

West Nile virus epizootics in the Camargue (France) in 2015 and reinforcement of surveillance and control networks

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Summary

West Nile virus (WNV) infection is a non-contagious disease mainly transmitted by the bites of infected mosquitoes from the genus *Culex*. The virus is maintained in a mosquito–bird–mosquito cycle, and can accidentally be transmitted to mammalian hosts. Among mammalian hosts, equines and humans are the most sensitive to WNV infection and can develop severe meningoencephalitis. As WNV infections are zoonotic and can be severe in humans and equines, West Nile fever is considered to be a public and animal health concern. After a silent period of almost ten years, WNV re-emerged in France at the periphery of the Camargue area during the summer of 2015, underlining the fact that the Camargue area creates favourable conditions for WNV emergence and amplification in France. The French Network for Epidemiological Surveillance of Equine Diseases (*Réseau d'Épidémiologie-Surveillance en Pathologie Équine* [RESPE]) facilitated the early detection of WNV cases in horses. In total, 49 horses were found to be infected; among them, 44 presented clinical signs, 41 with meningoencephalitis and three with hyperthermia only. Six horses among the 41 with nervous symptoms died from the disease or were euthanised (a case fatality rate of 14.6%). The authors describe the characteristics of the 2015 WNV epizootics, the early detection of the first WNV equine cases via the RESPE network and the coordination of WNV surveillance in France.

Keywords

Camargue – Epizootic – Equine – France – French Network for Epidemiological Surveillance of Equine Diseases – Human – Réseau d'Épidémiologie-Surveillance en Pathologie Équine – RESPE – West Nile virus.

Introduction

West Nile virus (WNV) is a member of the Japanese encephalitis (JE) serocomplex and belongs to the genus *Flavivirus* in the *Flaviviridae* family, which includes other major animal and/or human pathogens, such as the Saint Louis encephalitis, JE, yellow fever, Zika and dengue viruses. West Nile virus was first isolated from the blood of a febrile woman in Uganda in 1937 (1) and currently has a worldwide distribution that ranges from Africa, the Middle East, Europe, Asia and Oceania to South and North America.

West Nile virus is maintained in an enzootic cycle between *Culex* mosquitoes and birds but can also infect and cause disease in other vertebrate animals, including horses and humans (2). In most infected equines, WNV infection is subclinical, but approximately 5–20% of infected animals may develop symptoms of WNV disease, ranging from West Nile fever (fever, weakness, lethargy, anorexia) to meningoencephalitis (behavioural impairment, including hyperaesthesia, aggressiveness and somnolence; ataxia, paresis or paralysis; muscle tremors). Given that WNV is a zoonotic pathogen and is associated with potentially severe or fatal diseases in infected horses and humans, WNV neuroinvasive disease is a notifiable disease that must be reported to national and international organisations, and in particular to the World Health Organization (WHO) (human cases) and to the World Organisation for Animal Health (OIE) (equine cases). Members of the European Union (EU) must also notify the European Commission.

The introduction and circulation of WNV have been demonstrated on multiple occasions in southern Europe and the Mediterranean Basin since the 1960s. WNV activity has dramatically increased over the last five years and it has spread to eastern territories without previous WNV records (3). In 2015, 106 human cases of neuroinvasive disease were reported in EU countries (4), a situation similar to that observed in 2014; nevertheless, unlike previous years, WNV activity was primarily reported in western Mediterranean countries (in Italy, France and Portugal in humans and equines, and in Spain in equines).

The first reported WNV outbreak in France occurred in 1962 in the Camargue region, a natural wetland on the Mediterranean coast located south of the city of Arles, within a triangle defined by the two arms of the Rhône River and the Mediterranean Sea (5). More recently, WNV epizootics in France were reported in this same region in 2000 and 2004, as well as in Var and the Eastern Pyrenees, two other French 'departments' bordering the Mediterranean Sea, in 2003 and 2006, respectively (6). After a silent period of more than ten years in the region, WNV re-emerged at the periphery of the Camargue area during the summer

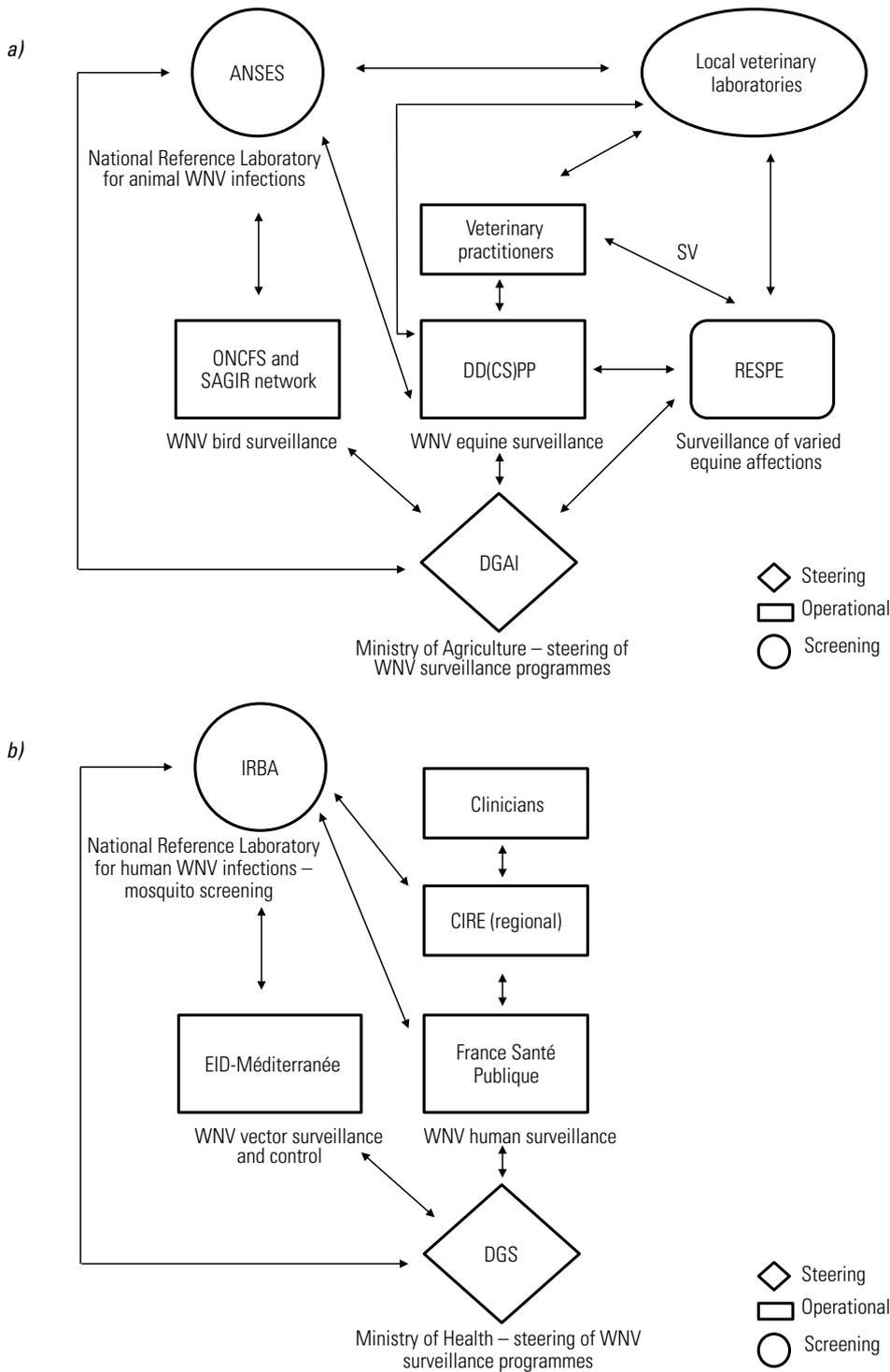
of 2015, underlining the fact that the Camargue area creates favourable conditions for WNV amplification. The characteristics of the 2015 French WNV epizootics are presented. The authors emphasise the effectiveness of the French Network for Epidemiological Surveillance of Equine Diseases (*Réseau d'Épidémiologie-Surveillance en Pathologie Équine* [RESPE]) for the early detection of equine WNV cases, as well as the importance of integrated and coordinated monitoring in humans, animals and vectors and concerted measures implemented by the multiple actors involved in WNV surveillance and control.

Materials and methods

Reporting of West Nile virus suspect cases in equines through the RESPE network and local Veterinary Services

West Nile virus surveillance in equines in France (Fig. 1a) is based on clinical surveillance of neurological syndromes, with veterinary practitioners expected to report clinically suspect cases of West Nile fever to local Veterinary Services. Owner and horse data (location, clinical data) are systematically collected by veterinarians upon their first visit and provided to local Veterinary Services, while samples are sent to local laboratories approved for WNV diagnosis. The first samples found positive in a newly affected department are analysed for confirmation at the French Agency for Food, Environmental and Occupational Health and Safety (*Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail* [ANSES]) in Maisons-Alfort, which is the National Reference Laboratory for equine West Nile disease. All positive results are reported to local and national Veterinary Services.

French veterinary practitioners are supported by the RESPE network in the identification of the causative agent. The network is a passive surveillance system based on the declarations of 594 voluntary sentinel veterinarians (SVs) distributed all across France and its overseas territories in 96 of its 101 departments (Fig. 2). Only three metropolitan departments and two departments from the Caribbean do not have SVs. Accordingly, the network covers all the major French horse-breeding regions, as well as areas with numerous sports or recreational equestrian activities. Aetiological investigation of diverse pathological conditions in horses (e.g. neurological, acute respiratory, gastrointestinal [in the foal only] infections and abortions, as well as piroplasmosis-like syndrome, atypical myopathy and genetic abnormalities) is conducted following standardised protocols which include a list of mandatory laboratory tests. The RESPE network provides testing in partner laboratories (LABEO Frank Duncombe and ANSES).



ANSES: French Agency for Food, Environmental and Occupational Health & Safety (National Reference Laboratory for equine West Nile virus infections)
 CIRE: Regional surveillance unit
 DD(CS)PP: French local Veterinary Services
 DGAI: French national Veterinary Services
 DGS: Ministry of Health
 EID-Méditerranée: Interdepartmental Agreement for Mosquito Control on the Mediterranean Coast

France Santé Publique (formerly InVS, Institut de Veille Sanitaire): French Institute for Public Health Surveillance
 IRBA: Army Biomedical Research Institute (National Reference Laboratory for human West Nile virus infections)
 ONCFS: French National Hunting and Wildlife Agency
 RESPE: French Network for Epidemiological Surveillance of Equine Diseases
 SAGIR: Network for the Epidemiological Surveillance of Wildlife Diseases
 SV: Sentinel Veterinarians

Fig. 1
Organisation of the French West Nile virus surveillance system, (a) in animals and (b) in humans and vectors
 The role of West Nile virus surveillance institutes is depicted at bottom right

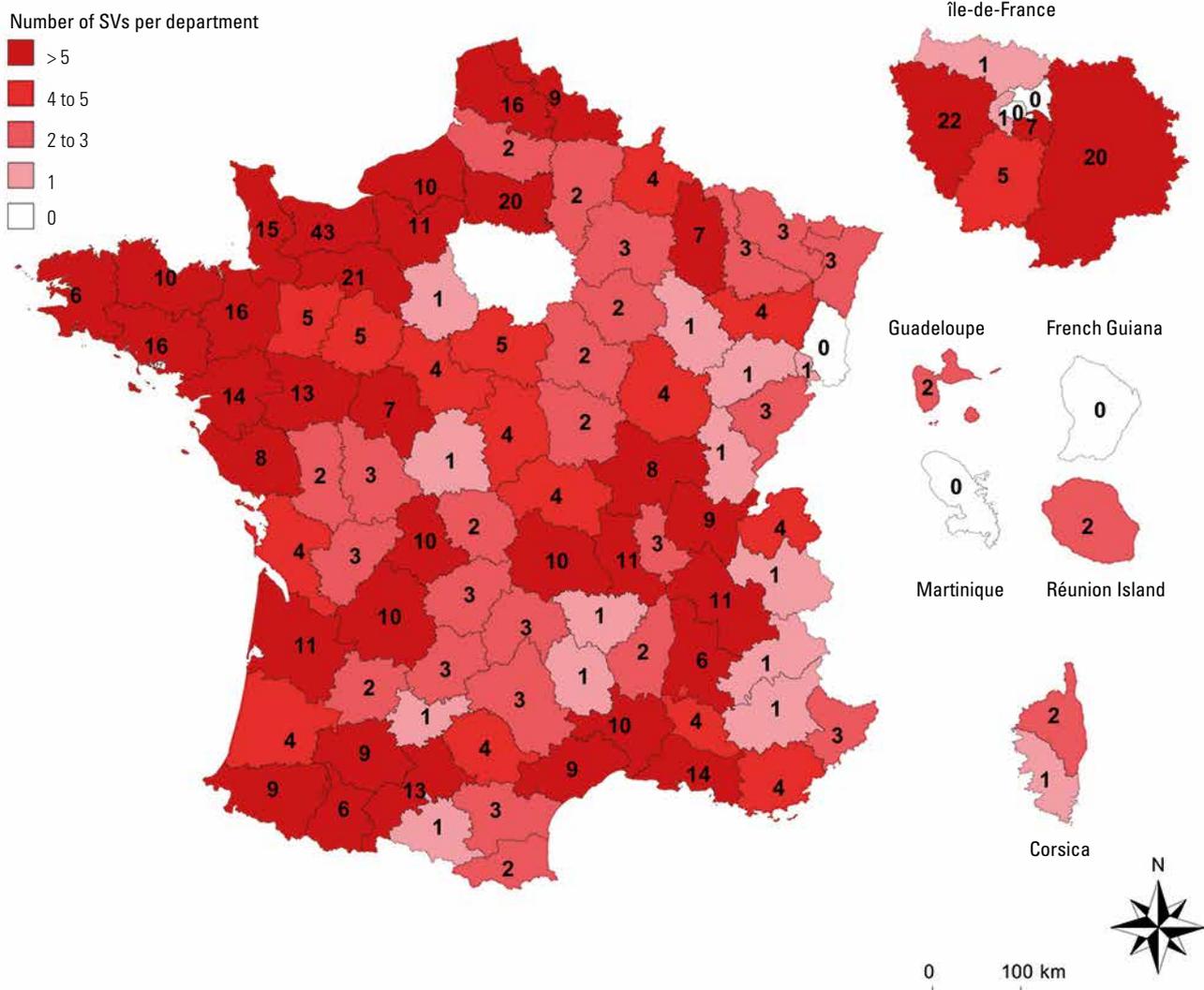


Fig. 2
Distribution of RESPE sentinel veterinarians (SVs) in French departments, December 2014

The number of SVs per department is presented. There are 96 departments in metropolitan France (including eight departments in the Ile-de-France region) and five overseas departments (Guadeloupe, Martinique and French Guiana in the Caribbean, and Reunion Island and Mayotte in the Indian Ocean)

More specifically, the RESPE neurological syndrome sub-network, which was created in 2003, is involved in the surveillance of West Nile neuroinvasive disease and of the neurological form of equine herpesvirus infection (EHV-1). With the exception of traumatic conditions, all neurological abnormalities, including behavioural, alertness, posture or gait problems, as well as decubitus, can be reported through this sub-network. All cases with neurological symptoms reported to the RESPE network are tested both for WNV antibody, by competition (immunoglobulin [Ig]G + IgM) enzyme-linked immunosorbent assay (ELISA) (on serum), and for EHV-1, by reverse-transcription polymerase chain reaction (RT-PCR) (on nasal swabs, blood and/or cerebrospinal fluid [CSF]). The SVs' online submissions include general information on the affected

horse (name, age, sex, location, vaccination and travel history, activity, etc.), as well as its complete clinical picture, comprising the initial assessment and a description of the evolution of general features and neurological signs. A syndromic surveillance approach was recently developed by the RESPE neurological syndrome sub-network and alerts are triggered when numerous cases with neurological signs are reported in France (at least four cases reported during the same week or at least three cases reported per week for two consecutive weeks).

Sentinel veterinarians reporting suspect cases to the RESPE neurological syndrome sub-network proceed with standardised sampling protocols: two serum blood collection tubes, plus two ethylenediaminetetra-

acetic acid (EDTA) whole blood collection tubes, or one nasopharyngeal swab, or one CSF sample. In addition to such sampling protocols, veterinary practitioners reporting directly to local Veterinary Services can send one serum and one EDTA whole blood collection tube to local laboratories. In order to identify and confirm WNV infections in live animals, indirect serological diagnostic tools are primarily used (7). Blood was systematically sampled in serum collection tubes from suspect equine clinical cases, allowed to clot at ambient temperature and centrifuged at 1,500–2,900 g for a maximum of 10 min before the serum was collected. Sera were stored at +4°C for one month at most, or at –20°C for long-term archiving.

Reporting of West Nile virus human suspect cases

The surveillance of WNV infections in humans at countrywide level (Fig. 1b) relies on the human National Reference Laboratory for arboviruses (hNRL). This laboratory, which confirms all WNV infections, is based in Marseille at the Armed Forces Biomedical Research Institute (*Institut de recherche biomédicale des armées* [IRBA]). In areas at risk for WNV, namely departments from the Mediterranean area (Alpes-Maritimes, Aude, Bouches-Du-Rhône, Corsica, Eastern Pyrenees, Hérault, Gard, Var), enhanced seasonal surveillance is implemented every year between 1 June and 31 October. It aims to detect West Nile neuroinvasive disease in humans. Suspect cases, defined as patients over 15-years old presenting with fever ($\geq 38.5^{\circ}\text{C}$) and clinical signs of viral meningitis or encephalitis, should be reported to local health authorities and screened for WNV.

In addition, active surveillance, both retrospective and prospective, is triggered as soon as WNV infection is detected in humans or animals (equines or birds). In 2015, once the first two equine WNV cases had been notified, a retrospective survey was conducted in hospitals from Bouches-du-Rhône and Gard departments with the aim of identifying suspect patients that had not been tested for WNV.

Samples were obtained from human patients with neurological symptoms (meningitis, encephalitis, polyradiculoneuritis). Blood samples in serum and EDTA collection tubes, as well as CSF samples whenever possible, were sent to the hNRL for WNV diagnostics. Serum was processed as previously described for equine samples, while EDTA blood and CSF were stored at –20°C until analysis.

West Nile virus surveillance in wild birds

Surveillance was carried out in Mediterranean departments at risk for WNV circulation from May to October through the SAGIR network, which is a network for the epidemiological surveillance of wildlife diseases set up

by the National Hunting and Wildlife Agency (*Office National de la Chasse et de la Faune Sauvage* [ONCFS]) and the National Federation of Hunters (*Fédération Nationale des Chasseurs* [FNC]). Mortality was monitored in wild birds that have been described as the most sensitive to WNV infection, e.g. corvids, passerids and turdids (order Passeriformes) and species of the order Falconiformes (8, 9) (Fig. 1a). Surveillance was reinforced in wild birds in early September, allowing dead birds from the selected families to be collected through SAGIR, as soon as one specimen was found dead or moribund. Dead birds were shipped to local veterinary laboratories for systematic autopsy and brain, heart, spleen and liver sampling. Organs from the birds were stored at –80°C and shipped frozen to ANSES for analysis.

Vector surveillance

Mosquitoes were captured in CO₂ traps for identification and monitoring of abundance from mid-April to mid-October 2015 by the Interdepartmental Alliance for Mosquito Control on the Mediterranean Coast (*Entente Interdépartementale de Démoustication* [EID] *Méditerranée*), within the framework of regular French vector surveillance programmes (Fig. 1b). Thirty-three CO₂-baited traps (modified from the Centers for Disease Control [CDC] Miniature Light Traps Model 512, without light source) baited with ± 1 kg dry ice (10) were used for specimen collection along the Mediterranean coast. Additional specimens were trapped during the first two identified equine outbreaks, during the first half of September. Mosquitoes were frozen on dry ice, identified, pooled according to species (≤ 50 mosquitoes/pool) and stored at –80°C until analysis at IRBA.

West Nile virus diagnostic methods

For equine suspect cases reported through either RESPE or local Veterinary Services, sera were first screened for anti-WNV antibodies by competition ELISA (IDScreen® West Nile competition, IDVet, Montpellier, France) in local veterinary laboratories approved for WNV indirect diagnosis (Bouches-Du-Rhône, Calvados, Gard, Hérault). Competition ELISA-positive sera were further analysed by MAC (M-antibody capture)-ELISA for IgM detection (IDScreen® West Nile IgM capture, IDVet) in these same laboratories and at ANSES (11). The WNV infection was then confirmed at ANSES by virus neutralisation tests (VNTs), as prescribed by the *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*) (12), as well as by flavivirus Luminex assay, as described by Beck *et al.* (11). Given the long persistence of anti-WNV IgG antibodies in horses, a horse found positive by competition ELISA but negative by IgM capture ELISA was regarded as an unconfirmed case, but assumed to be carrying markers of previous infection (or vaccination).

Organs of wild birds suspected of WNV disease were ground in tubes containing 1 ml Dulbecco's Modified Eagle's Medium (Invitrogen, Thermo Fisher Scientific) and ceramic beads (Lysing Matrix D, MP Biomedicals) and placed into a ribolyser. Extraction of RNA was performed on an aliquot of 250 μ l of the obtained supernatant with the RNeasy mini-kit (Qiagen, Hilden, Germany), while the residual supernatant was stored at -80°C for isolation assays. The RNA extracts were screened at ANSES for the presence of WNV genomic RNAs by real-time RT-PCR on Applied Biosystems thermocyclers (Foster City, California, USA), as described by Linke *et al.* (13). Diagnostic tests performed at IRBA on human samples consist of an identical real-time RT-PCR protocol for the detection of the WNV genome (13) and in-house ELISAs (indirect IgG and MAC-ELISAs) using precipitated and inactivated virus (14).

Mosquito samples were analysed at IRBA for WNV genome detection. Mosquito pools (≤ 50 mosquitoes/pool) were crushed by mechanical homogenisation in 500 μ l–1 ml

phosphate-buffered saline–bovine serum albumin 3%. The RNA extraction was performed with a QIAamp viral RNA mini-kit (Qiagen, Hilden, Germany), according to the manufacturer's recommendations. The real-time RT-PCR for the detection of WNV genomic RNAs was performed as previously described (13).

Results

West Nile virus epizootics in 2015

By 17 November 2015, through the implementation of initial ELISA tests and MAC-ELISA in local veterinary laboratories, as well as confirmatory serological tests at ANSES, 39 equine outbreaks had been confirmed in three departments surrounding the Camargue area, namely Bouches-du-Rhône, Gard and Hérault (Fig. 3). In the outbreak area, 65 animals exhibited meningoencephalitis. Serological tests for WNV ruled out WNV aetiology in

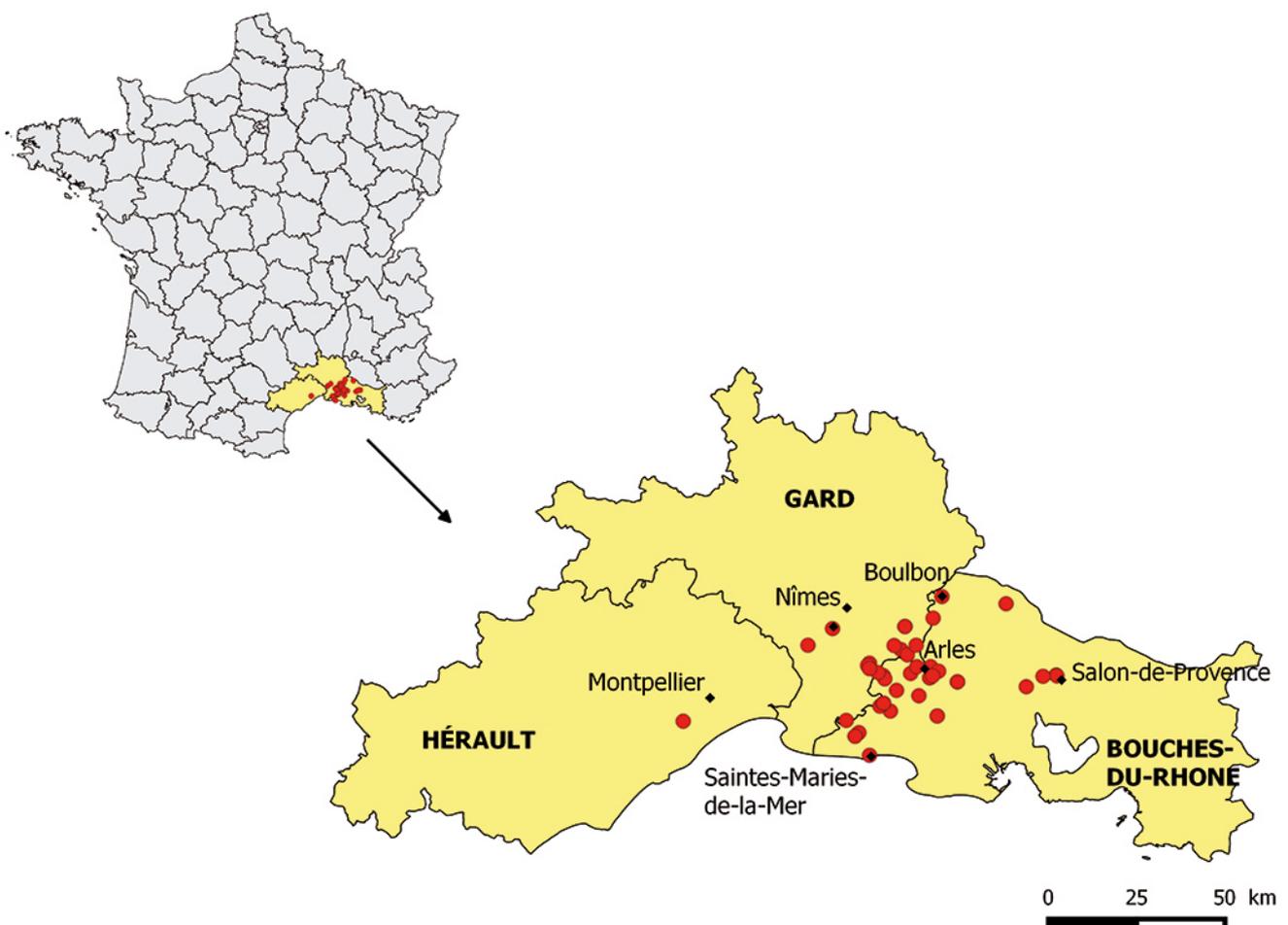


Fig. 3
Distribution map of equine West Nile virus outbreaks in south-eastern France (30 October 2015)

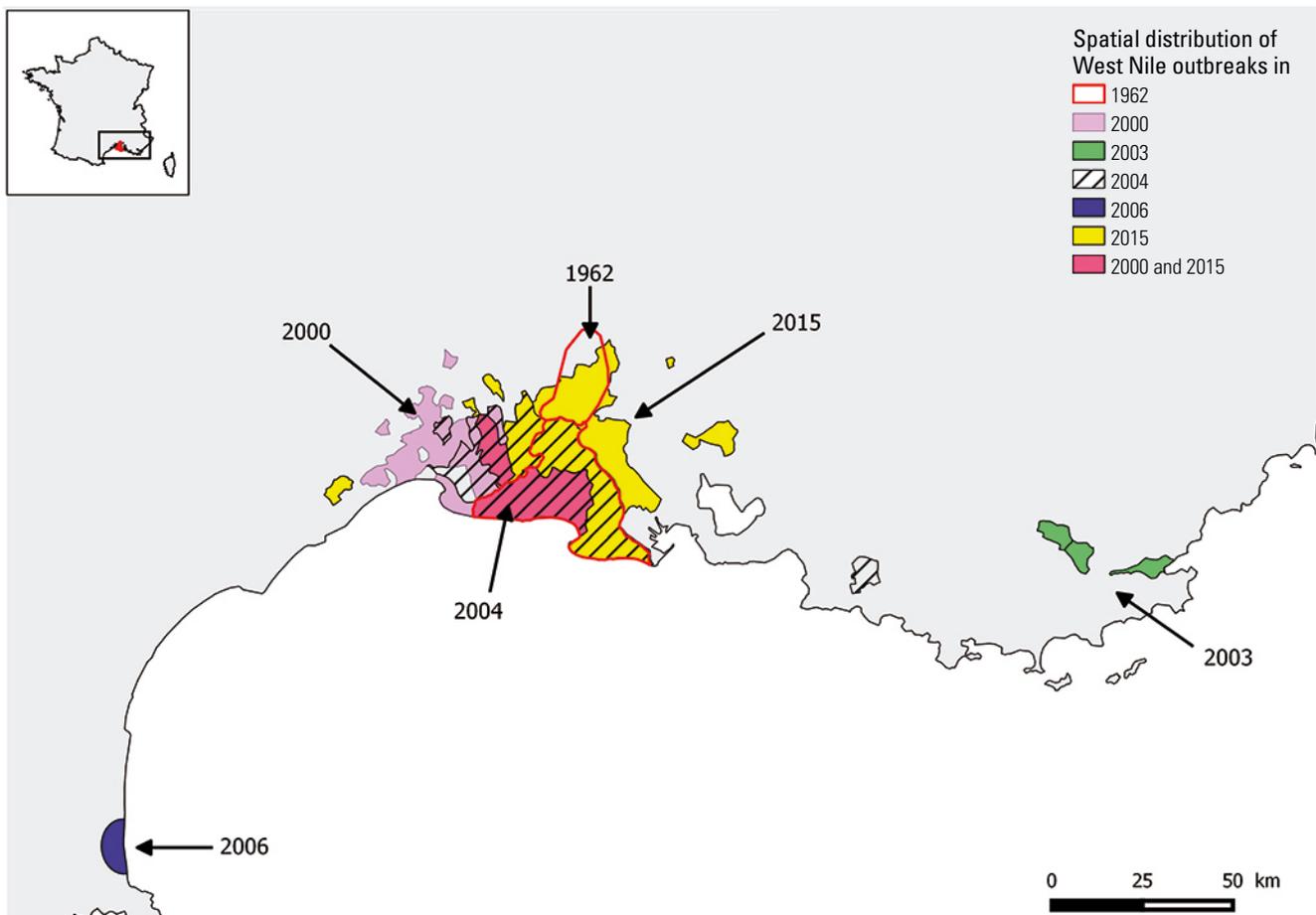


Fig. 4
West Nile virus epizootics on the French Mediterranean coast
 Only equine clinical cases are represented

24 equines (four animals with WNV IgG only and 20 animals with no WNV antibody response). In total, 49 equines were found to be infected with WNV (positive in WNV competition and MAC-ELISAs, as well as in WNV VNT); among them, 41 exhibited a neuroinvasive form, three a mild febrile form and five were asymptomatic. The five asymptomatic infections were identified through systematic screening of all the equines from three recognised outbreaks and through retrospective analysis of equine sera collected in the vicinity of the outbreaks. Neuroinvasive forms of WNV in equines were observed between 11 August and 30 October. Six equines died or were euthanised subsequent to severe WNV neuroinvasive disease and prolonged decubitus; this corresponds to a case fatality rate of 14.6% (6/41).

Equine outbreaks of WNV were mainly located in the Camargue area, on the border of the Bouches-du-Rhône and Gard departments (Fig. 3). They were centred in a 60-km-diameter circle between the communes of Saintes-Maries-de-la-Mer in the south, Boulbon in the north, Salon de Provence in the east and Milhau in the west. Only one

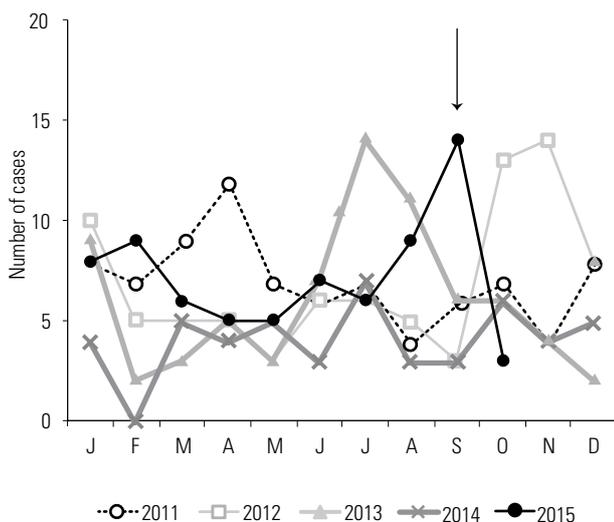
remote case was reported in Hérault department (50 km away from the nearest outbreak in Gard), in a horse with no travel history. Outbreaks of WNV were described as early as 1962 in the region and more recently in 2000 and 2004 in equines only, suggesting that conditions in the Camargue area facilitate WNV circulation (5, 6). France has also reported WNV episodes in other Mediterranean departments, in 2003 in Var (four equine cases) and in 2006 in the Eastern Pyrenees (five equine cases) (Fig. 4). Interestingly, the 2015 outbreak area partially overlapped with those of the 1962, 2000 and 2004 outbreaks (particularly the 1962 and, to a lesser extent, the 2004 outbreaks), while extending further west than previously reported WNV circulation areas.

Early detection of West Nile virus cases in horses

West Nile virus has been monitored through the RESPE neurological syndrome sub-network for more than five years. Unusual increases in the reporting of neurological syndromes to RESPE can be readily detected. Through analysis of monthly reports submitted to the RESPE

neurological syndrome sub-network from 2011 to 2014, notifications were shown to peak in autumn and early winter, from October to January (with six to eight reports each month, on average, while for the rest of the year three to six cases per month were generally observed), when EHV-1 outbreaks are also most frequently detected (Fig. 5). However, in 2013 and 2015, remarkably high numbers of neurological syndromes were reported in July–August and September, respectively. Although the neurological cases that occurred in summer 2013 were investigated, WNV and EHV-1 aetiologies were not confirmed, and no other pathogens, and in particular no arbovirus, could be identified through extensive molecular screening of available samples (next-generation sequencing, RNA array).

However, in 2015, WNV infections were rapidly detected through the RESPE network. The first two WNV cases were reported to RESPE on 17 August, identified at LABEO Frank Duncombe Laboratory as WNV IgM-positive on 26 August and confirmed at ANSES on 29 August. Signs of meningoencephalitis were manifest in both horses: ataxia, convulsions, alertness or behavioural modifications, hyperaesthesia and decubitus were described and had started on 11 August and 17 August. After confirmation of the first two WNV cases, the RESPE network immediately informed every SV about WNV circulation in France, encouraging veterinary practitioners to be more vigilant for neurological signs in horses and to report suspect cases to Veterinary Services. An increase in the number of reports in September 2015 probably reflects the enhanced vigilance of equine veterinary practitioners (Fig. 5, arrow).



RESPE: French Network for Epidemiological Surveillance of Equine Diseases

Fig. 5
Monthly reports of equines with neurological signs received by the RESPE neurological syndrome sub-network from 2011 to 2015
 An unusually high number of reports of neurological cases were reported in September 2015 (arrow)

While ensuring early detection of WNV and participating in the sensitisation of SVs to WNV circulation in the Camargue area, it is important to note that RESPE registered a total of only 26 clinically suspect cases in August–October 2015, quite uniformly distributed throughout France, including three confirmed WNV cases (Fig. 6a). After confirmation of the first WNV cases, most horses in south-eastern France suspected of having meningoencephalitis were not analysed through the RESPE network but were reported directly to local Veterinary Services (75 suspect cases reported to local Veterinary Services only, versus nine suspect cases reported through RESPE). They were mostly located in the three departments with confirmed WNV cases (Bouches-du-Rhône, Gard and Hérault) (Fig. 6b).

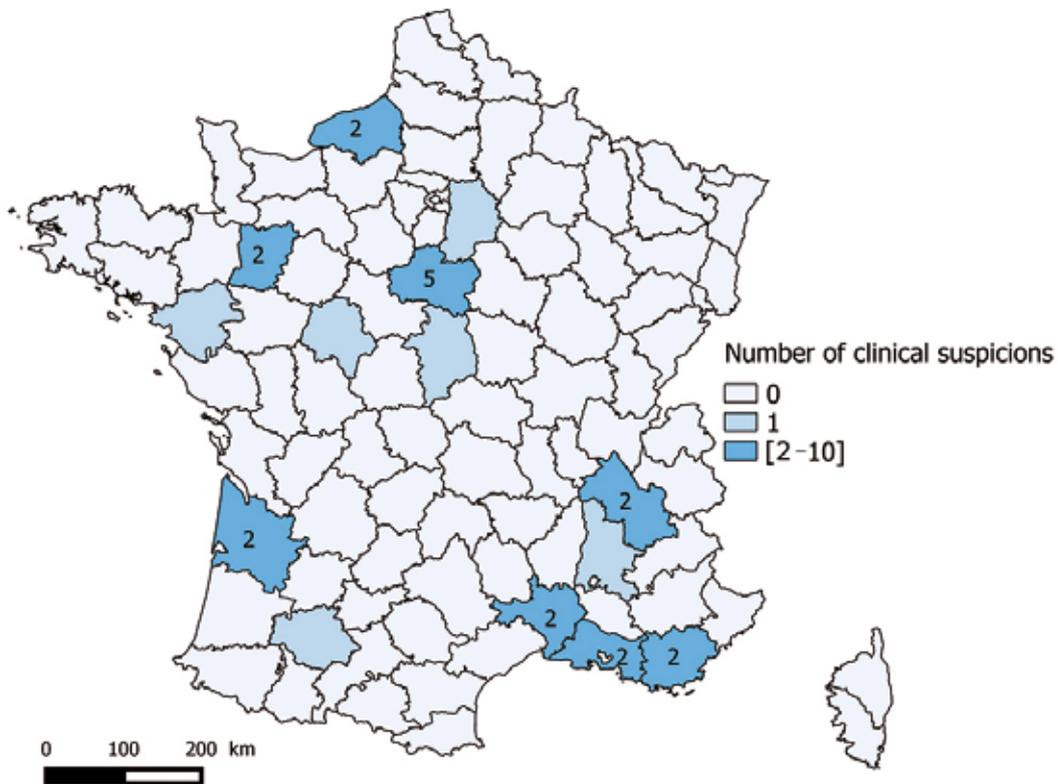
Coordination and reinforcement of West Nile virus surveillance activities in France

As soon as the first equine WNV cases had been confirmed, the French national Veterinary Services (*Direction Générale de l'Alimentation* [DGAL]) informed the numerous contributors to WNV surveillance in equines, humans, wild avifauna and vectors through a telephone conference organised on 31 August. Animal surveillance was reinforced, with DGAL, the French National Society of Veterinary Technical Groups and ONCFS/FNC prompting veterinarians and the SAGIR network to increase vigilance for WNV-induced disease or mortality in horses and wild birds, respectively (on 2 and 4 September). Even though SAGIR was rapidly informed of WNV circulation in the Camargue, only 12 dead birds were reported in the area from July to October 2015, and none of them tested positive for WNV, a situation similar to that described during the 2000 and 2004 WNV outbreaks in the region.

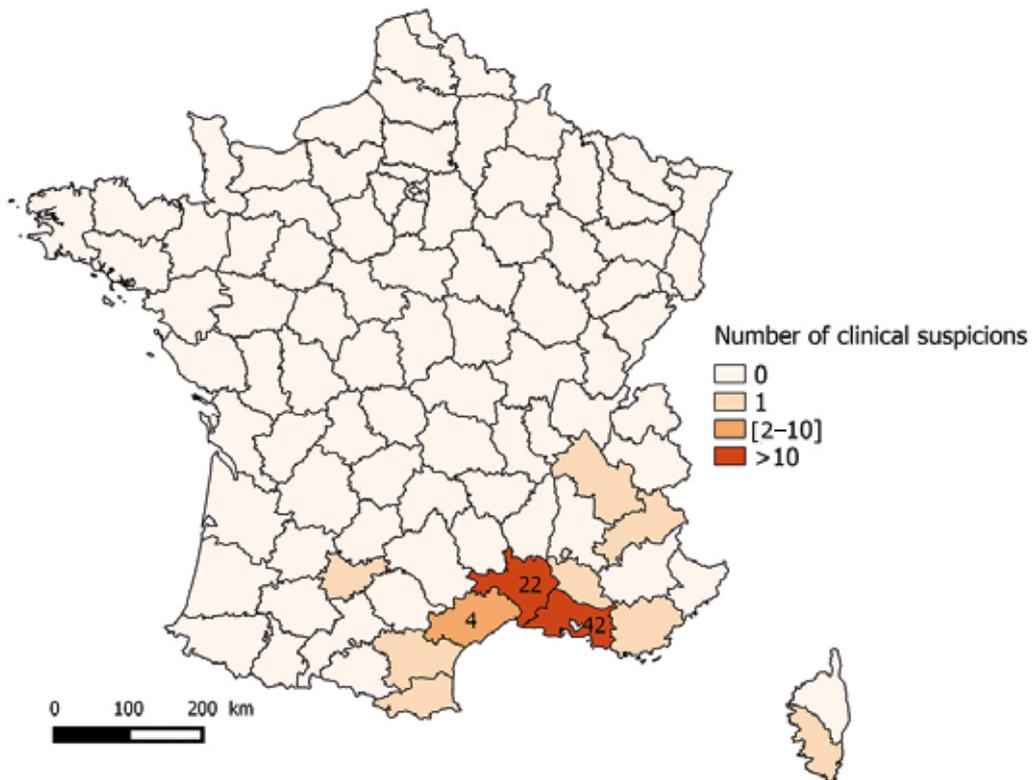
Updated information on equine outbreaks of WNV was shared with WNV surveillance contributors on a weekly or bi-weekly basis on the ESA platform (a French platform for animal epidemiological surveillance [www.plateforme-esa.fr]) and the RESPE Internet site (www.respe.net). A WNV management unit was rapidly implemented on the initiative of the DGAL, in order to evaluate animal surveillance data and to gain insight into the extent of WNV circulation through the establishment of a dedicated prospective survey.

West Nile virus surveillance was also rapidly strengthened in humans and mosquito vectors, under the guidance of the Ministry of Health. The usual enhanced surveillance of West Nile neuroinvasive disease in humans, implemented on 1 June, identified 96 suspect cases, all of which were negative for WNV. Once the first two equine WNV cases had been notified, a retrospective survey was conducted in hospitals in the Bouches-du-Rhône and Gard departments from August to September 2015 to identify patients presenting with unexplained meningitis or encephalitis and not tested for WNV. No WNV infection was diagnosed among the 71 patients identified.

a)



b)



RESPE: French Network for Epidemiological Surveillance of Equine Diseases

Fig. 6

Number of horses suspected of having meningoencephalitis reported (a) through the RESPE network or (b) through local Veterinary Services

The number of clinical suspicions per department is given when greater than one

Discussion

The 2015 WNV transmission season in France was characterised by intense and localised WNV amplification cycles associated with equine WNV neuroinvasive cases. It is, however, difficult to fully infer the amplitude and distribution of WNV circulation in 2015 without implementing specific serosurveys in animals or in humans (15, 16), because most WNV infections are asymptomatic in infected mammals (17). In total, 49 WNV infections were confirmed in equines (positive IgM response), including 41 animals with meningoencephalitis, which corresponds to the third most important WNV epizootic reported in France. West Nile virus had been described in the Camargue area as early as 1962, when at least 50 cases with neurological signs were observed (5). More recently, in 2000, the largest WNV epizootic ever reported in the Camargue occurred from August to early November, with 131 suspect cases presenting with meningoencephalitis, 58 confirmed (positive IgM response), 18 probable infections (positive IgG response only) and 21 deaths (18). At the end of summer 2004, another WNV epizootic was reported in an area close to the 2015 WNV outbreaks, with 57 suspect cases in horses, 32 confirmed cases and seven deaths (6). The case fatality rate of 14.6% during the 2015 WNV epizootic was found to be much lower than usually reported (20–57%) (19). Such findings could be attributed to the low pathogenicity of the circulating WNV strain(s) (analysis in progress), to improved therapeutic measures implemented by equine veterinarians or to enhanced vigilance of practitioners; the last hypothesis is supported by the fact that horses with mild neurological signs (rare stumbling events, for example) were reported and tested using WNV diagnostic tests. It is interesting to note that no abnormal fatalities in wild birds were observed in the Camargue in 2015, a situation similar to that reported in the most recent French WNV outbreaks and in European outbreaks due to lineage 1 WNV strains (20). Moreover, no severe neuroinvasive forms were described in humans.

Disease control mainly relied on informing the public on how to reduce mosquito populations (essentially through destruction of mosquito breeding sites) and limit exposure of animals to mosquito bites. Entomological investigation identified ponds and water tanks that were breeding sites for *Culex pipiens* and *Culex modestus*, and those which could not be emptied were treated with Bti (*Bacillus thuringiensis* serovar *israelensis*, Bt H-14). Since 2009, inactivated or recombinant vaccines have been made available for use in equines in Europe. In France, animal vaccination is performed upon the request of horse owners, and apparently only a small proportion of equines from the Camargue were vaccinated during the outbreak (ongoing investigation).

The spread of WNV in 2015, as assessed by equine WNV surveillance data, was found to mostly overlap with the distribution of equine WNV cases in 1962 and in 2004. Ecological, land use and landscape features in the Camargue area have been shown to support WNV amplification cycles on multiple occasions: large natural reserves, large wetlands, as well as substantial drainage and irrigation efforts for rice production, varied landscapes and lack of adult mosquito controls promote substantial species diversity in WNV bird reservoirs as well as in vectors. The 2015 epizootic was localised in high-risk areas for WNV circulation, as previously assessed by Pradier and co-workers on the basis of landscape structural features (21). Such regular WNV outbreaks in the Camargue area suggest either endemic circulation of the virus, with WNV cycling in most years between birds and *Culex* mosquitoes only, or frequent virus introductions that would more systematically lead to WNV outbreaks. Analysis of the viral strain responsible for the 2015 WNV episode may help in prioritising these hypotheses. Virus could not be recovered from samples collected from living horses with confirmed WNV infection during the 2015 epizootic but was finally isolated and characterised from the central nervous system of a dead animal; the 2015 WNV epizootic was caused by a lineage 1 strain. Interestingly, maintenance of WNV in the absence of equine or human cases has been demonstrated in two bird serosurveys in the Camargue area, conducted in 2007 and in 2009–2010 (22, 23).

Equine WNV surveillance allowed early detection of WNV infections in 2015, as it did during the previous French outbreaks. In Europe, modelling WNV circulation has indicated that clinical surveillance in horses is a cost-effective and sensitive system (24). With reporting of every suspect equine case, identification of the first WNV-positive clinical cases in horses should precede the identification of WNV in mosquitoes or of seroconversion events in birds or horses by a few days, and, at a maximum, two weeks. However, in practice, in countries endemic for WNV such as Italy or Greece, early detection or assessment of WNV spread is usually achieved by vector or sentinel bird surveillance (25, 26).

In 2015, the first two equine cases were reported through the RESPE network, while clinically suspect cases detected subsequently were more readily reported to local Veterinary Services. The two reporting systems appeared to be complementary. The RESPE network, which was created in 1999, was the first network of its kind in Europe. Since 2008 it has become an independent association connecting the equine medicine and industry sectors, and has been recognised as essential for the detection, follow-up and control of equine diseases at the national and international levels (iRESPE initiative). In particular, it ensures comprehensive surveillance of infectious diseases that cause major economic losses for the equine industry or

pose significant risks to public health (zoonotic pathogens). Besides providing support, differential diagnostics and epidemiological information, RESPE also facilitates the submission of samples for WNV diagnostics, and thus enables WNV surveillance. Indeed, following the 2000 French WNV outbreaks, during which strict control measures had been implemented in the Camargue, including banning horse movements and equestrian events, many horse owners were reluctant to report WNV cases. Therefore, RESPE has been useful in maintaining a minimum level of vigilance in the Camargue area. The RESPE neurological syndrome sub-network should also enable more effective and rapid monitoring for encephalitis-causing endemic or emerging pathogens through syndromic surveillance (27). Syndromic monitoring in animals or humans consists of early alerts triggered by unusual spatio-temporal increases of non-specific health indicators. Syndromic surveillance of neurological presentations has already proved useful for the early identification of WNV cases in a retrospective analysis of the French 2006 WNV epizootic (28). However, during the 2015 outbreaks, the alert protocol recently defined for RESPE syndromic surveillance (at least four cases with neurological signs reported during the same week or at least three cases reported per week for two consecutive weeks) was probably too stringent and was not activated in August or prior to confirmatory laboratory analysis.

It is likely that WNV will re-emerge in France, because many European countries have reported increased WNV outbreaks since 2010 (29). This rise in WNV outbreaks has generally been associated with WNV endemisation in southern European countries such as Italy or Greece (30, 31). It can therefore be anticipated that WNV outbreaks will be reported in the next few years in France, so the French WNV surveillance system will have to be adjusted accordingly and collaboratively.

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Épizootie due au virus de West Nile survenue en Camargue (France) en 2015 et renforcement des réseaux de surveillance et de contrôle

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Résumé

L'infection par le virus de West Nile est une maladie non contagieuse essentiellement transmise lors de piqûres de moustiques infectés appartenant au genre *Culex*; le virus se maintient dans la nature au moyen d'un cycle moustique-oiseau-moustique; la transmission à des hôtes mammifères a lieu de manière accidentelle. Parmi les mammifères hôtes, les plus sensibles à l'infection par le virus de West Nile sont les équidés et l'homme, chez qui l'infection peut se manifester sous forme d'une méningo-encéphalite sévère. Les infections par le virus de West Nile étant des zoonoses potentiellement graves chez l'homme et chez les équidés, la fièvre de West Nile doit être considérée comme une priorité de santé publique et animale. Resté silencieux pendant plus d'une décennie, le virus de West Nile est réapparu en France à l'été 2015 en bordure de la Camargue, confirmant que les conditions de cette région sont favorables à l'émergence et à l'amplification du virus. Le réseau français d'épidémiosurveillance en pathologie équine (RESPE) a contribué à la détection précoce du virus de West Nile chez les chevaux. Au total, 49 chevaux étaient infectés, parmi lesquels 44 présentaient des signes cliniques, correspondant à une méningo-encéphalite pour 41 d'entre eux et à une hyperthermie seule pour les trois autres. Six chevaux parmi les

41 qui présentait des signes neurologiques ont succombé à la maladie ou ont été euthanasiés (taux de létalité de 14,6 %). Les auteurs de cet article décrivent les principales caractéristiques de l'épizootie de 2015 due au virus de West Nile ainsi que la détection précoce des premiers cas équinés grâce au réseau RESPE et la coordination des activités de surveillance du virus en France.

Mots-clés

Camargue – Épizootie – Équidés – France – Homme – Réseau d'épidémiosurveillance en pathologie équine – RESPE – Virus de West Nile.



Epizootia causada por el virus West Nile en la Camarga (Francia) en 2015 y refuerzo de las redes de vigilancia y control

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Resumen

La infección por el virus West Nile es una enfermedad no contagiosa que se transmite básicamente por la picadura de mosquitos infectados del género *Culex*. El virus, que se instala en un ciclo mosquito-ave-mosquito, también puede transmitirse accidentalmente a mamíferos, de entre los cuales los más sensibles a la infección son los equinos y el ser humano, que pueden contraer graves meningoencefalitis. Puesto que las infecciones por este virus son zoonóticas y pueden revestir gravedad en personas y equinos, se considera que la fiebre West Nile es una enfermedad de importancia sanitaria y zoonosológica. En Francia, tras un periodo silente de más de diez años, el virus reapareció en verano de 2015 en la periferia de la zona de la Camarga, poniendo así de manifiesto que esta zona genera condiciones propicias al surgimiento y la amplificación del virus en el país. La red francesa de vigilancia epidemiológica de patologías equinas (*Réseau d'Épidémiologie-Surveillance en Pathologie Équine*: RESPE) facilitó la rápida detección de caballos infectados por el virus West Nile. Se detectaron en total 49 animales infectados, entre ellos 44 con signos clínicos, de los que 41 sufrían meningoencefalitis y tres solo presentaban hipertermia. Seis de los 41 caballos que mostraban signos neurológicos murieron a causa de la enfermedad o fueron sacrificados con métodos de eutanasia (lo que supone una tasa de letalidad del 14,6%). Los autores describen las principales características de la epizootia causada por el virus West Nile en 2015, la pronta detección de los primeros casos de caballos infectados gracias a la red RESPE y la coordinación de las labores de vigilancia del virus en Francia.

Palabras clave

Camarga – Epizootia – Equino – Francia – Humano – Réseau d'Épidémiologie-Surveillance en Pathologie Équine – RESPE – Virus West Nile.



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