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TECHNICAL REPORT

Vector control practices and strategies against West Nile virus

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Contents

Figures

Tables

Abbreviations

Bti	*Bacillus thuringiensis israelensis*
US CDC	United States Centers for Disease Control and Prevention
ECDC	European Centre for Disease Prevention and Control
EU/EEA	European Union/European Economic Area
ENP	European Neighbourhood Policy
IRR	Incidence rate ratios
IVM	Integrated vector management
LV	Low volume
MLE	Maximum Likelihood Estimates
ULV	Ultra-low volume
WNV	West Nile virus

Glossary

Biological larviciding = control of mosquito larvae by introducing natural enemies/predators and/or microbial agents applied in the air and on the ground.

Chemical larviciding = control of mosquito larvae using chemical larvicides applied in the air and on the ground.

ULV adulticiding = ultra-low volume (ULV) application involves applying low rates of insecticides (with active ingredients that degrade rapidly in the environment) in the form of a fine aerosol spray, using an efficient droplet size range to target the flying adult vector in outdoor settings. This method is applied in the air and on the ground.

LV surface spray adulticiding = low volume spraying of insecticides (with long residual effect), applied directly onto surfaces/refugia where adult mosquitoes rest. This method is applied on the ground.

Executive summary

West Nile virus (WNV) is a vector-borne virus maintained and amplified in nature in an enzootic cycle between birds and mosquitoes which, under certain conditions, can spill over to humans and equines. To mitigate the risk of WNV being transmitted to humans and animals, European countries have been investing significant resources in vector surveillance and control interventions, but with little common knowledge on their effectiveness and no EU-wide strategy or technical guidance to properly apply these methods and evaluate their efficacy. To help address these gaps, ECDC has conducted a survey to collect information on the current WNV surveillance and control capacities across European Union/European Economic Area (EU/EEA) countries, European Neighbourhood Policy (ENP) partner countries[1] and EU candidate/potential candidate countries[2] to identify the major challenges faced by public health authorities when implementing WNV control strategies. In addition, ECDC has also carried out a scoping literature review to collate existing knowledge and operational experience on the effectiveness of vector control practices in reducing WNV risk.

Eighty-three percent of the EU/EEA countries, ENP partner countries and EU candidate/potential candidate countries have implemented at least one method of WNV surveillance. The most common passive WNV surveillance method for all countries is the detection of human cases, followed by surveillance of dead animals. The most common method of active WNV surveillance is mosquito screening, followed by sentinel bird screening and sentinel equid screening for EU/EEA countries, ENP partner countries and EU candidate/potential candidate countries. The majority of the countries conduct routine vector surveillance (abundance monitoring). However, less than 15% of the EU/EEA countries, ENP partner countries and EU candidate/potential candidate countries perform pesticide resistance testing, and even in those countries that do, this is not implemented systematically. The majority of the EU/EEA countries that do not implement vector control have no history of autochthonous WNV human cases. Among countries that implement vector control (58%), the most widely-adopted methods are biological larviciding and public education, while the least widely-adopted method is source reduction through environmental management. Among ENP partner countries and EU candidate/potential candidate countries, the most widely-used methods are public education, followed by biological larviciding, chemical larviciding and low volume (LV) surface spray adulticiding. A substantial number of countries include adulticiding in their vector management response strategies, including ground-level ultra-low volume (ULV) space spraying, aerial ULV adulticiding and ground-level LV surface spraying. EU/EEA countries apply larviciding interventions (in any form) in response to WNV vector abundance (larval density) data, however the most important trigger by far for any ULV adulticiding treatment is the occurrence of autochthonous WNV human cases. In ENP partner countries and EU candidate/potential candidate countries, all available vector control tools (including adulticiding) are routinely implemented in response to vector abundance data.

The scoping literature review targeted published, peer-reviewed manuscripts on controlled studies assessing the impact of operational WNV vector control strategies using entomological indicators (adult vector abundance and WNV prevalence in mosquitoes), and/or veterinary indicators (enzootic circulation in sentinel/wild animals), and/or assessing the impact of vector control directly on human cases. Twelve studies were identified that satisfied the above criteria, the majority of which were conducted in the United States. According to the available scientific evidence, aerial ULV adulticiding is currently the only method directly linked to (a) reduction in WNV circulation levels using entomological/veterinary indicators, (b) interruption of WNV enzootic amplification in a natural-wetland environment and (c) interruption of WNV transmission in urban areas resulting in fewer human cases. Source reduction (when feasible) and larviciding interventions are justifiably the first and most important step in reducing and sustaining vector populations at low levels. There is, however, a critical absence of evidence linking the impact of preventive intervention (such as larviciding) to reductions in WNV circulation and transmission levels.

In general, survey participants identified limited availability of insecticidal active substances, lack of long-term registration for products/methods, a complex regulatory framework for the use of biocidal products, and lack of EU-wide technical guidelines as significant barriers to effective vector control operations. To address the above issues, the first and most important step is to increase communication between all relevant stakeholders. Moreover, studies are needed on the impact of vector control measures in order to better inform public health policy decisions on strategies for WNV management.

[1] The European Neighbourhood Policy partner countries are: Algeria, Armenia, Azerbaijan, Belarus, Egypt, Georgia, Israel, Jordan, Lebanon, Libya, Moldova, Morocco, Palestine*, Syria, Tunisia and Ukraine.

* *This designation shall not be construed as recognition of a State of Palestine and is without prejudice to the individual positions of the Member States on this issue.*

[2] Under the terms of the EU enlargement policy, the EU candidate countries are Albania, Republic of North Macedonia, Montenegro, Serbia and Turkey and the potential candidate countries are Bosnia and Herzegovina and Kosovo†

† *This designation is without prejudice to positions on status and is in line with UNSCR 1244 and the ICJ Opinion on the Kosovo Declaration of Independence.*

1. Background

West Nile virus (WNV) infection represents a serious burden to human and animal health worldwide because of the capacity of the virus to disperse quickly and adapt to a large variety of environments, causing large epidemics. In Europe, the virus was detected for the first time in 1958 in Albania and sporadically recorded until 1996, when a major outbreak of infection occurred in Romania, characterised by a high fatality rate (10%) with 393 confirmed cases [1]. Since then there has been a continuous increase and spread of reported WNV cases in humans and horses across Europe. This can partly be attributed to improved surveillance and diagnostic capacities, but also to the introduction and establishment of WNV lineage 2 strains which have been responsible for major outbreaks in central European and eastern Mediterranean countries, such as Greece, Serbia, Italy, Hungary and Austria [2]. In 2018, an extraordinary increase in human cases of WNV across the EU/EEA and neighbouring countries was observed, with the total number of human infections (n=2 083) far exceeding the total for the previous seven years (n=1 832) [3]. Furthermore, the distribution of the virus expanded northwards, with the first equine outbreak reported in Germany in 2018, followed by autochthonous human cases in 2019 [3,4]. This unprecedented rise in disease incidence, combined with the rapidly growing geographic expansion of the virus indicates the urgent need for an increased capacity to address and contain the threat across Europe.

In 1999, WNV invaded the United States (USA) and within four years spread rapidly across the country, despite intensive organised vector control efforts to prevent invasion [5]. A total of 51 747 WNV human cases and 2 381 deaths (as of January 2020) have been recorded since the introduction of the virus, which is currently considered to be the leading cause of mosquito-borne disease in the continental USA [6,7]. Significant research efforts have been dedicated to deciphering the complex ecology of the virus in order to better predict and contain outbreaks, while significant improvements have been made to vector surveillance and control programmes across the country. However, the primary factors preventing the reduction of WNV in the USA have been identified as the uneven distribution of adequate surveillance and control capacities (leaving gaps in preparedness and response) [8], inconsistent vector surveillance activities and delays in acting upon surveillance indicators [9].

There is a plethora of laboratory-based and small-scale field studies demonstrating the impact of vector control methods on the abundance of WNV vectors. However, controlled, large-scale, operational studies assessing the impact of control methods on vector abundance, while making the link between entomological efficacy and epidemiological impact, are scarce. Moreover, the vast majority of these studies have been conducted in the USA [10]. Such studies are very demanding in terms of resource requirements (technologies, tools, expertise), committed funding and interdisciplinary collaboration (entomologists, epidemiologists, regulators, physicians and vector control professionals in academia, state institutions, industry and the private sector). The knowledge and experience accumulated in the USA from these few studies is valuable. Nevertheless, it is important to understand the operational context of vector control studies in order to properly relate, compare and interpret them within a European context. The operational context takes into account the environmental, financial, regulatory and societal parameters defining a specific region. The impact of any vector control tool applied at an operational level, even when it is applied by the most experienced professionals with the best available technologies, is always limited by the following factors:

- environmental (i.e. a treatment can only be effective if it reaches the target at the effective dose and physical, or other obstructions commonly prevent homogeneous treatments);
- financial (i.e. treatment cost and budget availability directly affects the scale and frequency of interventions);
- regulatory (i.e. legislation can restrict access to larval habitats, or limit frequency of treatments), and societal (i.e. societal acceptability of methodologies).

European national (country-based) and local (region/municipality-based) public health agencies are important actors in the defence against mosquito-borne disease. National and regional WNV surveillance and response plans are in place in some countries [11-13], with a variety of WNV risk mitigation strategies and vector control interventions.

This report aims to identify and evaluate the operational challenges each country is facing in implementing vector control and to prioritise the needs to be addressed in order to facilitate national public health authorities in developing and/or enhancing their national WNV response capacities. In addition, existing knowledge and operational experience on the effectiveness of vector control practices in reducing WNV risk was collated through a scoping literature review. This review aimed to identify, present and discuss studies on applied aspects of vector control under specific operational scenarios of WNV management, to establish what worked, where and why, and equally, to ascertain what did not work and why.

2. Methods

Survey

The survey was administered online via the EU Survey tool and targeted all EU/EEA countries (n=31), EU candidate and potential candidate countries (n=7) and ENP partner countries (n=16). The survey was distributed via the local focal points of ECDC's Emerging and Vector-borne Diseases (EVD) network and representatives from the ENP partner countries and EU candidate and potential candidate countries. The focal points were responsible for reaching out to national vector control authorities and assembling the information requested by the survey. Where applicable, sub-national/regional representatives also completed the survey. The survey consisted of 13 questions (Table 1) addressing four main topics:

- WNV surveillance capacities (vector abundance, WNV surveillance through mosquito and sentinel animal screening, dead animal surveillance, human case detection, pesticide resistance testing);
- WNV vector control capacities;
- criteria for implementing WNV vector control;

challenges and constraints relating to vector control implementation.

The last question (number 13) was a general open question, allowing respondents to elaborate on their general experience in relation to WNV vector management in their countries or regions. For the complete questionnaire, see Annex 1.

Table 1. Summary of questions asked in the survey

WNV status	
1	What is the current WNV risk level applied to your country/region?
Surveillance	
2	Is WNV surveillance conducted at national, regional or local level?
3	Who conducts surveillance in your country?
4	Who funds WNV surveillance in your country?
5	Which WNV surveillance activities are performed in your country?
Vector control	
6	Who conducts vector control in your country?
7	Who funds vector control in your country?
8	What specific control measures are implemented, depending on different risk levels?
9	What are the most important constraints for implementing each vector control measure?
10	Vector control methods: evaluation
Guidelines	
11	Do you need technical guidelines for WNV mosquito vector surveillance?
12	Do you need technical guidelines for WNV mosquito vector control?
Open question	
13	Additional information issues/challenges.

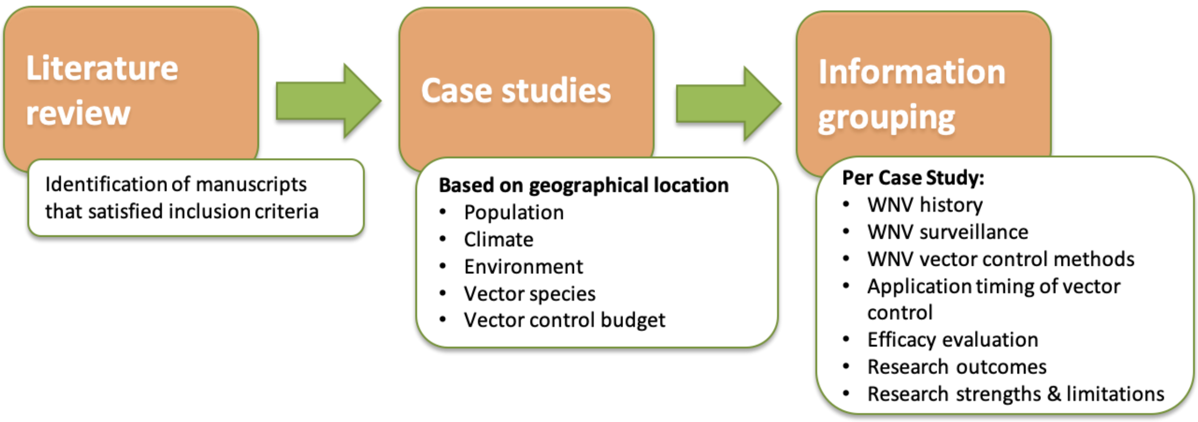
Literature review

The scoping review focused on peer-reviewed, controlled studies published within the last two decades. The following inclusion criteria were used: (a) studies assessing the impact of operational WNV vector control strategies using entomological indicators (adult vector abundance in parallel to WNV prevalence in mosquitoes); (b) studies assessing the impact of operational WNV vector control strategies on enzootic circulation of WNV (sentinel/wild animals); (c) studies assessing the impact of operational WNV vector control strategies on human cases. Only studies applied under field conditions – defined as any community or environment where WNV vectors naturally occur with ongoing WNV transmission – were included. Due to the limited studies available in Europe demonstrating the impact of vector control on WNV circulation levels, an additional search was conducted for studies with efficacy estimates solely based on entomological outcomes in the European region, demonstrating the impact of larviciding/adulticiding methods against adult *Culex* populations under operational settings. Non-operational studies, or studies assessing the impact of control methods based solely on larval density (without making a link to the impact on adult vector populations) were excluded. The most recent effort to review WNV vector control measures in Europe was published in 2014 [14], covering studies published until 2011 and our efforts therefore focused on the post-2011 period.

A literature review was performed by searching bibliographic databases (PubMed), Internet sources (Google Advanced search) and the websites of relevant public health and vector control authorities – i.e. US CDC, ECDC, the European Mosquito Control Association (EMCA), the American Mosquito Control Association (AMCA), ministries of health/local departments of public health, mosquito and vector control districts – to identify studies evaluating WNV vector control practices. Studies (including review articles) found were also used to identify other relevant studies and websites. The searches were limited to the English language and combined the concepts within the scope of this project: WNV vector management strategies, specific control methods/tools (e.g. larviciding, adulticiding) and their impact on WNV circulation levels. Search terms were in three main groups: disease-relevant terms, vector-relevant terms and intervention-relevant terms. For the disease group, the terms were 'West Nile virus', 'West Nile fever' and 'WNV'. For the vector group the terms were '*Culex*' and '*Culex pipiens*'. For the intervention category the terms were 'integrated vector management', 'larviciding', 'adulticiding' and 'source reduction'. To increase the focus on Europe, the above terms were combined (through the AND operator) with specific European countries having a long history of WNV outbreaks: Italy, Greece, France and Serbia.

The manuscripts that satisfied the inclusion criteria were further categorised into 'case studies' based on the geographic location of the interventions (more than one manuscript fitting the criteria could apply to one case study) (Figure 1). For each case study, an additional literature search was conducted to retrieve information on the history of WNV transmission, ecological parameters related to WNV transmission (environment, climate, vectors) and vector control capacities (resources, budget) associated with the specific geographical region. All relevant information was presented in the following format: study site, land area and population, climate (e.g. temperate, arid), environment (e.g. urban, agricultural), vector species targeted (e.g. *Culex pipiens*), budget of organised vector control programmes (where applicable), vector control methods applied (e.g. larviciding, adulticiding), WNV surveillance methods applied, timing of interventions (before or after human cases, or both), assessment/evaluation of efficacy (mosquito infection rates, and/or sentinel animal seroconversions, and/or impact on human cases, all of the above) and main conclusions. Where necessary, WNV management experts involved in the selected case studies were approached to provide additional information or clarifications relating to their research.

Figure 1. Methodological process for presenting information retrieved from the literature review



3. Results

The survey

Participating countries

In total, 47 responses were received (39 from national authorities, eight from regional authorities) from 25 EU/EEA countries, six ENP partner countries and six EU candidate/potential candidate countries (Table 2). Two of the participating countries submitted two questionnaires each at national level, however only one questionnaire per country was included in the analysis. One questionnaire form was only partially completed (responses provided for Questions 1, 11, 12) and therefore excluded from the analysis of the remaining questions. Data were analysed separately in two groups – one for EU/EEA countries and one for ENP partner and EU candidate/potential candidate countries, and the results are reported in two sections accordingly. For all questions, results are presented at country level (except for Questions 8 and 9 where responses from the eight regional authorities were included in the analysis).

Table 2. Participating countries in the survey per group

European Union/EEA		EU Neighbourhood Policy		EU Enlargement Policy	
Austria	√	Algeria		Albania	√
Belgium	√	Armenia	√	Bosnia Herzegovina	√
Bulgaria	√	Azerbaijan		Kosovo	√
Croatia	√	Belarus		Montenegro	√
Republic of Cyprus	√	Egypt	√	Republic of North Macedonia	√
Czech Republic	√	Georgia	√	Serbia	√
Denmark		Israel		Turkey	
Estonia		Jordan			
Finland	√	Lebanon			
France	√	Libya	√		
Germany	√	Moldova			
Greece	√	Morocco			
Hungary	√	Palestine	√		
Ireland	√	Syria			
Italy	√	Tunisia			
Latvia	√	Ukraine	√		
Lithuania	√				
Luxembourg	√				
Malta	√				
Netherlands	√				
Poland					
Portugal					
Romania	√				
Slovakia	√				
Slovenia	√				
Spain					
Sweden	√				
Iceland (EEA)					
Liechtenstein (EEA)	√				
Norway (EEA)	√				
United Kingdom (former EU member)	√				

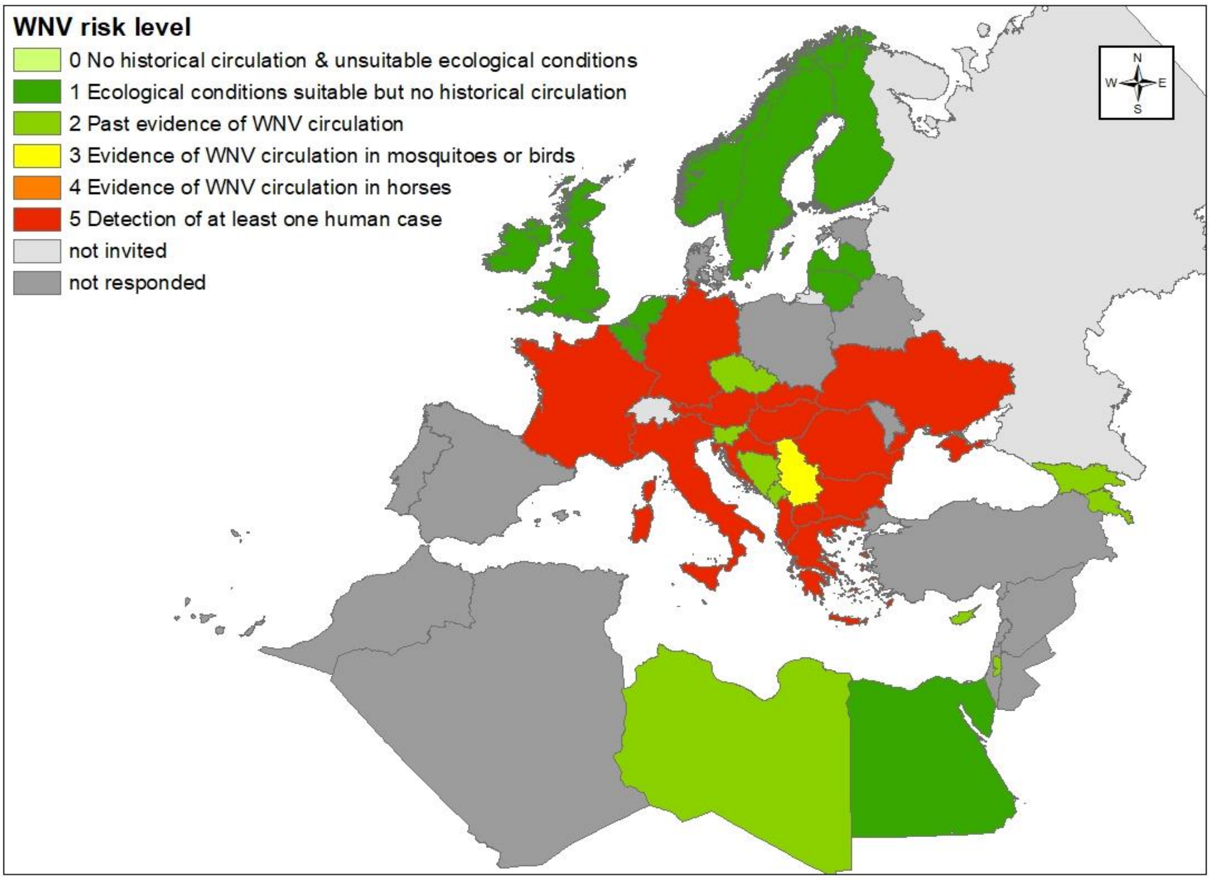
√ = responded

WNV risk level of participating countries (Question 1)

According to the responses received from 25 EU/EEA countries, 40% reported at least one WNV human case (according to EU case definition) in 2019 (risk level 5 according to ECDC's WNV risk assessment tool, Figure 2). In 12% of the countries there is past evidence of WNV circulation (risk level 2), while in 48%, risk level 1 is applicable (ecological conditions suitable but no historical transmission).

Four EU candidate/potential candidate countries declared that they had WNV circulation during 2019. Two of those countries declared detection of at least one human case (risk level 5), one declared WNV detection in horses (risk level 4) and one declared evidence of WNV circulation in mosquitoes or birds (risk level 3). Among the participating ENP partner countries, one reported at least one human case during 2019 (risk level 5). Four countries declared past evidence of WNV circulation (risk level 2) and one declared risk level 1 as being applicable (ecological conditions suitable but no historical circulation).

Figure 2. WNV risk level for EU/EEA, EP, ENP partner countries, 2019*



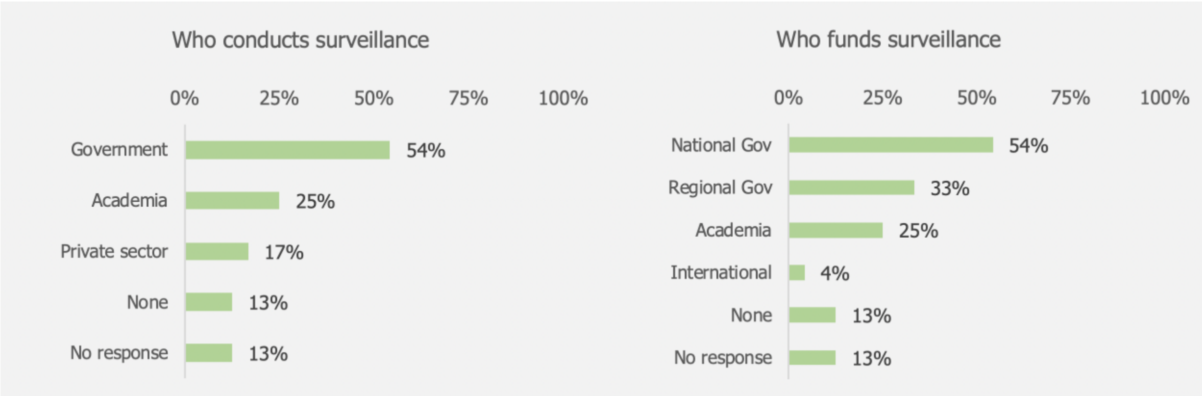
**The risk levels are defined in accordance with ECDC's WNV risk assessment tool available at:*
https://www.ecdc.europa.eu/sites/default/files/media/en/publications/Publications/west-nile-virus-risk-assessment-tool.pdf

Section 1. EU/EEA countries

WNV surveillance implementation and funding (Questions 2, 3 and 4)

WNV surveillance is implemented by the state, academia, and private sector. In 54% of responses, the state is involved in surveillance implementation, followed by academia and the private sector (25% and 17%, respectively). In most countries, surveillance activities are funded by national and/or regional sources (54% and 33%, respectively). In 25% of the countries, academia is involved in funding surveillance (Figure 3).

Figure 3. WNV surveillance implementation and funding sources (N=24)

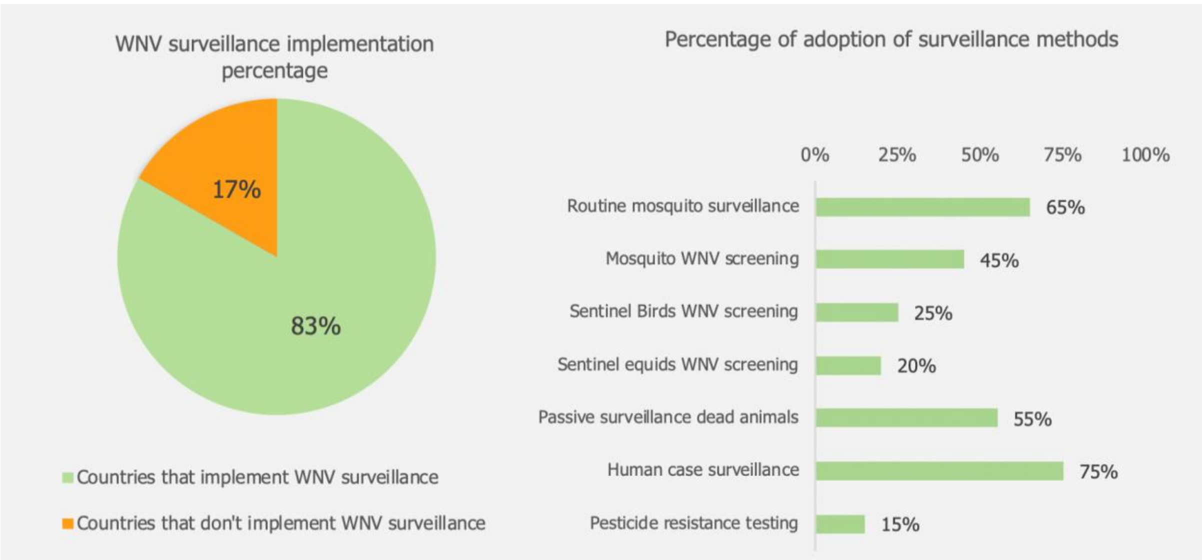


WNV surveillance capacities (Question 5 & 11)

Most countries (83%) implement at least one method of active or passive WNV surveillance (Figure 4). The most common passive WNV surveillance method is the detection of human cases (75%), followed by surveillance of dead animals (55%). The most common method of active WNV surveillance is mosquito screening, followed by sentinel bird screening and sentinel equid screening. The percentage of countries that implement routine mosquito surveillance (mosquito abundance monitoring) is 65%. Human WNV infection surveillance is performed in 75% of the EU/EEA countries. Only 15% of the countries perform pesticide resistance testing. Sixty-eight percent of the countries indicated that there is a need for technical guidelines on WNV surveillance.

Figure 4. WNV surveillance methods among EU/EEA countries (N=24)

Left panel: Percentage of countries that implement at least one surveillance method.
Right panel: Percentage of adoption – surveillance methods among countries implementing surveillance.



WNV vector control implementation and funding (Questions 6 & 7)

In the EU/EEA, 58% of countries implement at least one vector control method. Vector control activities are funded and implemented by a combination of state (national, regional) and private institutions (Figure 5). In 21% of the countries vector control is conducted and funded exclusively by the state, whereas in 8% of the countries vector control is conducted exclusively by the private sector.

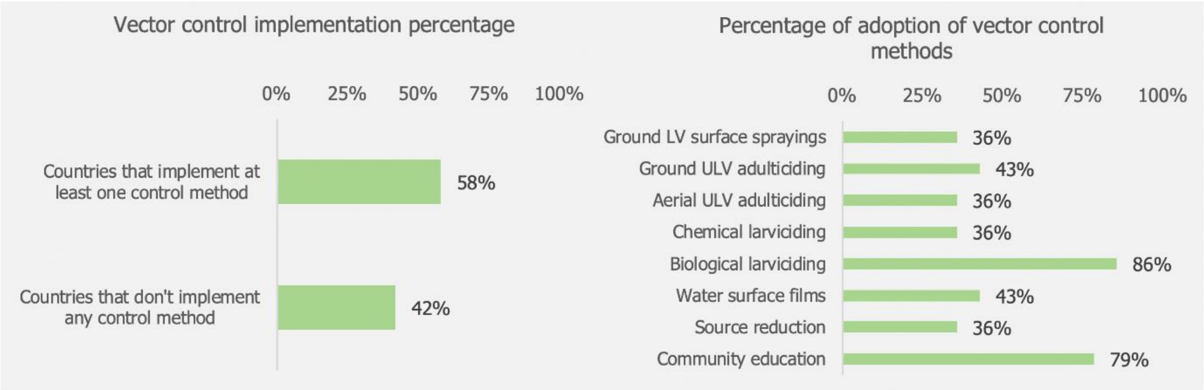
Figure 5. WNV vector control implementation and funding sources among EU/EEA countries (N=24)

WNV vector control capacities and implementation triggers (Question 8)

The most common control methods in EU/EEA countries are biological larviciding (86%) and public campaigns (79%), followed by ground ULV adulticiding (43%), application of water surface films (43%), aerial ULV adulticiding (36%), ground LV surface sprayings (36%), chemical larviciding (36%) and source reduction (36%, Figure 6). In total, 84% of respondents agreed that vector control guidelines were needed.

Figure 6. Vector control capacities among EU/EEA (N=24)

Left panel: Percentage of countries implementing vector control.
Right panel: Percentage of adoption – vector control methods.



For any adulticiding treatment (ground ULV, aerial ULV or ground LV), the most important trigger by far is the detection of WNV human cases, followed by the detection of WNV-positive mosquitoes/birds and a high adult vector population (Table 3).

Larviciding by chemical, biological, or mechanical means (water surface films) is applied in response to high vector abundance (larval indices) by 71%, 83%, and 83% of the countries/regions (Table 3). All larviciding methods are intensified in response to WNV human cases. Larval source reduction is a strategy adopted by 90% of the countries/regions in response to high vector abundance (Table 3). Community outreach interventions become progressively more intensive as the WNV risk level increases; 60% of the countries/regions routinely perform community outreach activities in response to high vector populations and intensify their efforts depending on the WNV risk level (100% of the countries/regions perform community outreach in response to WNV human cases).

Table 3. Percentage of EU/EEA countries implementing specific vector control in response to four different triggers

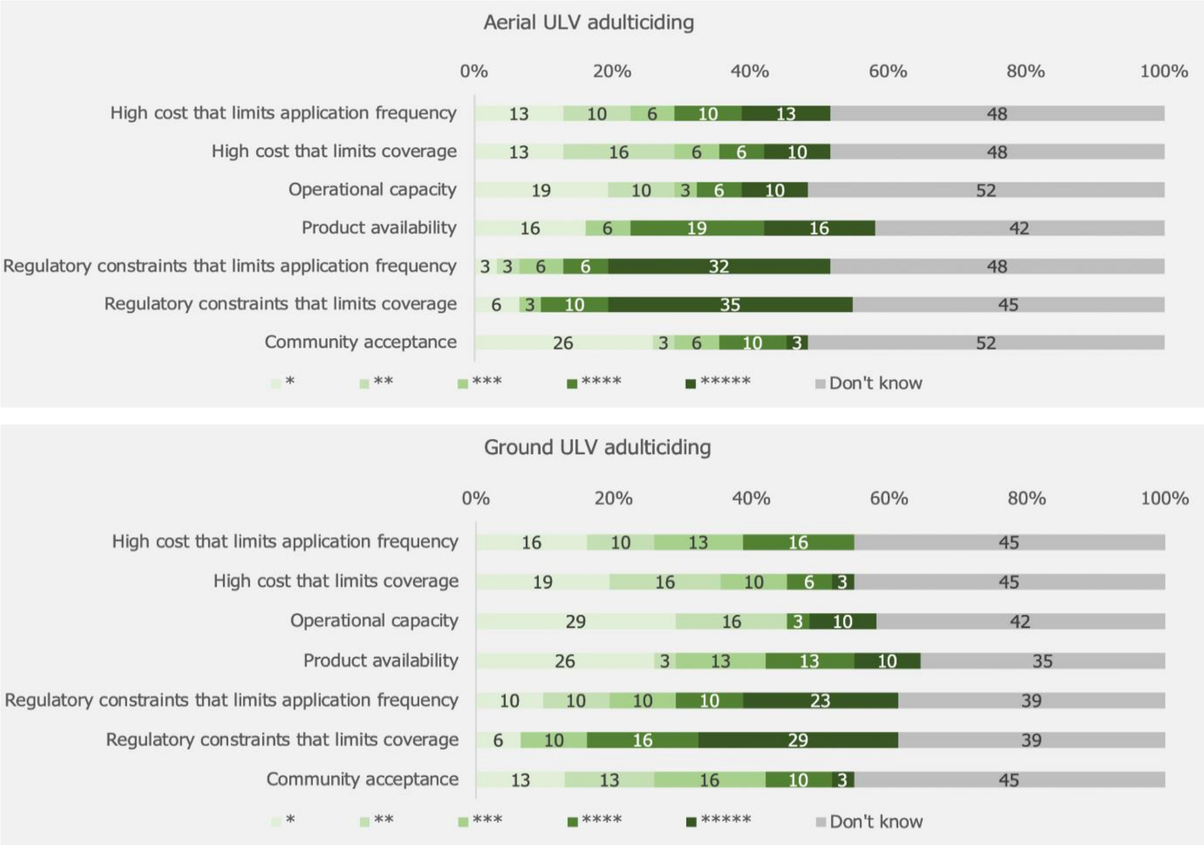
Vector control target	Vector control intervention	Trigger for vector control implementation			
		High vector population	WNV-positive mosquitoes or birds	WNV-positive horses	WNV human cases
Adults	Aerial ULV	50%	50%	17%	100%
	Ground ULV	33%	33%	17%	100%
	Ground LV	58%	58%	50%	100%
Immature stages	Chemical larviciding	83%	92%	83%	92%
	Biological larviciding	71%	76%	71%	82%
	Water surface films	83%	83%	75%	100%
	Source reduction	90%	80%	90%	100%
	Community outreach	60%	80%	73%	100%

Constraints and limitations relating to vector control (Question 9)

Question 9 aimed to identify the specific constraints/challenges (such as regulatory constraints, limited availability of products, operational capacity, cost) faced by each country/region in implementing the various WNV vector control methods. The respondents were asked to rate the constraints associated with each method from one to five (with five being the strongest level of constraint, and one being the weakest). If the participants had no experience of the method, they were asked to leave it blank.

More than 40% of the respondents declared lack of knowledge relating to the constraints or limitations in implementing all vector control methods. More than 40% of the respondents agreed that the most important challenges for adulticiding are regulatory constraints, limiting either the frequency of the treatments or the size of the treated areas (Figure 7). Product availability, followed by high cost are also considered to be important constraints for the application of aerial and ground ULV adulticiding in some countries. For chemical and biological larviciding the most important constraints are product availability and a limited number of products with long residual activity (Figure 8). High cost appears to be a more important constraint for biological larviciding than chemical larviciding (on average 18% of the respondents declared cost to be a concern for biological larviciding compared to 7% for chemical larviciding). Operational capacity followed by community acceptance, are the most important limitations to the implementation of larval source reduction and communication outreach/education campaigns.

Figure 7. Constraints and limitations relating to the implementation of adulticiding methods among EU/EEA countries



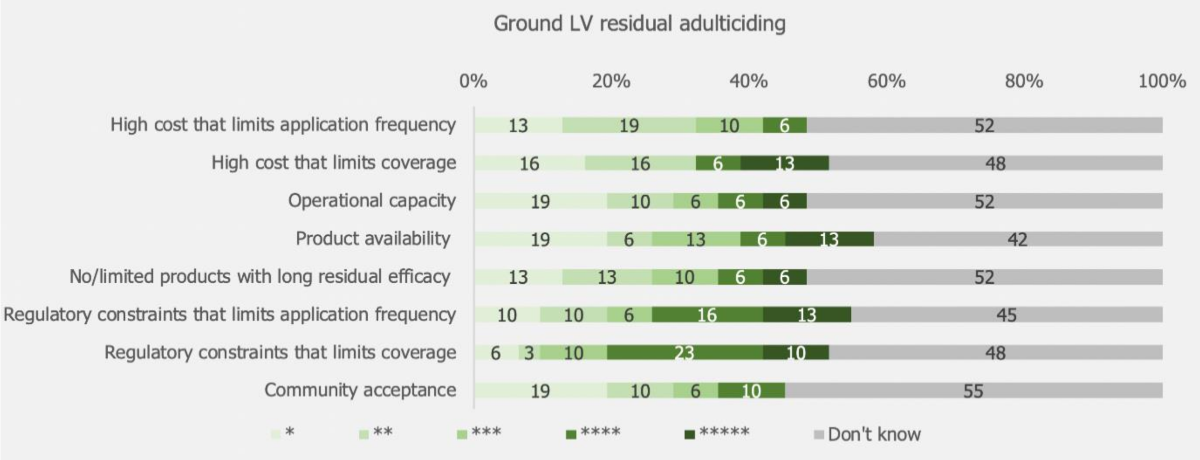
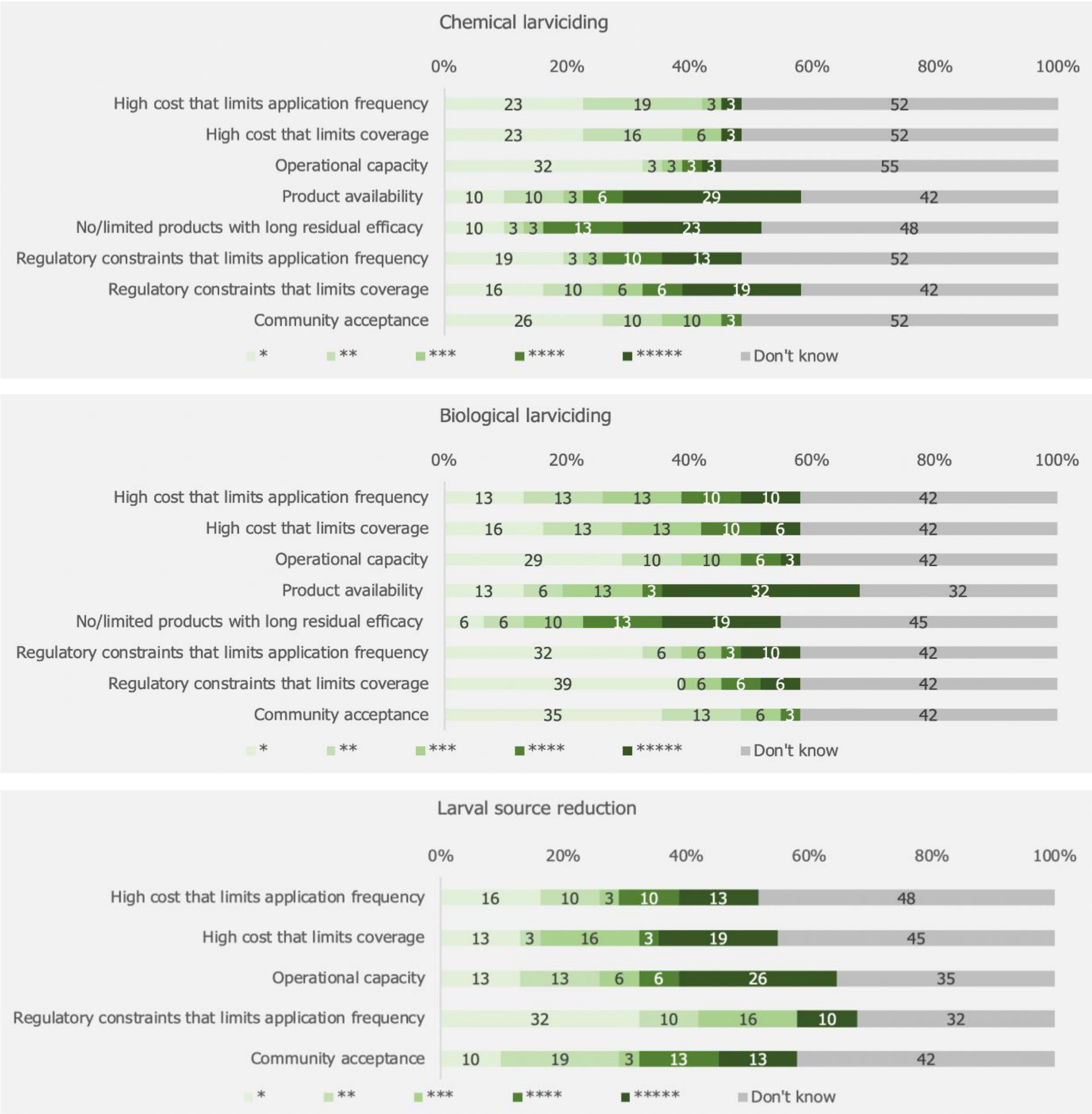
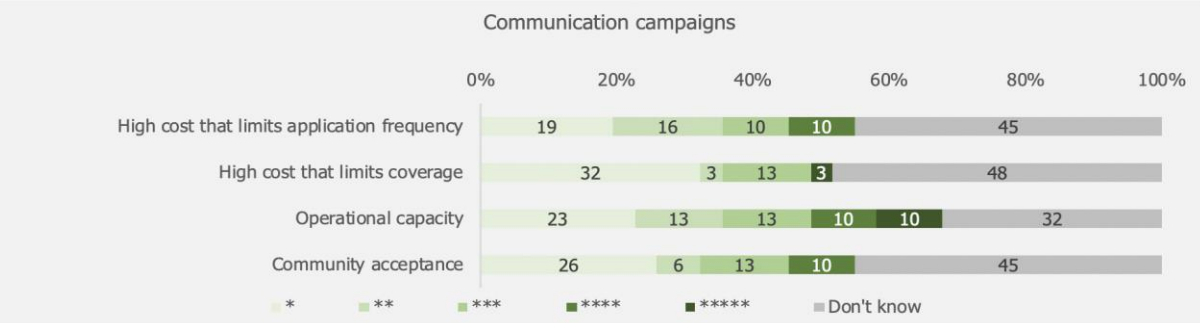


Figure 8. Constraints and limitations relating to the implementation of larviciding (biological, chemical), source reduction and community outreach campaigns among EU/EEA countries





Additional issues/challenges relating to WNV surveillance and control (Question 13)

In the last survey question, the respondents were asked to provide any additional information on issues or challenges that they are facing in WNV vector surveillance and control in their countries/regions. Among the 20 responses from EU/EEA countries, at least half were clear and detailed enough to provide insight on how certain issues affect surveillance and vector control at local level. The most frequently recurring issues are summarised below.

Regulatory constraints

Regulatory constraints were brought up repeatedly in many of the responses. Issues that were reported include limitations on the application parameters for the larvicides currently available, preventing areas from being treated, or receiving sufficiently frequent treatment. Large areas of natural wetlands may be excluded from treatment because of their protected status. Restrictions on application parameters of biological larviciding with *Bacillus thuringiensis israelensis* (*Bti*) (applications restricted to every 10 days and no more than eight applications per season) prevent the effective implementation of vector control programmes in some countries.

> ‘The current regulations for larviciding exempt large productive areas from being treated (NATURA). These areas are productive in WNV vectors and in proximity to residential areas. More than 20 000 hectares of productive natural wetlands are found in our region.’
>
> ‘Current regulations for *Bti* limit the number and frequency of applications. We can only make eight applications per area with a 10-day interval. Our needs are much greater than this, especially during the months of July and August when re-application is required every five days in some regions.’

Restrictions on adulticiding treatments are also mentioned. Respondents reported that the current regulatory framework limits aerial ULV adulticiding treatments to 2 km buffer zones from residential areas. Buffer zones also apply to ground ULV adulticiding treatment in some countries (100 m from residential areas). The respondents were concerned that these restrictions were affecting the efficacy of the treatment and also expressed their doubts about the scientific merit of these decisions.

> ‘Current regulations for aerial ULV (a very effective method in reducing *Cx. pipiens* abundance and WNV infection rates) limit the applications to 2 km distance from residential areas - thus decreasing the impact this method can have near people who are at risk of WNV infection.’
>
> ‘The current regulations for ground ULV applications are being restricted to 100 m from residential areas. We do not understand how these buffer zones are chosen, looks like a completely arbitrary decision.’

One respondent commented that the current regulatory framework is inflexible and does not account for environments with a high level of complexity and vector abundance, underlining the fact that all available vector control methods are needed in order to overcome the challenges in the field.

> ‘Dense vegetation and lack of access may prevent areas from being treated effectively with larvicides. ULV space sprays are susceptible to weather conditions and are affected by densely populated areas (tall, dense buildings) that may obstruct the treatment. It becomes clear that the challenges in the field (practical challenges) are such that they require a diverse set of methods and products. Also, realistic regulations are needed to facilitate the application of all available methods.’

Biocides availability

Concerns about the restricted number of registered products (larvicides and adulticides) and their limitations in efficacy were raised. For a wide range of very productive larval habitats, such as rice-fields and natural wetlands, the only registered product is *Bti*, which is known to be less effective in rich organic environments. Concerns about the development of pesticide resistance development, especially to pyrethroids, are also expressed, emphasising the need for a wider range of products and active ingredients to cope with this.

> ‘The most important challenge for us is the current regulatory framework that limits the number of products available for vector control. For example, we have only one product (*Bti*) available for larviciding.’

> 'The field context often limits the efficacy of the various control methods. For example *Bti* is not as effective in a heavily organic environment against *Cx. pipiens*.'

> 'For our adulticiding treatments we only have one group of chemicals (pyrethroids). With this limited number of active ingredients we are concerned about the development of insecticide resistance. We need a wide range of products that would allow for rotations to help prevent the development of resistance.'

Constraints related to budget and expertise

The issue of lack of funding was raised in multiple questionnaires. Some countries consider WNV surveillance/control not to be a high priority and therefore no investment is made in this area. Comments were also provided regarding the lack of expertise in the entomological and vector control sector.

> '(WNV) is not seen as a public health issue by many health authorities.'

> 'In our opinion, the main constraints for implementing a complete surveillance system on WNV are the lack of funding, alongside the shortage in human resources.'

> 'Limited local expertise, limited human resources, no local entomologists.'

> 'Lack of a network of entomologists with responsibilities for vector surveillance and control.'

Vector control budgeting issues were also identified by respondents and attributed to the low number of larvicides available on the market. In particular, the only product currently registered for use in natural wetlands and rice fields is costly and has a short residual effect, requiring recurrent application. This may have a significant effect on the sustainability of the larviciding programmes. Concerns were also raised about the creation of a 'monopoly' in Europe's biocide market.

> 'This product has a short residual impact (24 hours in liquid form) and re-application is needed, significantly raising the cost of our programme.'

> 'We are also concerned about the creation of a monopoly in the field of public health and we are worried for the ramifications this may have for the future of vector control in our country.'

Societal constraints

The unwillingness of the community to cooperate was identified as an important constraint. Difficulties in gaining access to private houses and reluctance to follow instructions provided by vector control personnel were reported.

> 'An important source of *Cx. pipiens* breeding sites are private houses – we spend significant resources to access private property and educate citizens, however often no access is granted to our personnel and also citizens often do not cooperate, despite receiving information and educational material about reducing vector breeding sites. We need to find a way to compel citizens to take action.'

> 'Even though we perform regularly door-to-door educational campaigns it is often difficult to gain access in private property. Also, bad practices in agriculture (e.g. intentional flooding for animal grazing) further increases the complexity of vector control.'

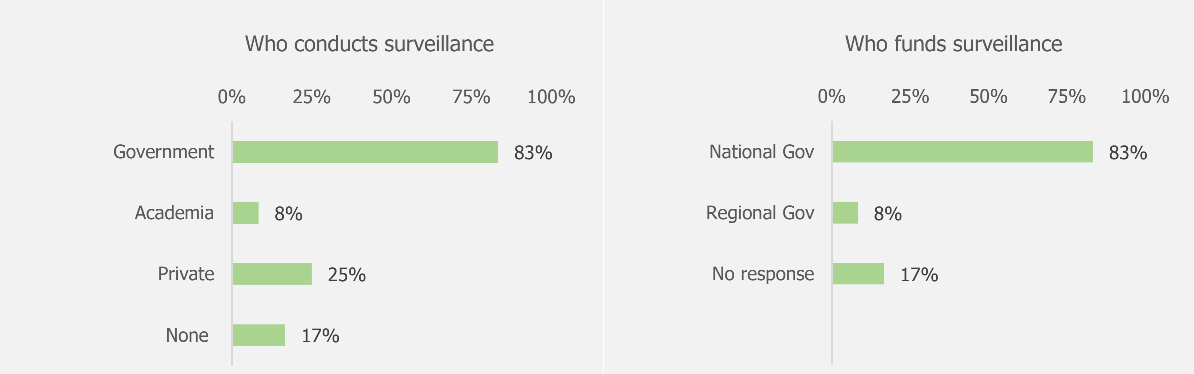
> 'Another challenge is raising public awareness and enthusiasm about domestic source reduction and personal protective measures.'

Section 2. EU Enlargement and ENP partner countries

WNV surveillance implementation and funding (Questions 2, 3 & 4)

WNV surveillance is implemented by the state, academia and the private sector (Figure 9). In 83% of cases the state is involved in surveillance implementation, followed by the private sector (25%) and academia (8%). Surveillance activities are funded exclusively by national and/or regional sources (83% and 17%, respectively).

Figure 9. WNV surveillance implementation and funding sources among ENP partner countries and EU candidate/potential candidate countries (N=12)

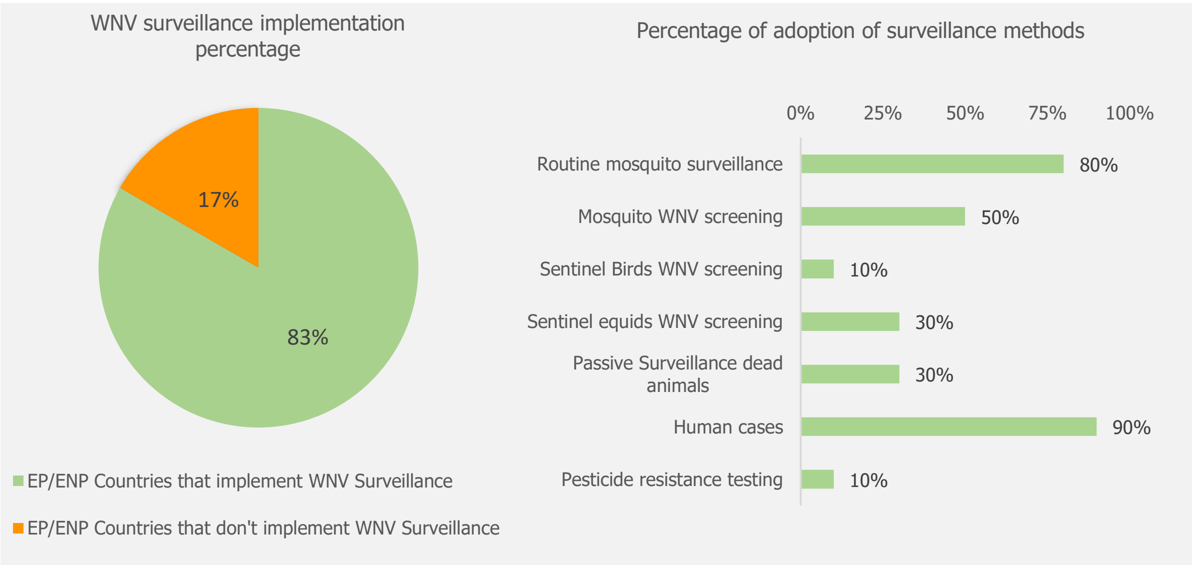


WNV surveillance capacities (Questions 6 & 7)

Most countries implement at least one method of active or passive surveillance (83%, Figure 10). The most common passive WNV surveillance method is the detection of human cases (90%), followed by surveillance of dead animals (30%). The most common method of active WNV surveillance is mosquito screening, followed by sentinel surveillance of equids (30%) and sentinel surveillance of birds (10%). Eighty percent of the countries within this group implement routine mosquito surveillance. Pesticide resistance testing is performed in 10% of the countries (Figure 10). All twelve countries in this group indicated a need for technical guidelines on WNV surveillance.

Figure 10. WNV surveillance methods among ENP partner countries and EU candidate/potential candidate countries (N=12)

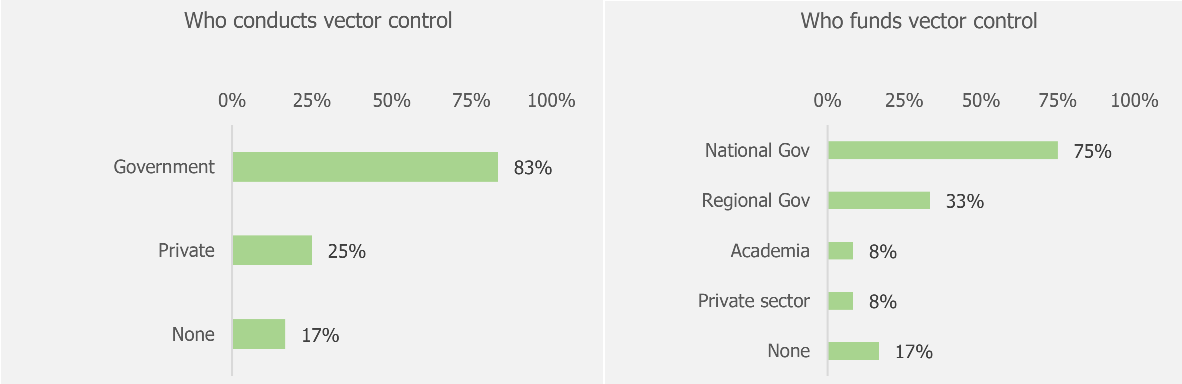
Left panel: Percentage of countries that implement at least one surveillance method.
Right panel: Percentage of adoption of surveillance methods among countries implementing surveillance.



WNV vector control implementation and funding

Vector control is conducted by the state in 83% of the countries, whereas the private sector is involved in 25% of cases. Vector control is funded by national and/or regional sources in 75% and 33% of the countries, respectively. In two countries, vector control is funded by academia, and in another two countries it is funded by the private sector (Figure 11).

Figure 11. Vector control implementation and funding sources among ENP partner countries and EU candidate/potential candidate countries (N=12)

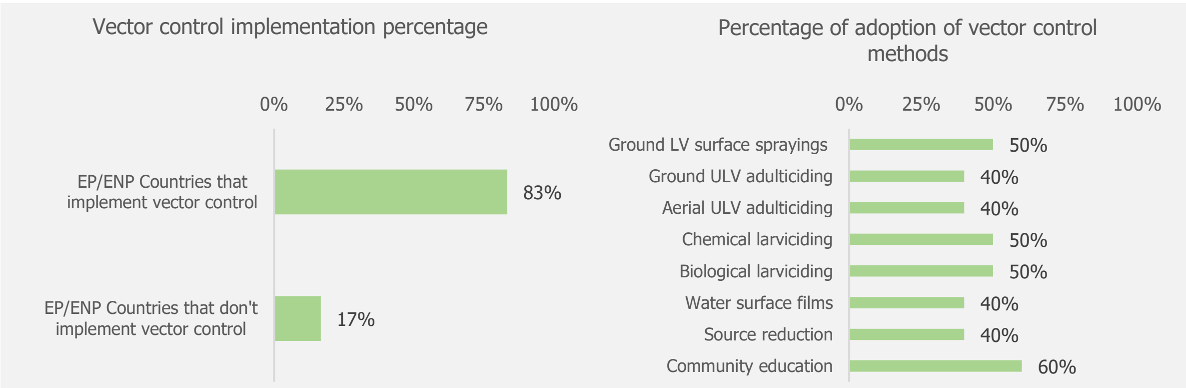


WNV vector control capacities and implementation triggers

Eighty-three percent of the ENP partner countries and EU candidate/potential candidate countries implement at least one vector control methodology (Figure 12). The most common vector control method is communication campaigns (60%), followed by chemical larviciding (50%), biological larviciding (50%) and ground LV surface spraying (50%). Aerial ULV adulticiding, ground ULV adulticiding, application of water surface films and larval source reduction are implemented in 40% of the countries. Eleven of the twelve countries confirmed the need for vector control guidelines.

Figure 12. Vector control capacities among ENP partner and EU candidate/potential candidate countries (N=12)

Left panel: Percentage of countries implementing vector control.
Right panel: Percentage of adoption – vector control methods.



Larviciding using chemical or biological means, larval source reduction and communication campaigns are performed in response to high vector abundance in all countries (100%, Table 4). Ground LV, aerial ULV and ground ULV adulticiding treatments are triggered in response to high vector population in 80%, 75% and 67% of the countries, respectively. All vector control interventions appear to be applied routinely on the basis of high vector abundance, irrespective of the WNV risk level.

Table 4. Percentage of countries implementing specific control in response to four different triggers

Vector control target	Vector control intervention	Trigger for vector control implementation			
		High vector population	WNV-positive mosquitoes or birds	WNV-positive horses	WNV human cases
Adults	Aerial ULV	75%	25%	25%	25%
	Ground ULV	67%	67%	33%	67%
	Ground LV	80%	40%	20%	40%
Immature stages	Chemical larviciding	100%	50%	25%	50%
	Biological larviciding	100%	25%	25%	25%
	Water surface films	75%	50%	25%	50%
	Source reduction	100%	50%	25%	50%
	Community outreach	100%	33%	17%	33%

Constraints and limitations relating to vector control (question 9)

More than 60% of the respondents declared a lack of knowledge relating to the constraints or limitations on implementing all vector control methods. Twenty-five percent of the respondents agreed that the most important challenge for both ground and aerial ULV adulticiding is high cost, limiting either the frequency of the treatment or the size of the treated areas (Figure 13). Furthermore, regulatory constraints are considered to be an important factor limiting the application of aerial ULV adulticiding in some countries. For chemical and biological larviciding the most important limitations are regulatory constraints and high cost, respectively (Figure 14). High cost and operational capacity are the most important limitations in the implementation of larval source reduction and communication outreach/education campaigns, respectively.

Figure 13. Constraints and limitations relating to the implementation of adulticiding methods

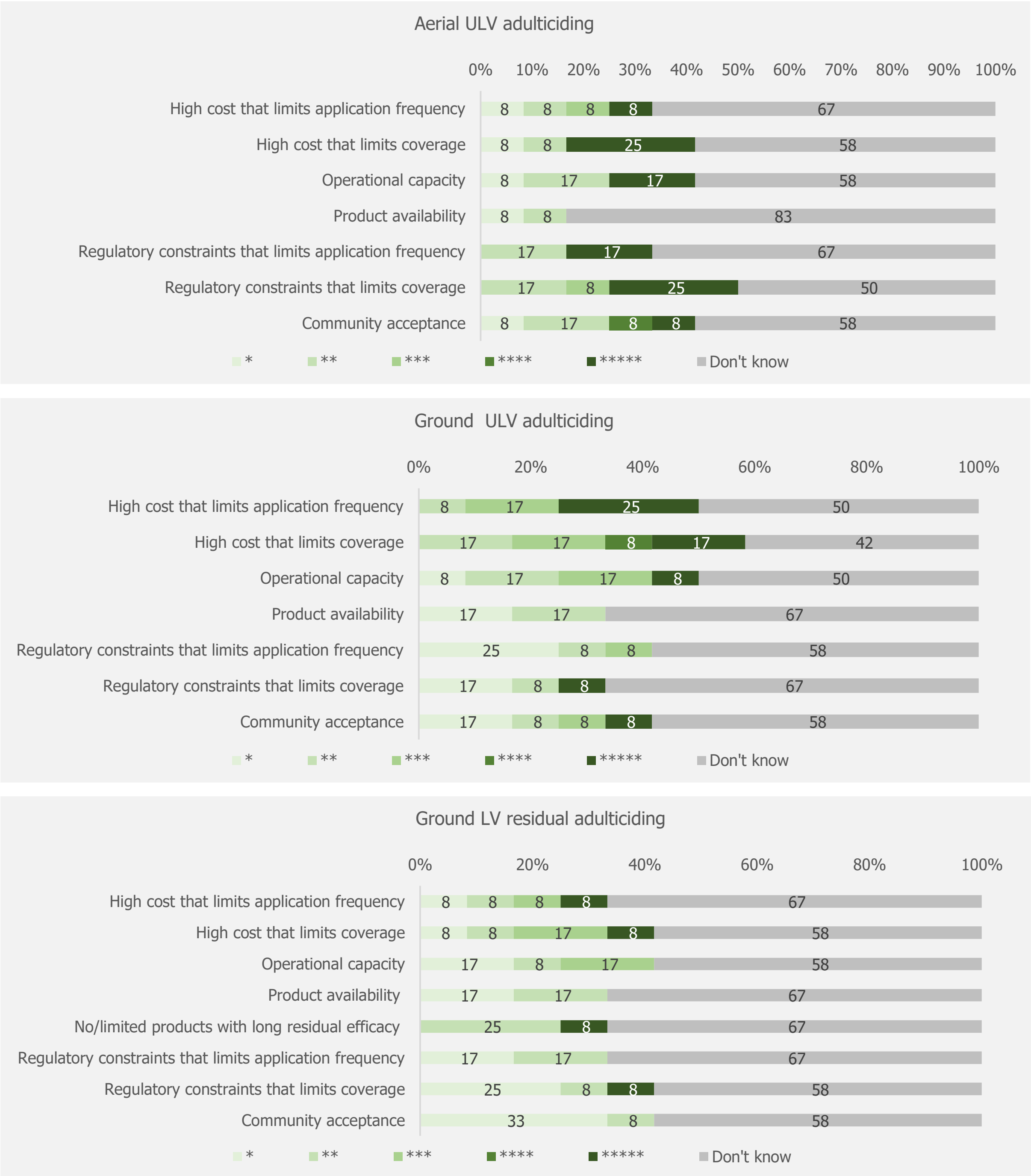
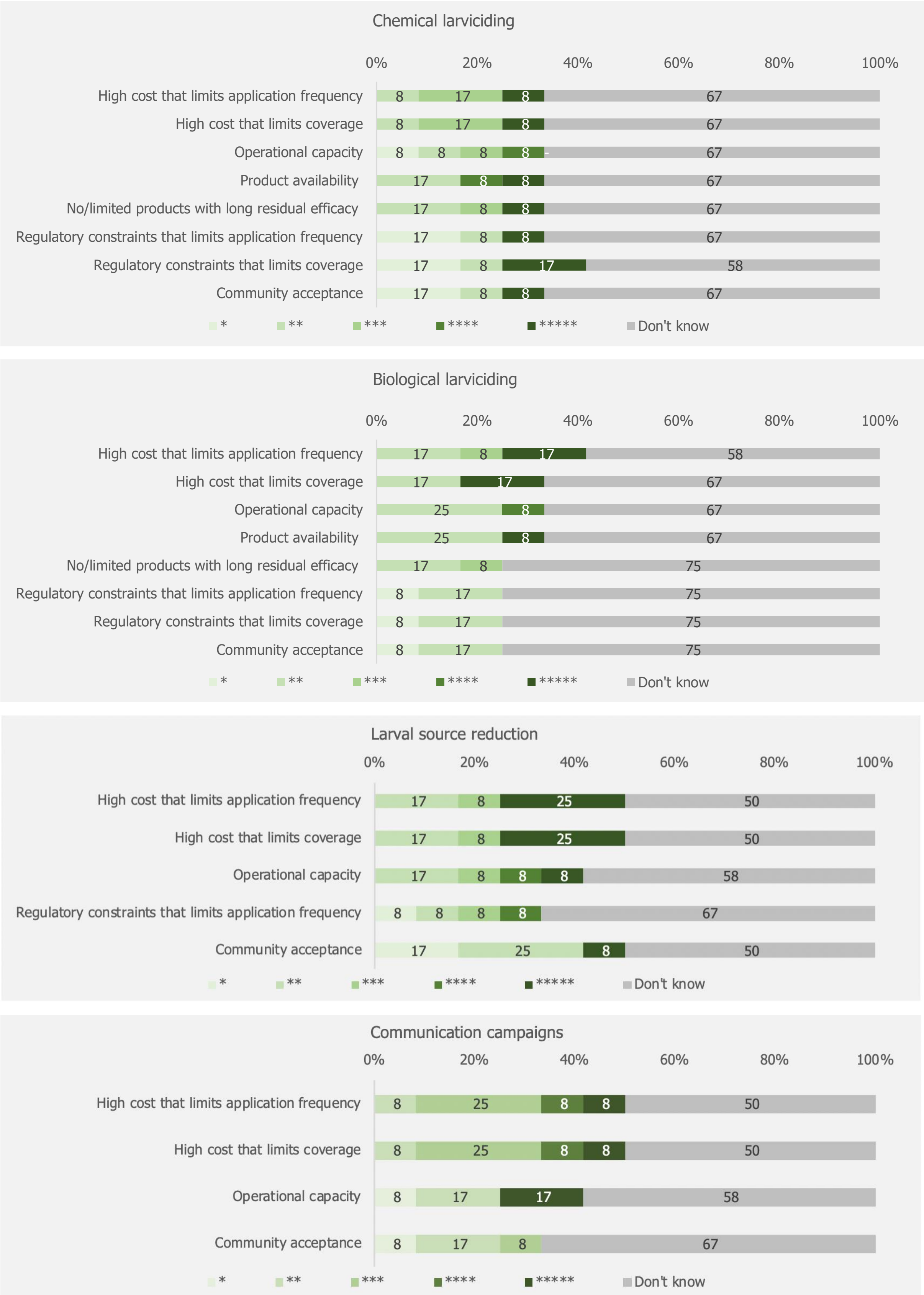


Figure 14. Constraints and limitations relating to the implementation of larviciding (biological and chemical), source reduction and community outreach campaigns



Literature review

Search results

A total of 389 manuscripts were identified from the databases and websites. After removing manuscripts which were outside the scope of WNV vector management (n=332), 57 manuscripts remained for closer analysis. The majority of these were laboratory based/semi-field studies, larviciding efficacy studies demonstrating impact solely on larval mortality, WNV control review documents and WNV vector surveillance studies. An additional two manuscripts were identified from the reference list for these manuscripts. One operational study in the EU was identified [15] during the post-2011 period demonstrating the impact of larviciding and adulticiding methods against adult *Culex* populations. The study was primarily designed to target *Aedes albopictus* and although efficacy against adult *Cx. pipiens* populations was reported, there was no link to or assessment made of the WNV transmission risk. In total, twelve manuscripts satisfied all inclusion criteria. These were divided into eight case studies, according to their geographical regions. Seven of the regions are in the USA (California, Louisiana, Texas, Georgia, Colorado, and Illinois) and one is in Europe (Greece) (Table 5).

- Case study 1. St. Tammany Parish, LA [16]
- Case study 2. Sacramento-Yolo Counties, CA [17-20]
- Case study 3. Coachella Valley, CA [21]
- Case study 4. Central Macedonia Region, GR [22]
- Case study 5. North Central Texas, TX [23,24]
- Case study 6. City of Chicago, IL [25]
- Case study 7. Atlanta, GA [26]
- Case study 8. Fort Collins, CO [27].

In case studies 1-4, WNV vector management interventions were conducted under the umbrella of a centralised, organised vector control programme. For case studies 5-8, surveillance and/or control interventions were supported by city or municipality-based health departments. Table 5 below summarises the experiences gained from these eight separate case studies in relation to WNV management.

Table 5. Overview of the case studies included in the analysis of this report

Case study	Land area* (km²)	Population*	Climate (Köppen classification)	Environment	Research manuscripts identified	WNV vector species targeted	Treatments were conducted under the umbrella of a centralised state funded vector control programme (annual budget provided)**	Vector control funding per capita/per km²
1. St. Tammany Parish, LA, USA	2 188	258 111	Humid-sub-tropical	Urban/rural, natural wetlands (swamps, salt marshes, ponds)	[16]	*Cx. pipiens* s.l. *Cx. salinarius*	USD 8 100 000 (2017)[a]	USD 31.38/3 702 (2017)
2. Sacramento and Yolo Counties, CA, USA	5 122	1 760975	Mediterranean hot summer	Urban/rural, agricultural (rice fields), natural wetlands associated with river deltas	[17-20]	*Cx. pipiens* s.l. *Cx. tarsalis*	USD 15 060 623 (2018)[b]	USD 8.55/2 940 (2018)
3. Coachella Valley, CA, USA	1 728	387 737	Hot semi-arid	Urban/rural, agricultural, natural wetlands associated with the Salton sea lake	[21]	*Cx. pipiens* s.l. *Cx. tarsalis*	USD 10 849 764 (2019)[c]	USD 27.98/6 279 (2019)
4. Central Macedonia Region, Greece	18 811	1 564 736	Mediterranean hot summer	Urban/rural, agricultural (rice-fields), natural wetlands associated with river deltas	[22]	*Cx. pipiens* s.l. *Cx. modestus*	EUR 2 700 000 (2019)[d,e]	EUR 1.73/144 (2019)
5. North Central Texas (Collin, Dallas, Denton, Tarrant Counties) TX, USA	8 943	6 586 913	Humid-sub-tropical	Urban/rural	[23,24]	*Cx. pipiens* s.l.	N/A	N/A
6. City of Chicago, IL, USA	588	2 705 994	Monsoon influenced sub-arctic climate	Urban (roadside ditches, catch basins)	[25]	*Culex* spp.	N/A	N/A
7. Atlanta (Dekalb and Fulton Counties), GA, USA	2 054	1 807 000	Humid-sub-tropical	Urban (roadside ditches, catch basins)	[26]	*Culex* spp.	N/A	N/A
8. Fort Collins, CO, USA	141	167 830	Cold semi-arid	Urban/rural	[27]	*Cx. tarsalis*	N/A	N/A

** Information on land area and population estimates for 2019 were retrieved from https://www.census.gov/quickfacts/fact/table/US/PST045219 (for USA) and https://www.statistics.gr/el/demographic-data for Greece (2011 census data); **Information on budget was retrieved from official resources available online:*

[a] *https://app.lla.state.la.us/PublicReports.nsf/11F8F448A601D7F2862582DB005B5770/$FILE/0001A355.pdf*

[b] https://www.fightthebite.net/wp-content/uploads/2019/10/symvcd-18ar.pdf

[c] https://www.cvmosquito.org/sites/indiocacvm/files/uploads/2019_annual_report_final_0.pdf

[d] http://www.pkm.gov.gr/default.aspx?lang=el-GR&page=160&proclid=2143

[e] http://www.pkm.gov.gr/default.aspx?lang=el-GR&page=160&proclid=2142

Review of the case studies

1. St. Tammany Parish case study

Background

St. Tammany Parish is located in the south-eastern corner of the state of Louisiana, USA, on the north shore of Lake Pontchartrain and is characterised by a humid sub-tropical climate. It comprises 2 190 km^2 and has a population of 258 111. There are four major mosquito-producing habitats in the region: brackish and freshwater marshes, roadside ditches, woodland sloughs and creek beds, and thousands of artificial containers [16]. The first organised mosquito control efforts in the region began in 1968 and have expanded over the years to form what is known today as St. Tammany Parish Mosquito Abatement District (STPMAD). The district uses an integrated mosquito management plan with an annual budget of approximately USD 8 000 000, involving mosquito surveillance activities, arbovirus surveillance, ground and aerial larviciding (biological, chemical), ground and aerial adulticiding, source reduction and public education. WNV was first detected in the region in 2002, resulting in increased enzootic transmission and human cases (40 cases in total by the end of the 2002 transmission season). In anticipation of the virus, the district decided to intensify surveillance and control measures and assess the impact of those measures in reducing WNV transmission intensity. This was done in collaboration with Tulane University of Tropical Medicine. Specific information on the methodology, results and conclusions of these studies is summarised below.

Research objectives

The objective of the study was to assess the impact of intensified larviciding and adulticiding vector control activities by surveying *Culex* spp. abundance, mosquito positive pools and sentinel chicken seroconversions for the 2002 WNV transmission season. Links with human cases were also made and compared with other regions in the state and previous years.

Was WNV anticipated and what WNV surveillance methods were applied (if any)?

The STPMAD performs routine WNV vector surveillance which includes (a) weekly/twice a week monitoring for mosquito abundance and WNV screening, (b) weekly monitoring of sentinel chicken seroconversion for WNV virus, and (c) wild bird surveillance. In 2002, WNV was anticipated in the study area and WNV activity was recorded in late May with the detection of a positive dead crow. Soon after the first human case was reported in early June, in parallel with the first positive mosquito pool, the virus spread rapidly throughout the region within a month, as evidenced by positive sentinel chickens, positive mosquito pools, dead bird reports and human cases.

What WNV vector control interventions were applied?

The St. Tammany Parish applied routine vector control activities and intensified the frequency of those activities in all regions in response to the elevated WNV transmission levels. Specifically, ground larviciding to roadside ditches was increased by 46% compared to the 5-year average; ground and aerial ULV adulticiding treatments were increased by 63% and 450%, respectively, compared to the 5-year average. Ground ULV treatments were conducted with pyrethroid-based products, whereas aerial ULV treatments were conducted with an organophosphate-based product.

Application timing of WNV control interventions (in response to WNV vector surveillance indicators, or/and in response to human cases)

WNV management interventions were conducted and adjusted (intensified) in response to elevated vector abundance, clusters of dead American crows, elevated WNV mosquito infection rates, and human cases. The parish intensified their efforts in mid-March and sustained their larviciding activities for roadside ditches throughout the season. Following the first human cases and positive mosquito pool in June, ground ULV adulticiding and aerial ULV adulticiding were initiated and intensified for the remainder of the season. Ground ULV treatments targeted all accessible residential areas weekly, whereas locations for aerial ULV adulticiding were chosen based on positive mosquito pools, sentinel chicken seroconversions, dead bird reports and human cases.

Efficacy evaluation of interventions

The impact of ground and aerial adulticiding interventions was assessed by comparing weekly/monthly *Culex* abundance, and monitoring WNV in mosquito pools and sentinel chicken seroconversion across the 2002 season, before and after the onset of adulticiding treatments. Larval abundance indices were also calculated to assess the impact of larviciding interventions throughout the 2002 season. Furthermore, monthly adult and larval *Culex* abundance means from 2002 were compared with the five-year abundance mean for that period.

Summary of outcomes

Even though larviciding operations were intensified starting mid-March, WNV activity levels increased in early June and spread rapidly through the region. The intensive adulticiding treatments, following the first human case in June, in combination with the continuation of larviciding treatments resulted in a 10-fold reduction in *Culex* abundance between May and August. This reduction in abundance was not observed in the previous five years where no intensive interventions were applied (in contrast to the five-year monthly abundance means which remained fairly constant). Similarly, larval indices for the 2002 season showed an eight-fold decline, compared to the two-fold decline for the five-year average. The authors concluded that this rapid and widespread reduction in vector abundance contributed to a reduction in WNV transmission levels to humans, as evidenced by the subsequent reduction of human cases in August (this reduction was not observed in other parts of the state where transmission continued until December and where no intensified vector control was implemented).

Strengths and limitations of research

Vector control interventions were applied over large areas following standardised operational procedures. Use of both intervention and control (untreated) areas. Impact of treatment was assessed using multiple indicators (including mosquito infection rates and sentinel birds). Even though a reduction in human cases was observed in the region exposed to intensive interventions, this was not supported by statistical analysis.

2. Sacramento-Yolo case study

Background

Sacramento and Yolo are two neighbouring counties located in the state of California in the middle of the Central Valley – the state's single most productive agricultural region with a large variety of crops, including 46 000 acres = 18 600 hectares of rice fields (P. Macedo, personal communication, 2 March 2020). The counties cover 5 122 km^2 and support a human population of 1 760 975. A major inland river delta formed by the Sacramento and San Joaquin Rivers is located on the south-west arm of Sacramento County, serving as an important habitat for important wildlife, such as native and migrating birds. The climate is Mediterranean, characterised by mild wet winters and hot dry summers. Mosquitoes have historically been a problem in the region due to the prolific water sources and suitable climate. For this reason, a state-funded vector control programme at district level was established in the area in 1946 and has been growing in resources and technical/scientific capacities since then. Currently, the district runs a contemporary integrated vector control programme throughout the year (2018 annual budget = USD 15 060 623), with a diverse and intensive vector surveillance network and a wide variety of vector control methods and products, while investing in operational research partnering with local universities and research institutes [28]. WNV was first detected in the counties in 2004 with low-level transmission to humans and horses. In 2005, a major outbreak occurred with 177 human infections, 40 equine cases, 16 900 dead birds, and 53% seroconversion rate in sentinel chickens. WNV was anticipated in Sacramento County in 2005 and the district's sensitive surveillance capacities allowed for early detection in birds and mosquitoes with subsequent interventions, depending on the response level designated by the California Mosquito-Borne Virus Surveillance and Response Plan [29]. The efficacy of those interventions was assessed by a team of researchers from different disciplines (entomology, veterinary medicine, public health, environmental science) from a large variety of institutes (Sacramento-Yolo Vector Control District, California Department of Public Health, Montana State University, University of California) and was described in two different manuscripts [17,18]. An additional assessment was conducted during the 2006 and 2007 WNV transmission seasons to provide additional data on control efficacy [19,20], as well as an assessment of the human health risk of adulticiding interventions [20]. Specific information on the methodology, results and conclusions of these studies are summarised below.

Research objectives

The objectives of the studies were to assess the impact of vector control applications in reducing incidence of human disease [17], abundance of *Culex* spp. and WNV infection rates [18] for the 2005 season. These two studies were conducted in parallel; one study assessed the impact on human disease and the other assessed the entomological impact of the same interventions on abundance and infection rates. Research was repeated in the same region during the 2006 [19] and 2007 WNV transmission seasons [20] when, in addition to assessing treatment effect on mosquito abundance and WNV infection rates, an assessment of the risk to human health of adulticiding was also conducted [20].

Was WNV anticipated and what WNV surveillance methods were applied (if any)?

The Sacramento-Yolo mosquito control district performs routine WNV vector surveillance which includes (a) weekly monitoring for mosquito abundance and WNV screening, (b) weekly monitoring of sentinel chicken seroconversion for WNV virus, and (c) wild bird surveillance [28]. In 2005, WNV was anticipated in the study area and elevated WNV activity was detected before the onset of human cases in the form of clusters of dead birds, positive mosquito pools and chicken seroconversions. The same happened during the 2006 and 2007 season. The district had coordinated with aerial contractors in advance, and all administrative and regulatory processes were in place for immediate aerial adulticiding interventions in the event of elevated WNV transmission risk.

What WNV vector control interventions were applied?

The Sacramento-Yolo mosquito control district applied a sequence of control methodologies from their IVM plan [28] which included larviciding (biological, microbial and chemical), public education, and ground and aerial adulticiding treatments (ULV space sprays) with pyrethrin/pyrethroid based insecticides.

Application timing of WNV control interventions (in response to WNV vector surveillance indicators, or/and in response to human cases)

WNV management interventions were conducted and adjusted (intensified) in response to elevated vector abundance, clusters of dead American crows, elevated WNV mosquito infection rates and human cases. The combination of methods chosen and the intensity of their application (frequency, area coverage) were dependent on the risk level assigned to the region according to the state and local vector control plan. The district intensified their activities in March and sustained their larviciding and public education activities throughout the season, followed by ground ULV adulticiding efforts spot-treating regions with elevated mosquito infection rates. Despite the intensive larviciding, public education and ground adulticiding efforts, the district reached epidemic levels in late July/early August (the same pattern was observed in 2005, 2006, and 2007). The onset of human cases triggered the application of aerial ULV adulticiding using pyrethrin/pyrethroid-based insecticides over specific urban areas of the counties. Aerial treatments were conducted by professional contractors using modern aerial spray guidance technologies and a spray drift modelling system coupled with real-time weather recording for appropriate offset calculations. Despite a 60-year history of the aerial application of mosquito control products in California, this was the first instance in the state of aerial adulticiding over a large urban area.

Efficacy evaluation of interventions

Larviciding, public education and ULV ground adulticiding were routinely conducted throughout the county in areas where virus activity was detected. In addition, aerial adulticiding ULV treatments were conducted in specific designated blocks, leaving plenty of unsprayed regions that could serve as control areas and allow for direct comparisons between treated and untreated regions. For this reason, all studies only allowed quantitative analysis of the aerial ULV adulticiding methodology. Carney and colleagues [17] quantified the impact of operational, large-scale, aerial ULV interventions on human cases by comparing the proportion and incidence of cases in the treated (two treated blocks, North and South block) and untreated areas of Sacramento (the remainder of the county) in 2005 before and after aerial treatments. Simultaneously that same year, Elnaiem and colleagues [18] quantified the impact of the same aerial treatments by comparing *Cx. pipiens* and *Culex tarsalis* abundance and WNV infection rates between treated (North Block) and untreated areas (the remainder of the county), before and after aerial treatments. In addition to pre- and post-trapping they used sentinel caged mosquitoes deployed within the spray zone under open field, semi-open field, and dense vegetation conditions to assess the efficacy of the treatment across different levels of exposure to the insecticide. Macedo and colleagues [19,20] repeated the evaluation of aerial ULV interventions during the 2006 and 2007 transmission season, applying the same evaluation approach followed by Elnaiem and colleagues [18], while further optimising the number and placement of trapping stations.

Summary of outcomes

During all three seasons (2005, 2006 and 2007) [18-20] aerial adulticiding interventions significantly reduced abundance (*Cx. pipiens* population reduction ranged from 40.81–75%), and WNV infection rates in *Culex* spp. mosquitoes within the treatment areas. No such reductions were observed in untreated control areas where, on the contrary, an increase in abundance and infection rates was recorded. The assessment of the same interventions on human cases conducted by Carney and colleagues in 2005 [17], indicated that adulticiding reduced the number of human cases within the treatment sites compared to the untreated area of the county and that the odds of infection after spraying were six time lower in the treated areas than the treated ones. Barber and colleagues [30] conducted an economic cost analysis of the 2005 WNV outbreak in Sacramento county and concluded that only 15 WNV neuroinvasive cases would have to be prevented to make the emergency spray cost-effective. The entomological line of evidence produced by Elnaiem and colleagues [18], together with the epidemiological line of evidence produced by Carney and colleagues [17] (two independent groups of researchers with different scientific backgrounds), confirm the conclusion that the aerial ULV adulticiding interventions were effective in disrupting the WNV transmission cycle and reducing human illness. The fact that these interventions were replicated for an additional two years with similar entomological outcomes (reduction in abundance and WNV infection rates) is strong evidence that, if applied appropriately, aerial ULV can measurably reduce the entomological indicators of WNV transmission activity, as well as the number of human WNV cases. Based on the risk assessment study, human risk from exposure to the ULV adulticiding applications was below regulatory levels of concern, and the risks from aerial ULV adulticiding are lower than those for ground (truck-mounted) ULV adulticiding [20].

Strengths and limitations of research

Vector control interventions were applied over large areas following standardised operational procedures. Use of both intervention and control (untreated) areas. Impact of treatments was assessed using multiple indicators (including mosquito infection rates, sentinel birds, incidence of human cases). In addition to trapping in order to monitoring the abundance of wild mosquito populations, caged sentinel mosquitoes were used for direct assessment of insecticide toxicity and spray coverage. Observations were made over several seasons and experiments were replicated by different groups of researchers. In addition to efficacy, the human health risk of spraying interventions was also assessed. No major limitations were identified.

3. Coachella Valley case study

Background

Coachella Valley, characterised by a semiarid climate, is 25 km wide and extends north-westwards for 70 km from the Salton Sea (a shallow saline lake) through Riverside County to the San Gorgonio Pass, Southern California, USA. The Coachella canal, built in 1949, provides irrigation to approximately 388 km^2 of productive agricultural land. The construction of the canal brought an abundance of water to the valley and benefitted the agricultural economy immensely, but at the same time resulted in the formation of mosquito larval habitats as a result of irrigation run-off. Due to the increasing mosquito nuisance and public health concerns, a mosquito control department was formed for the first time in 1951 to combat mosquitoes, in addition to the already active programmes for combatting eye gnats (Diptera: Chloropidae). Currently, vector control activities are conducted by the Coachella Valley Mosquito & Vector Control District with an annual budget of USD 10 849 764 (2019). Similar to Sacramento-Yolo, the district runs a contemporary integrated vector control programme throughout the year with a diverse and intensive vector surveillance network and a wide variety of vector control methods and products, while investing in operational research partnering with local universities and research institutes. Low level WNV enzootic transmission, but no human cases, was first detected in the valley during 2003 and seemed confined to the rural areas in the lower valley at the Salton Sea. The small wetlands associated with the north-eastern shore of the Salton Sea seem to be the early transmission/amplification foci for WNV, as well as other viruses (St. Louis encephalitis, Western equine encephalitis) in the region, with subsequent dispersal northward and upland away from the Salton Sea. In 2004, it was anticipated that WNV would occur in the region and this was detected during the district's arbovirus surveillance activities. The unique topography of the region – with Salton Sea (early WNV transmission focus) at the extreme south-east corner of the valley (lower valley) and only one narrow corridor of wetlands and agriculture connecting this area to the rest of the valley (upper valley) – provided an opportunity to investigate whether intensive early mosquito control interventions, targeting early season virus amplification, could interrupt or delay amplification by limiting dispersal and subsequently prevent outbreaks of disease. To test this hypothesis, the University of California Davis, in collaboration with Coachella Valley Mosquito & Vector Control District, conducted a longitudinal study over three years (2004, 2005 and 2006) applying different strategies for vector control intervention [21]. Specific information on the methodology, results and conclusions of these studies is summarised below.

Research objectives

The objective of the study was to assess the impact of early season and late season vector control strategies on *Culex* spp. abundance, WNV infection rates and sentinel chicken seroconversions for the 2004, 2005 and 2006 WNV transmission seasons.

Was WNV anticipated and what WNV surveillance methods were applied (if any)?

The Coachella Valley Mosquito & Vector Control District performs routine WNV vector surveillance which includes (a) weekly/bi-weekly monitoring for mosquito abundance and WNV screening, (b) bi-weekly monitoring of sentinel chicken seroconversion for WNV virus (discontinued as of 2016), and (c) wild bird surveillance (because of the low number of corvids present in the region very few birds are tested annually). In 2004 WNV was anticipated in the study area and WNV activity was detected before the onset of human cases through positive mosquito pools and chicken seroconversions, primarily confined to the lower valley near the Salton Sea. The same happened during the 2005 and 2006.

What WNV vector control interventions were applied?

The Coachella Valley Mosquito & Vector Control District applied routine vector control activities, such as larviciding (biological, chemical), and public education. The arrival of WNV necessitated enhanced suppression, leading to ground and aerial adulticiding treatments (ULV space sprays) with pyrethroid insecticides reported in the study by Lothrop and colleagues [21]. Although larviciding operations were applied simultaneously at the study sites (including larviciding over the Salton Sea wetlands), the suppression levels achieved by those treatments alone was insufficient to prevent amplification and dispersal of the virus.

Application timing of WNV control interventions (in response to WNV vector surveillance indicators, or/and in response to human cases)

The study investigated the efficacy of single and combination adulticiding operations applied reactively (after the onset of human cases) and pro-actively (before the onset of human cases). Other routine vector control activities (i.e. larviciding) were conducted throughout the study region. Three vector control scenarios were tested across the three seasons, as described below.

1. In 2004, the virus was first detected in April, routine vector control was in place, and in addition, intensive ground ULV adulticiding treatment was conducted one month after initial detection of WNV.
2. In 2005, the virus was detected in May – one month later than in 2004 – and spread rapidly across the valley. Routine vector control was in place, and in addition, ground and aerial ULV adulticiding was conducted in response to the elevated and widespread WNV transmission in May and June, respectively.
3. In 2006, the virus was detected in April and, unlike the previous seasons, ground and aerial adulticiding was conducted almost immediately after first detection starting in late April and continued with consecutive treatments until early June (the emphasis was on aerial ULV with ground ULV filling in some limited areas).

Efficacy evaluation of interventions

The impact of ground and aerial adulticiding interventions was quantified by comparing *Cx. pipiens* and *Cx. tarsalis* abundance and WNV infection rates for treated and untreated (control) areas, before and after adulticiding treatments (sentinel mosquito cages and droplet spinners for collecting droplets were also used to monitor spray coverage). The wetland region on the southern shore of the Salton Sea was chosen as a control (untreated) area. Furthermore, vernal geometric means of *Cx. tarsalis* abundance were compared across the four years (2003–2006) with different vector control strategies. Application parameters of aerial adulticiding treatments (flight path and flight height) were improved in 2006, based on the knowledge gained in 2005, for better spray coverage and treatment precision.

Summary of outcomes

In 2004, despite the additional intensive ground adulticiding treatments in early spring (May), virus dispersal could not be prevented and eventually WNV was detected throughout the valley in positive mosquito pools and through chicken seroconversion, with human cases recorded in the urbanised upper valley. In 2005, the virus spread fast and could not be contained, but researchers were able to test and optimise the application parameters of ground and aerial adulticiding. Tests with caged sentinel mosquitoes indicated that landscape and wind can significantly affect the efficacy of these treatments on mosquito mortality and therefore repeated treatments are needed to overcome possible gaps in efficacy. In 2006, the almost immediate application in mid-April – upon detection of the first positive mosquito pools – of aerial adulticiding targeting the early transmission focus of Salton Sea resulted in a reduction of *Cx. tarsalis* abundance and WNV activity, as evidenced by the reduced WNV infection rates in mosquitoes and seroconversion rates in sentinel chickens. This reduction apparently delayed virus amplification and prevented tangential transmission in the urban areas of the upper valley, as evidenced by the absence of WNV detections by the human WNV surveillance network, and the absence of human cases. Furthermore, during 2003, 2004 and 2005, without intensive adult control *Cx. tarsalis* vernal geometric means were significantly higher than in 2006, when intensive early adulticiding was conducted. These patterns were not observed in *Cx. tarsalis* populations from the control area, where *Culex* spp. abundance and WNV activity was greater in 2006 than in previous years.

Strengths and limitations of research

Vector control interventions were applied across large areas following standardised operational procedures. Use of both intervention and control (untreated) areas. Impact of treatments was assessed using multiple indicators (including mosquito infection rates and sentinel birds). In addition to trapping to monitor the abundance of wild mosquito populations, caged sentinel mosquitoes were used for direct assessment of insecticide toxicity and spray coverage. Observations were made over several seasons. This is the only study identified where aerial ULV adulticiding was applied proactively (before the onset of human cases). Even though no human cases were reported during the year of early and intensive interventions (compared to the previous years), this was not supported by statistical analysis.

4. Central Macedonia Region, Greece case study

Background

In 2010, a major WNV outbreak occurred in Central Macedonia, northern Greece. This was the first outbreak of WNV in the country and the second largest outbreak in Europe, with 197 neuroinvasive WNV disease cases and 25 fatalities. The epicentre of the outbreak was located in the agricultural region of West Thessaloniki (the regional capital of Central Macedonia, with a population of 1.5 million people). This region is characterised by a Mediterranean climate and comprises significant natural wetlands, rivers, and ~20 000 hectares of rice-fields with prolific mosquito larval habitats [31]. Local vector control programmes funded by the state have been implemented in the region since 1997 (2019 annual budget = EUR 2 700 000). Before the introduction of WNV in the region, the only vector control approach targeting *Culex* populations was larviciding. As a result of the WNV threat, an exemption was issued for the first time in 2010 by the Ministries of Agriculture and Health to allow for aerial and ground ULV adulticiding using a pyrethroid-based product. The aerial ULV treatments were optimised locally for efficacy, precision, and non-target effects in a three-year longitudinal study (2008–2010) prior to receipt of the registrations [31,32]. During 2011, in anticipation of WNV, a group of scientists from European and US research institutes, in collaboration with local vector control authorities, set up a WNV vector and sentinel chicken surveillance network in the region to gauge virus activity and provide timely guidance and assessment of vector control interventions [22]. Specific information on the methodology, results and conclusions of these studies is summarised below.

Research objectives

Chaskopoulou and colleagues [22] aimed to evaluate the usefulness of a newly-established WNV surveillance network in providing early warning indicators for WNV activity and to assess the efficacy of vector control measures applied in response to those indicators.

Was WNV anticipated and what WNV surveillance methods were applied (if any)?

WNV was anticipated in the region and a WNV surveillance network was activated during 2011 using weekly mosquito trapping (for abundance indicators and WNV mosquito screening) and weekly monitoring of enzootic transmission through sentinel chicken seroconversions.

What WNV vector control interventions were applied?

The local vector control plan involved aerial larviciding (targeting rice-fields/flooded fields and natural wetlands), ground larviciding (targeting irrigating canals and other residential/urban breeding sites), and ground residual adulticiding with pyrethroid-based products (targeting public residential areas with high abundance of resting adult mosquitoes). In addition to these routine measures, an exemption was given in 2011 to allow for aerial ULV adulticiding applications in areas of increased WNV circulation. Aerial guidance technologies incorporated with spray fate/drift models were transferred from the US to allow for precise coverage of the target sites.

Application timing of WNV control interventions (in response to WNV vector surveillance indicators, or/and in response to human cases)

Routine larviciding and public outreach activities targeting agricultural, suburban and urban areas of the Thessaloniki region were initiated in May. Despite the routine vector control activities, WNV circulation levels increased significantly, leading to the first positive mosquito pool and chicken seroconversions in late June and the first reported human cases in late July. Decisions to apply aerial adulticiding were only made after the onset of human cases and treatments were conducted in August over the agricultural region of West Thessaloniki where high WNV activity was recorded across all surveillance systems.

Efficacy evaluation of interventions

Efficacy of aerial adulticiding treatments was evaluated by comparing *Culex* spp. abundance and sentinel chicken seroconversions before and after the treatments for treated (rural agricultural areas of West Thessaloniki) and untreated areas (rural areas of East Thessaloniki).

Summary of outcomes

Aerial ULV adulticiding interventions significantly reduced *Culex* spp. abundance (80% population reduction after treatments) and sentinel chicken seroconversion rates in the treated areas. No such reductions were observed in the untreated areas of the city, where, on the contrary, an increase in abundance was observed during the same period (44% increase), followed by an increase in chicken seroconversions. *Culex* spp. infection rates were too low to allow for comparisons.

Strengths and limitations of research

Vector control interventions were applied over large areas following standardised operational procedures. Use of both intervention and control (untreated) areas. Impact of treatment was assessed using multiple indicators (including mosquito infection rates, sentinel birds). Prior to operational treatments, spray applications were tested for efficacy and non-target impact in a three-year longitudinal study. Unfortunately, due to the low number of positive mosquito pools, impact on vector infection rates could not be determined.

5. The North-Central Texas case study

Background

During 2012, Texas, USA, experienced a major WNV outbreak, with neuroinvasive disease incidence at least 1.6 times higher than any previous year since WNV disease cases were first reported in the state in 2002. Forty-two percent of the reported cases was concentrated in four adjacent Texas Counties: Collin, Dallas, Denton and Tarrant (an area 7 112 km^2 with a population of 5 700 000). The climate of the area is humid and sub-tropical and the 2012 epidemic year was considered a high outlier in terms of winter temperature, total rainfall in winter and early spring, and summer heat. There is no centralised county-based vector control at district level; nonetheless, surveillance and contract-specific vector control operations are conducted in coordination with local health departments and municipalities. In response to the outbreak in these four counties, surveillance and control activities were stepped up. The surveillance efforts and efficacy assessment of these interventions is described in two different manuscripts [23,24]. The impact of aerial adulticiding applications on the incidence of emergency department visits in Dallas (main concerns: skin rash and respiratory distress) was also assessed [24]. Specific information on the methodology, results and conclusions of these studies is summarised below.

Research objectives

The objective of Ruktanonchai and colleagues [23] was to assess the impact of vector control applications in reducing incidence of human disease using data from all four counties. Chung and colleagues [24], in parallel with Ruktanonchai and colleagues [23], closely monitored WNV transmission activity in vectors in one of the four counties (Dallas), with the aim of investigating whether a vector index could be used to predict the onset of human cases. Furthermore, the impact of aerial adulticiding on human health was evaluated in Dallas [24].

Was WNV anticipated and what WNV surveillance methods were applied (if any)?

WNV activity has been recorded in the region since 2002 and activity was expected. A dense WNV vector surveillance network, with weekly trapping of adult mosquitoes, was put in place from May until December in Dallas County. No information on the other three counties is reported in the published manuscripts.

What WNV vector control interventions were applied?

In response to increased WNV transmission levels, ground larviciding and ground ULV adulticiding were conducted in July. Due to the continuous increase in human cases, a decision was made to perform aerial ULV adulticiding in August.

Application timing of WNV control interventions (in response to WNV vector surveillance indicators, or/and in response to human cases)

All vector control activities were conducted in response to human cases. A significant increase in vector index was recorded in Dallas County early in the season, but adulticiding interventions were conducted and intensified only after the increase in human cases.

Efficacy evaluation of interventions

Efficacy of aerial adulticiding interventions, conducted in two out of the four counties, was evaluated by calculating incidence rate ratios (IRRs) in treated (Dallas and Denton) and untreated (Collin and Tarrant) counties by comparing incidence before and after aerial spraying.

Summary of outcomes

Aerial ULV adulticiding implemented for WNV control was associated with a reduction in WNV neuroinvasive disease. WNV neuroinvasive disease incidence before and after spraying was 7.3/100 000 population and 0.28/100 000 population, respectively; the IRR was 26.42. In untreated areas, the before and after incidence was 4.8/100 000 population and 0.45/100 000 population, respectively; the IRR was 10.57. As expected, given that aerial spray events were conducted late during the outbreak, disease incidence decreased during the post-spray period in both treated and untreated areas. However, the relative change was significantly greater in areas sprayed aerially. Furthermore, aerial ULV adulticiding was not associated with increases in emergency department visits for respiratory symptoms [24].

Strengths and limitations of research

Vector control interventions were applied over large areas following standardised operational procedures. The direct efficacy of insecticide interventions in reducing incidence of human disease was assessed by comparing clinical case distribution within treated and untreated control areas. However, the impact of interventions on vector population was not assessed.

6. Chicago case study

Background

WNV cases were first reported in the state of Illinois, USA, in 2002, with 884 human cases [6], 80% of which were reported from the Chicago metropolitan region in seven urban counties: Cook, DuPage, Kane, Kendall, Lake, McHenry, and Will. Transmission continued in the following years, and from 2002 through 2009, a total of 321 human cases were reported in the city of Chicago (urban environment, monsoon influenced sub-arctic climate, population of 2 716 000). In anticipation of WNV transmission in 2005, the city of Chicago responded with ground ULV adulticiding applications, targeting areas with high mosquito infection rates [25]. Specific information on the methodology, results and conclusions of these studies is summarised below.

Research objectives

To assess the impact of ground ULV adulticiding applications during the 2005 WNV transmission season by monitoring abundance and WNV infection rates of *Culex* spp. before, during and after adulticiding treatments in Cook County, Chicago. The authors investigated whether sequential adulticiding treatments had an additive effect and compared efficacy depending on the number of treatment rounds, ranging from zero to four treatment rounds.

Was WNV anticipated and what WNV surveillance methods were applied (if any)?

WNV was anticipated and WNV vector surveillance was in place, with weekly trapping of adult *Culex* spp. from April until September.

What WNV vector control interventions were applied?

Ground ULV adulticiding treatments with pyrethroid insecticides were conducted by professional contractors. Large blocks (80.9–159.4 km^2) in northern and southern parts of the county received treatments. Four sequential treatment rounds were applied across the season. Non-sprayed areas of the county were used as control sites.

Application timing of WNV control interventions (in response to WNV vector surveillance indicators, or/and in response to human cases)

Measures were applied in response to *Culex* spp. abundance and infection rates. Study sites were chosen on the basis of persistent WNV-positive mosquitoes.

Efficacy evaluation of interventions

Efficacy was assessed by comparing mosquito density changes and WNV infection rates in mosquitoes pre and post treatments and across treated and control areas.

Summary of outcomes

Significant *Culex* population reductions were observed post ULV treatments. Specifically, two sequential ULV treatments decreased mosquito abundance by 54%, whereas mosquito abundance increased by 153% in the non-sprayed areas. Overall, the highest reduction in mosquito populations was observed at sites receiving four sequential treatments. However, Maximum Likelihood Estimates (MLE) of WNV minimum infection rates varied widely across treated and untreated areas and no evidence of a significant effect of adulticide treatments on minimum infection rates was found.

Strengths and limitations of research

Vector control interventions were applied over large areas following assessment using multiple indicators (including mosquito infection rates). Different treatment scenarios (single versus serial applications) were tested. According to the authors, the study was conducted during an unusually hot and dry summer season that may have lessened the impact of the treatments.

7. Atlanta case study

Background

Since the introduction of WNV in Georgia, USA, in 2001, a total of 473 humans cases have been reported [6]. In Atlanta, the capital and most populated city in the state, WNV cases are uncommon, nonetheless surveillance and control activities in response to WNV are most intensely conducted in the most epidemiologically relevant counties, of Dekalb and Fulton (population of 1 807 114, humid sub-tropical climate). Vector surveillance and control services are provided by local health departments and/or private contractors. The main vector control strategy applied in both counties is larviciding treatments on roadside catch basins from late June until October in an attempt to control WNV transmission. When WNV positive pools are detected, neighbourhood outreach campaigns are performed near the detected infections and larviciding is intensified within a buffer distance around the WNV infection location. Neither county has budget to monitor efficacy of larviciding operations in larval or adult populations. To address this knowledge gap, McMillan and colleagues [26] performed a study to assess the impact of these strategies on WNV transmission level during the 2015 and 2016 season. Specific information on the methodology, results and conclusions of these studies is summarised below.

Research objectives: Given the lack of information about the population-level impact of larviciding, McMillan and colleagues [26] aimed to quantify the impact of larviciding catch basins on *Culex* spp. adult female abundance and WNV infection prevalence in urban environments of Atlanta, Georgia.

Was WNV anticipated and what WNV surveillance methods were applied (if any)?

WNV was anticipated in the region and WNV vector surveillance activities were in place via weekly trapping (starting April/March and continuing for the duration of the study) to calculate *Culex* spp. abundance indices and WNV infection rates.

What WNV vector control interventions were applied?

Larviciding applications targeting catch-basins were the only method applied. A combination of larviciding products were used in accordance with label instructions.

Application timing of WNV control interventions (in response to WNV vector surveillance indicators, or/and in response to human cases)

Two different application timing scenarios were tested across the two consecutive years. (1) In 2015, larvicides were applied during the epidemic period of WNV (July–September) following larviciding timing similar to routine spray applications of Fulton and DeKalb. (2) In 2016, larvicides were applied at the beginning of *Culex* spp. breeding season in Atlanta (March–May). Larvicides were applied weekly or bi-weekly according to manufacturer's label instructions for each product.

Efficacy evaluation of interventions

Four sites (urban parks) were used for the analysis (two were assigned as treatment sites and two as untreated control sites). Efficacy of treatments was assessed by (a) comparing abundance of larvae, pupae and resting adults associated with a sub-set of the treated catch basins before, during and after the applications in comparison with abundance data from an equivalent sub-set of untreated (control) catch basins; (b) comparing abundance of *Culex* spp. trapped in treated versus untreated areas before, during and after the applications, in an attempt to link larval productivity to adult population collections, and (c) comparing WNV minimum infection rates in adult *Culex* spp. (from both catch basins and traps) in treated and untreated areas before, during and after treatments.

Summary of outcomes

Larviciding in catch basins resulted in more than 90% reduction in larval/pupal collections in treated sites. Even though larvicides were effective in suppressing larval and pupal populations, no significant reduction was observed in adult *Culex* spp. collected with traps in the proximity of the treated catch basins or in adults collected resting in catch basins. In addition, WNV infection prevalence between treated and untreated sites was similar. The authors conclude that, on the scale and frequency applied in this study (0.21–0.37 km^2 study sites, spray periods 8–14 weeks), larval control alone may not lead to meaningful reductions in adult populations and WNV prevalence.

Strengths and limitations of research

Vector control interventions were applied following standardised operational procedures. Use of both intervention and control (untreated) areas. Impact of treatments was assessed using multiple indicators (including mosquito infection rates). Observations were made over several seasons. Despite the fact that the control strategy followed by the authors was pre-specified in terms of spatial extent and approach to mimic operational interventions, a larger spatial coverage would have possibly resulted in greater impact.

8. Fort Collins case study

Background
WNV cases were first reported in the state of Colorado, USA, in 2002 and since then human cases have been reported every year, accumulating to a total of 5 526 cases as of 2018 [6]. Fort Collins serves as the seat of Larimer County, which is one of the counties with consistent WNV circulation. In 2017, a team of researchers from USA and European institutions developed and tested under laboratory conditions an endectocide-treated feed as a systemic endectocide (an antiparasitic drug that is active both against endoparasites and ectoparasites) for birds to target blood-feeding *Cx. tarsalis* [27]. Ivermectin was the most effective endectocide and showed strong potential in laboratory studies. In order to assess the potential of the treatment under field conditions, the authors performed a small-scale field study in urban and suburban areas of Fort Collins during the 2017 WNV transmission season [27]. Specific information on the methodology, results and conclusions of the field study is summarised below.

Research objectives
The efficacy of the new IVM-treated bird feed in reducing *Culex* abundance and WNV infection rates was tested under field conditions. Furthermore, the impact of the treatment on the health of the local fauna was investigated.

Was WNV anticipated and what WNV surveillance methods were applied (if any)?
WNV was anticipated and a WNV surveillance network with weekly monitoring of vector abundance and infection rates had been in place since 2006.

What WNV vector control interventions were applied?
Field sites in areas with historically high WNV circulation levels were treated with ivermectin-treated bird feed stations. The ivermectin-treated bird feed was used at a concentration that proved to be the most effective (greater than 80% mortality on *Cx. tarsalis* mosquitoes) and safe under laboratory conditions (chickens and wild Eurasian collared doves exhibited no signs of toxicity when feeding on this concentration). Six sites were chosen in suburban/urban open areas of east Fort Collins (three treated and three untreated control sites). At each site, an array of three bird feed stations was placed in an approximately triangular pattern around a mosquito trap at a distance of 50 m. The feed was changed daily to account for any ivermectin degradation effects due to environmental exposure. Motion-activated trail cameras were used to document bird visits to the feeders, and blood samples from wild birds were collected for ivermectin detection and quantification.

Application timing of WNV control interventions (in response to WNV vector surveillance indicators, or/and in response to human cases)
Field treatments commenced in early June and lasted until early September in areas with high WNV circulation.

Efficacy evaluation of interventions
The efficacy of the ivermectin-treated bird feed stations in reducing WNV transmission levels was evaluated by (a) comparing *Culex* abundance between control and treated sites; (b) comparing *Culex* abundance between treated sites and historical data from the same sites for the period 2006–2016 (years without treatment), and (c) comparing WNV infection rates and the number of WNV-positive pools between treatment and control sites.

Summary of outcomes
Nearly all birds captured and tested around the treated bird feeders had detectable levels of ivermectin in their blood (87% of the tested sera was positive for ivermectin). However, entomological data showed that WNV transmission was not reduced around the treated bird feeders. *Culex* spp. abundance was similar between treated and untreated sites and between treated and untreated seasons. No significant difference was observed in the number of positive mosquito pools.

Strengths and limitations of research
This a proof of concept study assessing the impact of a novel control approach against vector abundance and infection rates. Although the scale of field interventions may have been too small to have an effect on vector abundance and infection rates, given the pilot nature of the study, this information will be valuable in informing future large-scale trials.

Table 6. Detailed information on WNV vector management experience presented by case study

Case Studies	Types of interventions	Timing of interventions	Efficacy evaluation (*Culex* spp.)	Impact on WNV circulation levels (circulation in mosquitoes, enzootic circulation, human cases)
1. St. Tammany Parish, LA, USA [16]	Intensive larviciding in combination with ground and aerial ULV adulticiding	2002 season - Intensive larviciding since March (46% increase compared to the five-year average). - Initiation of ground and aerial ULV adulticiding in June following the first human case (63% and 450% increase, respectively, compared to the five-year average).	- Comparing adult mosquito abundance, infection rates, chicken seroconversions before and after adulticiding treatments across the season. - Comparing mosquito larval abundance before and after larviciding treatments across the season. - Comparing monthly adult and larval mosquito abundance means from 2002 with the five-year abundance average for that period.	- Despite the early intensification of larviciding operations in March, WNV circulation levels increased in early June and spread rapidly across the region. - Larval abundance indices showed an eight-fold decline against the five-year average two-fold decrease. - Adulticiding treatments in combination with larviciding treatments resulted in a 10-fold reduction in *Culex* abundance from May to August. This reduction was not observed in the five-year average abundance curves, which in contrast remained constant. - Rapid decrease in human cases in August followed the rapid decline of *Culex* populations (this pattern was not observed in other regions of the state).
2. Sacramento and Yolo Counties, CA, USA [17-20]	- Intensive larviciding and public education. - Ground ULV adulticiding. - Aerial ULV adulticiding.	2005, 2006, 2007 seasons - Intensive larviciding and public education since March. - Initiation of ground ULV adulticiding June-July (in response to infection rates). - Aerial ULV adulticiding late July-August (in response to human cases).	2005, 2006, 2007 seasons - Mosquito abundance, infection rates and chicken seroconversions were used to monitor overall efficacy of control operations across the season. For larviciding/public education no control area was available (all areas with WNV activity were treated). Non-treated areas were available for aerial ULV adulticiding treatments, allowing for additional comparisons between treated and untreated blocks (pre- and post-trapping, sentinel cages). 2005 season - Aerial ULV adulticiding impact on human cases was quantified by comparing proportion and incidence of human cases between treated and untreated blocks.	- Despite the intensive larviciding, public education and ground adulticiding efforts (for three consecutive years) the district reached epidemic levels in late July/early August (as evidenced by high infection rates, chicken seroconversions and human cases). - Evidence for all three seasons (2005, 2006, 2007) indicated that aerial adulticiding interventions significantly reduced abundance, WNV infection rates and positive mosquito pools of *Culex* spp. mosquitoes within the treatment areas. - Evidence from the 2005 season indicated that aerial ULV adulticiding reduced the number of human cases within the treatment sites compared to the untreated sites and that the odds of infection after spraying were six times higher in the untreated areas compared to the treated ones.
3. Coachella Valley, CA, USA [21]	- Routine larviciding. - Ground ULV adulticiding and aerial ULV adulticiding (singly or in combination).	- Routine larviciding was conducted for all three seasons from early spring. - 2004 season: intensive ground ULV adulticiding started in June. - 2005 season: ground and aerial ULV adulticiding started in June and July, respectively, in response to human cases. - 2006 season: Early (April) aerial ULV adulticiding (with some spot ground ULV treatment) in response to positive mosquitoes.	- Comparing adult mosquito abundance and WNV infection rates between treated and untreated (control) areas, before and after ground and aerial ULV adulticiding treatments. - Geometric means of adult mosquito abundance were compared across four years (2003–2006) with different vector control strategies.	2004 season: Despite routine larviciding and additional intensive ground adulticiding treatments in early spring, WNV dispersed throughout the valley as evidenced by positive mosquito pools, chicken seroconversions and human cases. 2005 season: Interventions were conducted late and virus could not be contained but allowed for optimisation of aerial adulticiding methods. 2006 season: - Aerial ULV adulticiding treatments resulted in significant reductions of adult abundance and WNV activity, as evidenced by the reduced WNV infection rates in mosquitoes and seroconversion rates in sentinel chickens post-treatment. - Geometric abundance means in 2006 were significantly lower than in 2003, 2004, and 2005 seasons when early adulticiding was not conducted. These patterns were not observed in adult mosquito populations from the control area, where *Culex* sp. abundance and WNV activity was greater in 2006 than in previous years.

Case Studies	Types of interventions	Timing of interventions	Efficacy evaluation (*Culex* spp.)	Impact on WNV circulation levels (circulation in mosquitoes, enzootic circulation, human cases)
4. Central Macedonia Region, Greece [22]	- Routine ground and aerial larviciding targeting urban/suburban and rural/agricultural areas, respectively. - Aerial ULV adulticiding.	2011 season - Routine control treatments were initiated in May. - Despite the detection WNV circulation in late June (mosquitoes, sentinel chickens) aerial ULV was conducted in August in response to human cases.	- Comparing adult mosquito abundance and sentinel chicken seroconversions before and after the treatments between treated (rural areas of West Thessaloniki) and untreated areas (rural areas East Thessaloniki).	- Aerial ULV adulticiding interventions significantly reduced *Culex* spp. abundance and sentinel chicken seroconversion rates within the treatment areas. - No such reductions were observed in the untreated areas of the city where, on the contrary, an increase in abundance was observed immediately post-treatment followed by an increase in chicken seroconversions.
5. North Central Texas (Collin, Dallas, Denton, Tarrant Counties) TX, USA [23,24]	- Ground larviciding and ground ULV adulticiding. - Aerial ULV adulticiding.	2012 season - Ground larviciding and adulticiding were applied in response to increased WNV circulation in July. - Aerial ULV adulticiding was applied in response to human cases in August.	- Aerial ULV adulticiding impact on humans cases was evaluated by calculating incidence rate ratios (IRRs) in treated (Dallas, Denton) and untreated (Collin, Tarrant) counties by comparing incidence before and after aerial spraying.	- Aerial ULV adulticiding was associated with a reduction in WNV neuroinvasive disease. Given that aerial spray events were conducted late during the outbreak, disease incidence decreased during the after spray period in both treated and untreated areas. However, the relative change was significantly larger in areas sprayed by air.
6. City of Chicago, IL, USA [25]	Ground ULV adulticiding (single, versus sequential applications)	2005 season - Treatments were conducted in July-August in areas with high WNV infection rates in mosquitoes.	- Comparing adult mosquito abundance pre and post treatments and across treated and control areas.	- Significant *Culex* population reductions were observed post ground ULV treatments. Specifically, two sequential ULV treatments decreased mosquito abundance by 54%, whereas mosquito abundance increased by 153% in the non-sprayed areas. - The highest reduction was observed at the trap sites receiving four sequential treatments, indicating that serial treatments are more effective than single applications. - WNV minimum infection rates varied widely across treated and untreated areas, indicating that ground adulticide treatments had no direct effect on WNV infection rates.
7. Atlanta (Dekalb and Fulton Counties), GA, USA [26]	Larviciding applications targeting catch-basins.	Two different timing scenarios were tested: - 2015 season: larvicides were applied during the epidemic period of WNV (July–September). - 2016 season: larvicides were applied at the beginning of *Culex* spp. breeding season (March–May).	- Comparing abundance of mosquito larvae, pupae and resting adults associated with treated catch basins before, during and after treatments in comparison with abundance data from untreated (control) catch basins. - Comparing adult mosquito abundance trapped in treated versus untreated areas before, during and after the applications, in an attempt to link larval productivity to adult population collections. - Comparing WNV infection rates in adult mosquitoes (from both catch basins and traps) in treated and untreated areas before, during and after treatments.	- More than 90% reduction in larval/pupal collections in treated catch basins was recorded. - No significant reduction was observed in adult *Culex* spp. populations collected with traps in proximity to treated catch basins or in adults collected resting in catch basins. - WNV infection prevalence between treated and untreated sites was similar. - On the scale and frequency applied in this study, larval control alone did not lead to meaningful reductions in adult populations and WNV prevalence.
8. Fort Collins, CO, USA [27]	- Ivermectin-treated bird feed stations.	2017 season - Field treatments commenced in early June and continued until early September in areas with high circulation of WNV.	- Comparing adult mosquito abundance between control and treated sites. - Comparing adult mosquito abundance between treated sites and historical data from the same sites from 2006-2016 (no treatment years). - Comparing WNV infection rates/number of WNV-positive pools between treatment and control sites.	- Birds captured and tested around the treated bird feeders had detectable levels of ivermectin in their blood. - *Culex* abundance was similar between treated and untreated sites and between treated and untreated seasons. - No significant difference was observed in the number of positive mosquito pools between treated and untreated sites.

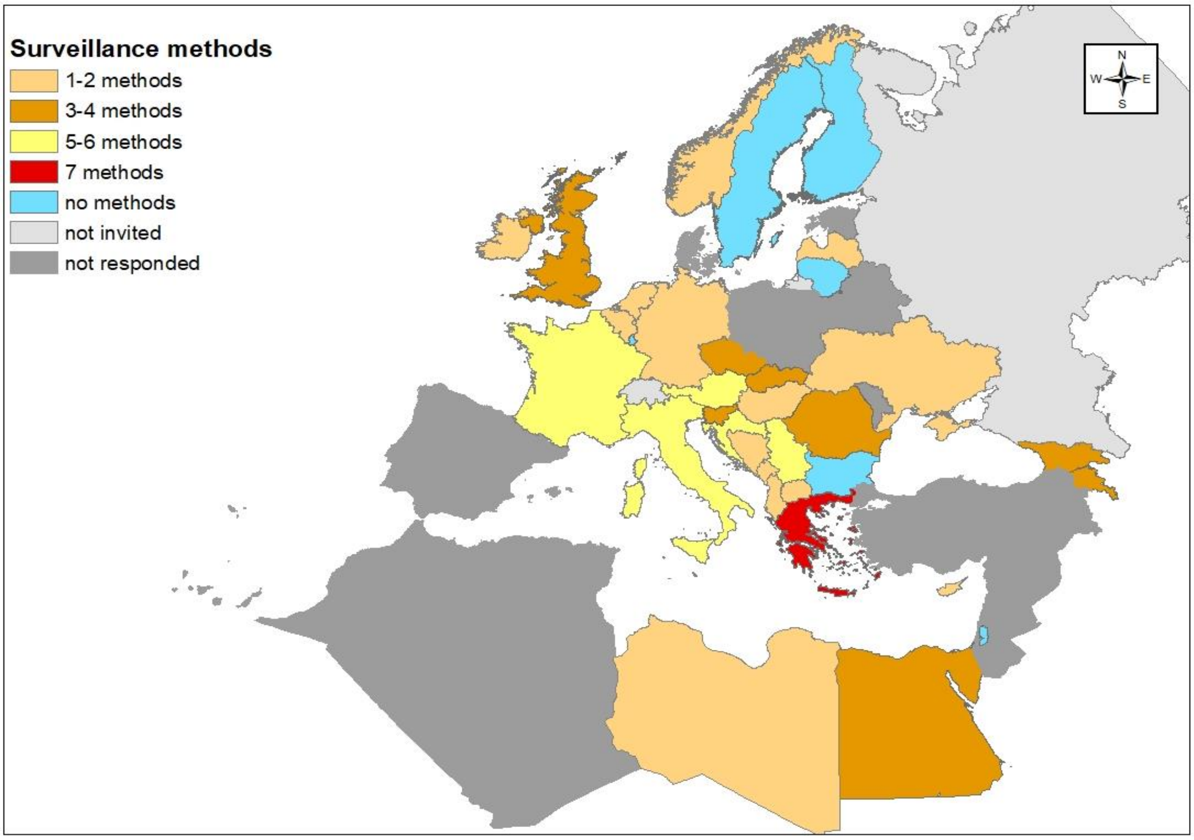
4. Discussion

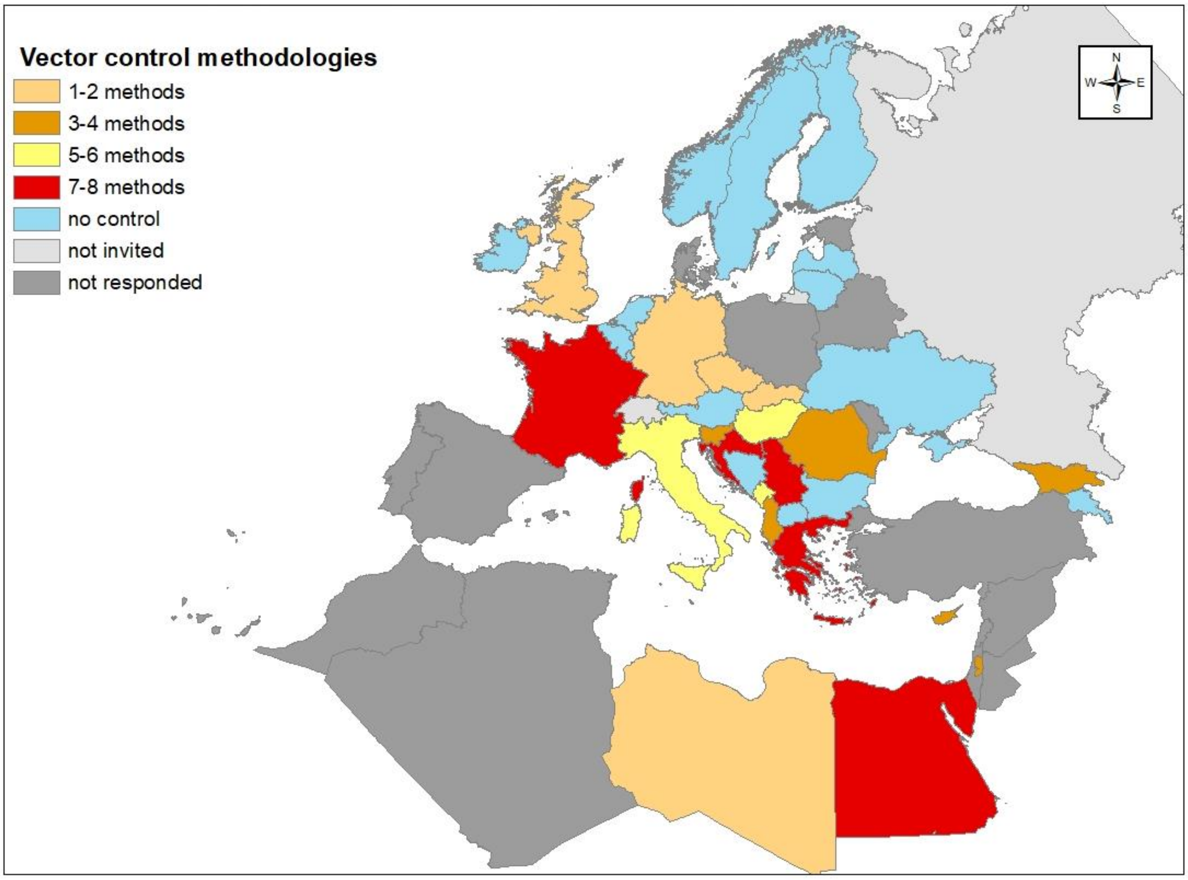
West Nile virus vector surveillance and management capacities in Europe

Through the survey administered to European national/regional state agencies involved in vector control operations, we were able to get an overview of the WNV vector surveillance and control activities in the EU/EEA, ENP partner and EU candidate/potential candidate countries. We also identified some of the major challenges related to the implementation of WNV management strategies. The majority of EU/EEA, ENP partner and EU candidate/potential candidate countries implement at least one method of WNV surveillance (Figure 15 top panel). The most common passive WNV surveillance method is the detection of human cases, followed by surveillance of dead animals. The most common method of active WNV surveillance is mosquito screening, followed by sentinel bird screening and sentinel equid screening for EU/EEA and ENP partner and EU candidate/potential candidate countries, respectively. Even though a large majority of countries perform routine vector surveillance (abundance monitoring), less than 50% of these countries perform mosquito screening for WNV. Furthermore, a much lower percentage (<15%) of countries perform insecticide resistance testing, and even in those cases it is not implemented systematically. The main challenge identified by respondents in relation to implementation and continuity of WNV surveillance methods is the lack of funding, alongside the shortage in human resources.

The majority of EU/EEA countries that do not implement any type of WNV vector control method have no history of autochthonous WNV human cases, indicating that the primary cause of the absence of control measures is the absence of WNV risk. A small percentage of ENP partner and EU candidate/potential candidate countries (17%) reported an absence of control measures against WNV vectors. However, due to the low response rate from ENP partner countries, this percentage may not be representative of the entire region.

Figure 15. Maps depicting the number of WNV vector surveillance and control methods applied per country





Among the EU/EEA countries that implement vector control, the most widely adopted method by far is biological larviciding, while the least adopted method is source reduction through environmental management. Among ENP partner and EU candidate/potential candidate countries, public education followed by biological larviciding, chemical larviciding, and LV surface spraying adulticiding are the most widely methods applied. The high adoption rate of biological larviciding across EU/EEA countries, which is not observed in ENP partner and EU candidate/potential candidate countries, is probably a result of the dominance of *Bacillus* active ingredients in the EU/EEA mosquito control market. This can be concluded from the responses to Questions 9 and 13, where EU/EEA respondents identified the limited number of registered biocidal products (biological, chemical) as the most important barrier towards effective vector control strategies. In fact, many of the respondents declared that only one biological larvicide was available, at high cost and with limited residual efficacy, affecting the sustainability of their larviciding operations. The respondents further stressed the need for a wider range of products and active ingredients to manage the increasing problem of pesticide resistance. Another important constraint identified was a complex regulatory framework that does not cater to environments with a high level of complexity and vector abundance.

Larviciding interventions (in any form) appear to be performed proactively in the various EU/EEA countries/regions, since the vast majority of programmes apply these methods in response to vector abundance data (larval densities). ULV adulticiding treatment is generally triggered by the occurrence of autochthonous WNV human cases among EU/EEA countries, with less than 50% of the respondents applying these methods in response to high infection rates in mosquitoes or sentinel animals. This method is therefore primarily used as an emergency, reactive response measure. The main flaw in this reactive approach is that by the time the first case is confirmed, WNV has already been amplified to epidemic levels in birds and mosquitoes and is therefore more difficult and costly to contain. When adequately implemented, some of the most effective WNV surveillance methods that are currently applied in several European countries (e.g. mosquito/sentinel animal screening) can provide warnings several weeks in advance of human cases. Therefore these methods should be used to support decision-making on when and where to deploy vector control (including adulticiding) to prevent or minimise the occurrence of human infections. ENP partner and EU candidate/potential candidate countries appear to apply all available vector control methods (including adulticiding) routinely in response to vector abundance data.

Finally, when asked whether they need vector control guidelines >84% of the countries responded 'yes', indicating that there is currently a need for more guidance on how to mitigate the threat posed by WNV.

West Nile virus vector management - what is the evidence of effect on epidemiological parameters?

In the absence of an effective vaccination for humans, vector control remains the primary line of defence in preventing and containing WNV outbreaks [5]. An integrated vector management (IVM) strategy utilises the appropriate combination of vector surveillance tools to guide vector control interventions at the right time and place, in response to WNV entomological/enzootic circulation indicators in order to reduce or even prevent WNV outbreaks [5,10,33]. An IVM strategy relies on a rational combination of effective and ecologically sound vector control methods (every method has its limitations by default and to help balance these limitations, a synergy of methods is often required) in a specific operational context, to reduce and sustain vector populations below thresholds where WNV transmission to humans is infrequent [10]. Integrated vector control is the most sound and comprehensive strategy for combating mosquito-borne diseases, however, there is currently limited evidence on the impact of IVM programmes in preventing WNV outbreaks.

Direct evidence from nine different studies, conducted by eight different groups of independent researchers in five different geographical regions indicates that targeted, surveillance-based aerial ULV adulticiding treatments can effectively reduce WNV circulation levels. Two of these studies [17,23] provide direct evidence that this vector control method is effective in disrupting the WNV transmission cycle and in reducing human illness at urban sites. One study provided evidence that ground ULV adulticiding can reduce abundance of vector populations, however, this reduction was not reflected in a reduction of WNV infection rates in *Culex* mosquitoes. In four case studies conducted in the context of an organised, central, integrated vector control programme (funding capacities ranging from USD 1.9–31.4 per capita), WNV was anticipated and early preventive measures were applied including intensive larviciding and public education. However, in all four case studies, WNV amplification could not be prevented, as evidenced by high infection rates in mosquitoes, and/or high seroconversion rates in sentinel animals, and also human cases. Adulticiding was not included in the prevention strategy and was only implemented after the onset of human cases.

Only one study was identified that aimed to link larval reduction (in catch basins exposed to regular larviciding treatments) to WNV prevalence in adult *Culex* mosquitoes [26], but no difference was observed between treated and untreated sites. Moreover, no link could be made between larval reduction and reduction of adult female mosquito populations within treatment sites. The scale of the treatments may have been too small, or the presence of larval habitats outside of and within the boundaries of the treatment sites may have limited efficacy estimates of larviciding in this study. *Cx. pipiens* adults are reported to travel for up to 1.5 km/night [34]. This, in combination with the evidence produced by McMillan and colleagues [26], indicates that the peri-domestic strategy of escalating vector control measures within a buffer of 200–500 m around a WNV human case, commonly applied in some European regions/countries [11], may not be sufficient to reduce transmission of WNV.

During the last decade, vector control research in Europe has been focusing on invasive mosquito species and in particular on *Ae. albopictus*. One study was identified [15] that, even though primarily designed to target *Ae. albopictus*, reported efficacy against adult *Cx. pipiens* populations in an urban environment: Caputo and colleagues [15] investigated the effects of operational larviciding interventions on road-side catch basins in combination with LV adulticiding against adult mosquito populations (two methods commonly applied in Italy) and reported significant reduction in both adult *Ae. albopictus* and *Cx. pipiens* populations in the treated areas. The authors could not determine whether the synergy of the two methods or adulticiding alone was responsible for the control levels achieved and concluded that the relative role of adulticides and larvicides should be investigated separately. Overall, it is clear that the impact of operational larviciding on wild adult *Culex* populations and WNV epidemiology remains largely understudied [14,26].

There is currently evidence that intensive mosquito-adulticiding efforts, applied once a WNV outbreak has started, can interrupt virus transmission and prevent human cases. However, evidence of vector control interventions preventing WNV outbreaks from occurring is limited and additional research under operational conditions is needed to better inform public health policy decisions relating to optimum vector control strategies for WNV management.

Limitations of the methodology

The results are representative only for those countries that responded to the questionnaire. One of the limitations of the survey is that it focused on national capacities of WNV surveillance and control, ignoring the intra-country variations that may be significant. The scoping literature review is not an exhaustive review and there may be studies (particularly in languages other than English) that were not detected.

5. Conclusions and recommendations

This report reviewed the WNV surveillance and control activities implemented in the EU/EEA, the EU candidate and potential candidate countries and ENP partner countries and collated existing evidence on the effectiveness of vector control practices in reducing WNV risk. The main conclusions are listed below.

- WNV surveillance and control activities are implemented in the majority of countries with a history of WNV transmission to humans and animals.
- Consistent WNV surveillance is the driver for vector control decisions and needs to be reinforced and acted upon.
- Source reduction and public education campaigns are essential vector control measures. However, as reported in the survey, it has been challenging for countries to raise public awareness about larval source reduction in the domestic environment. Establishing a mechanism to increase citizens' awareness and action is an important step forward.
- Larviciding is the primary method of response against WNV vectors (against mosquitoes in general, but that is outside the scope of this document). However, like any other method, it has limitations (e.g. patchy and non-homogenous applications due to vast and/or inaccessible areas, such as domestic breeding sites, may have a significant impact on the overall efficacy of larviciding efforts). Achieving reductions in mosquito populations to levels below thresholds of WNV transmission solely by larviciding has been challenging and was shown not to be feasible in the nine studies identified in this report.
- Other methods in addition to larviciding need to be applied in parallel, including adulticiding interventions, to reduce the risk of WNV. This is especially true when source reduction and larval control have failed or are not feasible.

Aerial ULV adulticiding is the only method for which scientific evidence is currently available showing that it can reduce the incidence of WNV human cases and can be considered a useful method for WNV response strategies. However, its successful application depends on complex insecticide application technology and suitable weather conditions. Therefore, it should only be applied by well-trained professionals using spray guidance technologies and following appropriate application parameters to achieve treatment precision with minimal health and environmental impact.

The implementation of vector control programmes is limited by a critical lack of evidence on the impact of vector control (primarily larviciding interventions) on preventing WNV outbreaks. Public health officials, veterinary authorities, academics and vector control operators in Europe and across the world should join forces in providing evidence from large-scale, controlled, operational trials on the efficacy of currently available vector control methods (singly and in combination under the umbrella of IVM) in reducing WNV circulation levels. Data on abundance reduction alone may be insufficient to shape public health policies. Due to the significant costs associated with the large scale of the studies required, it would make sense to identify areas with continuous WNV transmission and competent local surveillance and control infrastructure that could serve as research hubs.

WNV vector control constraints were highlighted by EU Member States in relation to the limited availability of biocidal products; issues with biocide registration and regulatory frameworks on vector control applications, and limited availability of local expertise and resources. Furthermore, the majority of EU countries expressed the need for EU guidelines on WNV vector control. To address the above issues the following actions can be considered:

- A dialogue needs to be established between all relevant actors (public health authorities, European and national biocide authorities, the insecticide industry, vector control operators) to ensure that products are developed and registered that suit the requirements for an effective vector control strategy. The limited number of active substances available in the EU, in combination with the increasing reports of insecticide resistance, further emphasise the need to maintain a vector control toolbox containing a wide variety of chemical and non-chemical interventions.
- EU-wide technical guidelines for WNV vector management should be established, taking into account the currently available scientific evidence. It is important to set application standards for the various vector control methodologies to ensure effective and safe (both for humans and the environment) applications. Absence of knowledge and lack of technical expertise can lead to unsafe and ineffective treatments. Standardised training and certification of vector control professionals, if not already present, should be performed to further ensure effective and safe insecticide applications.
- There is a need to further strengthen and expand WNV vector surveillance and control capacities across the EU/EEA and in EU candidate/potential candidate countries, while investing in a centralised technical support mechanism to assist those countries that may not be able to locate resources and expertise for sufficient vector control response in the near future.

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Annex 1.

Questionnaire survey about West Nile virus vector mosquito surveillance and control activities

Fields marked with * are mandatory.

The main objective of this survey is to collate information on the vector control strategies against West Nile virus (WNV) currently implemented for the prevention and response to WNV human outbreaks in EU member countries, EU enlargement countries and EU neighbourhood policy countries.

The survey collects key information on:

- Monitoring and surveillance of vectors of WNV
- Control of vectors of WNV
- Criteria for implementing methods
- Monitoring and evaluation of methods

The survey results will feed into a technical report on the topic of control of West Nile virus vectors, which will also be based on a literature review, expert knowledge and the discussion and report of an ECDC expert meeting reviewing vector control practices and strategies against West Nile virus, and will be prepared by a contractor for ECDC.

General Information about Respondent

Name (optional)

This information will be treated as strictly confidential.

Email (optional)

This information will be treated as strictly confidential.

* Affiliation

* Which country or region are you providing information for in this questionnaire?
Note: if you work in multiple sub-national regions, please fill in a separate questionnaire for each region.

In case of a sub-national region, please also provide the name of the country and the administrative level (e.g. NUTS2) of the region.

* Do you give ECDC and its contractor your consent to use the answers you provide in the report?

- Yes
- No

If you want ECDC to send you a copy of the report when finished (expected mid 2020), please re-enter your email address.

This information will be treated as strictly confidential.

WNV Risk Level

* 1. What risk level of West Nile virus applied to your country/region in 2019? Select the highest applicable level.
See also: https://www.ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/west-nile-virus-risk-assessment-tool.pdf

- 0. No historical circulation of WNV and unsuitable ecological conditions (e.g. absence of vectors)
- 1. Ecological conditions suitable for WNV circulation but no historical circulation of WNV
- 2. Past evidence of WNV circulation
- 3a. Evidence of WNV circulation in mosquitoes or birds in the second part of the current season (August-September-October)
- 3b. Evidence of WNV circulation in mosquitoes or birds in the first part of the current season (May-June-July)
- 4. WNV-specific IgM detected in local non-vaccinated horse(s) or WNV detected in a local horse
- 5. Detection of at least one human case according to the EU case definition

Questions about Surveillance

2. WNV vector surveillance in your country/region is currently conducted at: (check all that apply)

- National level
- Regional level
- Local level
- None (not conducted, go to question 6)

Comments

3. WNV vector surveillance in your country/region is currently conducted by: (check all that apply)

- ☐ Government
- ☐ Academia
- ☐ Private institutes
- ☐ Other (please provide details under comments below)

Comments

4. WNV vector surveillance in your country/region is funded by: (check all that apply)

- ☐ National government
- ☐ Regional/local government
- ☐ Academia
- ☐ Privately
- ☐ International funds
- ☐ Other (please provide details under comments below)

Comments

5a. Please check all WNV surveillance activities that are performed in your country/region and give the frequency

	Weekly	Every two weeks	Monthly	Other frequency (please specify in comments below)
Routine mosquito surveillance through standardized methodologies and species identification (do not select if mosquito surveillance is ad hoc or if species identification is not performed)	○	○	○	○
WNV surveillance through mosquito screening for WNV	○	○	○	○
WNV surveillance through sentinel birds	○	○	○	○
WNV surveillance through sentinel equids	○	○	○	○

5b. Please check all other WNV surveillance activities that are performed in your country/region

- ☐ WNV passive surveillance through sick/dead animals (birds, equids)

- [ ] WNV passive surveillance through detection of human cases
- [ ] WNV screening of human blood products
- [ ] Pesticide resistance testing in mosquitoes
- [ ] Other (please provide details under comments below)

Comments

Questions about Control

* 6. WNV vector control in your country/region is currently conducted by: (check all that apply)

- [ ] Government
- [ ] Academia
- [ ] Private institutes
- [ ] Other (please provide details under comments below)
- [ ] None (not conducted, go to question 8b)

Comments

7. WNV vector control in your country/region is funded by: (check all that apply)

- [ ] National government
- [ ] Regional/local government
- [ ] Academia
- [ ] Privately
- [ ] International funds
- [ ] Other (please provide details under comments below)

Comments

8a. What specific WNV vector control methods were implemented in your country/region in 2019?

- [ ] Adulticides: ULV (ultra low volume)– space spray (ground)
- [ ] Adulticides: ULV– space spray (aerial)
- [ ] Adulticides: LV (low volume) - surface spray (ground)
- [ ] Larvicides: Chemical (e.g. insect growth regulators)
- [ ] Larvicides: Biological (e.g. B.thuringensis var israelensis)
- [ ] Larvicides: Water surface films
- [ ] Larvicides: Physical source reduction
- [ ] Community outreach & educational campaigns: Door-to-door
- [ ] Community outreach & educational campaigns: Educational seminars
- [ ] Community outreach & educational campaigns: Media

- [ ] Other (specify in comments below)

Comments

8b. According to existing plans in your country/region, what specific WNV vector control methods (in rows) are to be implemented in your country/region depending on the WNV risk level (in columns)? Check any that apply to each (potential) risk level.

	High Vector population but no historical virus detection	Evidence of WNV circulation in the past	Evidence of WNV circulation in mosquitoes or birds in Aug.–Oct.	Evidence of WNV circulation in mosquitoes or birds in May–July	Evidence of WNV in one or more equids	Evidence of WNV in one or more humans
Adulticides: ULV (ultra low volume)– space spray (ground)	☐	☐	☐	☐	☐	☐
Adulticides: ULV– space spray (aerial)	☐	☐	☐	☐	☐	☐
Adulticides: LV (low volume) - surface spray (ground)	☐	☐	☐	☐	☐	☐
Larvicides: Chemical (e. g. insect growth regulators)	☐	☐	☐	☐	☐	☐

Larvicides: Biological (e. g. B. thuringensis var israelensis)	☐	☐	☐	☐	☐	☐
Larvicides: Water surface films	☐	☐	☐	☐	☐	☐
Larvicides: Physical source reduction	☐	☐	☐	☐	☐	☐
Community outreach & educational campaigns: Door-to-door	☐	☐	☐	☐	☐	☐
Community outreach & educational campaigns: Educational seminars	☐	☐	☐	☐	☐	☐

Community outreach & educational campaigns: Media (e.g. press, TV, radio, social media)	☐	☐	☐	☐	☐	☐
Other (specify in comments below)	☐	☐	☐	☐	☐	☐
None	☐	☐	☐	☐	☐	☐
I do not know / Not planned for	☐	☐	☐	☐	☐	☐

Comments

9a. Which are in your opinion the most important constraints/limitations for implementing **ULV (ultra low volume) ground adulticiding** for WNV vector control in your country/region? Rate the constraints from 1 to 5 stars with 5 stars being the strongest level of constraint, and 1 star being the weakest level. Leave blank if you do not know.

High cost that limits frequency of application/method	☆☆☆☆☆
High cost that limits the size of the area covered	☆☆☆☆☆
Operational capacity constraints (knowledge, people, resources, equipment)	☆☆☆☆☆
No or limited number of products registered for this use	☆☆☆☆☆
Regulatory constraints that limit the application frequency of products/methods	☆☆☆☆☆
Regulatory constraints that prevent areas from being treated	☆☆☆☆☆
Unwillingness of community to collaborate	☆☆☆☆☆

9b. Which are in your opinion the most important constraints/limitations for implementing **ULV (ultra low volume) aerial adulticiding** for WNV vector control in your country/region? Rate the constraints from 1 to 5 stars with 5 stars being the strongest level of constraint, and 1 star being the weakest level. Leave blank if you do not know.

High cost that limits frequency of application/method	☆☆☆☆☆
High cost that limits the size of the area covered	☆☆☆☆☆
Operational capacity constraints (knowledge, people, resources, equipment)	☆☆☆☆☆
No or limited number of products registered for this use	☆☆☆☆☆
Regulatory constraints that limit the application frequency of products/methods	☆☆☆☆☆
Regulatory constraints that prevent areas from being treated	☆☆☆☆☆
Unwillingness of community to collaborate	☆☆☆☆☆

9c. Which are in your opinion the most important constraints/limitations for implementing **LV (low volume) adulticiding** for WNV vector control in your country/region? Rate the constraints from 1 to 5 stars with 5 stars being the strongest level of constraint, and 1 star being the weakest level. Leave blank if you do not know.

High cost that limits frequency of application/method	☆☆☆☆☆

High cost that limits the size of the area covered	☆☆☆☆☆
Operational capacity constraints (knowledge, people, resources, equipment)	☆☆☆☆☆
No or limited number of products registered for this use	☆☆☆☆☆
Unavailability of products with long residual efficacy	☆☆☆☆☆
Regulatory constraints that limit the application frequency of products/methods	☆☆☆☆☆
Regulatory constraints that prevent areas from being treated	☆☆☆☆☆
Unwillingness of community to collaborate	☆☆☆☆☆

9d. Which are in your opinion the most important constraints/limitations for implementing **chemical larviciding** for WNV vector control in your country/region? Rate the constraints from 1 to 5 stars with 5 stars being the strongest level of constraint, and 1 star being the weakest level. Leave blank if you do not know.

High cost that limits frequency of application/method	☆☆☆☆☆
High cost that limits the size of the area covered	☆☆☆☆☆
Operational capacity constraints (knowledge, people, resources, equipment)	☆☆☆☆☆
No or limited number of products registered for this use	☆☆☆☆☆
Unavailability of products with long residual efficacy	☆☆☆☆☆
Regulatory constraints that limit the application frequency of products/methods	☆☆☆☆☆
Regulatory constraints that prevent areas from being treated	☆☆☆☆☆
Unwillingness of community to collaborate	☆☆☆☆☆

9e. Which are in your opinion the most important constraints/limitations for implementing **biological larviciding** for WNV vector control in your country/region? Rate the constraints from 1 to 5 stars with 5 stars being the strongest level of constraint, and 1 star being the weakest level. Leave blank if you do not know.

High cost that limits frequency of application/method	☆☆☆☆☆
High cost that limits the size of the area covered	☆☆☆☆☆
Operational capacity constraints (knowledge, people, resources, equipment)	☆☆☆☆☆
No or limited number of products registered for this use	☆☆☆☆☆

Unavailability of products with long residual efficacy	☆☆☆☆☆
Regulatory constraints that limit the application frequency of products/methods	☆☆☆☆☆
Regulatory constraints that prevent areas from being treated	☆☆☆☆☆
Unwillingness of community to collaborate	☆☆☆☆☆

9f. Which are in your opinion the most important constraints/limitations for implementing **larval source reduction through environmental management** for WNV vector control in your country/region? Rate the constraints from 1 to 5 stars with 5 stars being the strongest level of constraint, and 1 star being the weakest level. Leave blank if you do not know.

High cost that limits frequency of application/method	☆☆☆☆☆
High cost that limits the size of the area covered	☆☆☆☆☆
Operational capacity constraints (knowledge, people, resources, equipment)	☆☆☆☆☆
Regulatory constraints that limit the application frequency of methods	☆☆☆☆☆
Unwillingness of community to collaborate	☆☆☆☆☆

9g. Which are in your opinion the most important constraints/limitations for implementing **communication campaigns** for WNV vector control in your country/region? Rate the constraints from 1 to 5 stars with 5 stars being the strongest level of constraint, and 1 star being the weakest level. Leave blank if you do not know.

High cost that limits frequency of application/method	☆☆☆☆☆
High cost that limits the size of the area covered	☆☆☆☆☆
Operational capacity constraints (knowledge, people, resources, equipment)	☆☆☆☆☆
Unwillingness of community to collaborate	☆☆☆☆☆

Comments

10. Are you aware of any method to control West Nile virus vector mosquitoes being/having been **evaluated /tested** for **outcomes** and/or **cost** in your country/region in the last 5 years? Check all boxes that apply according to the suggested aim.

	For effect on mosquito abundance	For assessment of costs	Other objective (describe in comments below)

Aerial adulticide treatment	◯	◯	◯
Ground adulticide treatment	◯	◯	◯
Mass trapping	◯	◯	◯
Personal protection (Communication campaigns)	◯	◯	◯
Environmental management	◯	◯	◯
Chemical larvicides (e.g. Temephos, Triflumuron)	◯	◯	◯
Insect growth regulators larvicides (e.g. Pyriproxyfen, Methoprene)	◯	◯	◯
Non-biological non-chemical (e.g. Aquatain®)	◯	◯	◯
Biological larvicide (e.g. B.t.i.)	◯	◯	◯
Other biological method (e.g. fishes, copepods)	◯	◯	◯
Door-to-door campaigns	◯	◯	◯
Communication campaigns	◯	◯	◯
Other (provide detail below)	◯	◯	◯

Comments

Questions about Guidelines

* 11. In your opinion do you need new technical guidelines for WNV mosquito vector **surveillance** in your country/region?

- ◯ Yes
- ◯ No

* 12. In your opinion do you need new technical guidelines for WNV mosquito vector **control** in your country/region?

- ◯ Yes
- ◯ No

General

13. Please provide information on any additional **issues/challenges** you are facing in relation to WNV surveillance and WNV vector control in your country/region

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