriguer R, Figuerola J. Prevalence of West Nile virus neutralizing antibodies in Spain is related to the behavior of migratory birds. *Vector Borne Zoonotic Dis.* 2008;8:615–21. <http://dx.doi.org/10.1089/vbz.2007.0200>
26. Bernabeu-Wittel M, Ruiz-Pérez M, del Toro MD, Aznar J, Muniain A, de Ory F, et al. West Nile virus past infections in the general population of southern Spain. *Enferm Infecc Microbiol Clin.* 2007;25:561–5. <http://dx.doi.org/10.1157/13111181>

27. Human Animal Infections and Risk Surveillance (HAIRS) group. Qualitative assessment of the risk that West Nile virus presents to the UK human population 2017 [cited 2018 Mar 11].
<https://www.gov.uk/government/publications/hairs-risk-assessment-west-nile-virus>