

# First neuroinvasive human case of West Nile Disease in Southern Italy: Results of the 'One Health' approach

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## Abstract

**Background:** West Nile Disease (WND) is a zoonotic mosquito-borne infection involving viral pathogens, human and animal hosts, vectors and environment. Cooperation among medical, veterinary and entomological fields has been promoted by the Italian Public Health Authorities, and an integrated West Nile Virus (WNV) Surveillance Plan has been in force in Italy since 2016 to prevent the transmission risk of WND to humans through an early detection of viral circulation by animal and entomological surveillance. This managing model is unique in Europe.

**Objectives:** This survey aimed at presenting the 'One Health' approach applied in 2016 to the first autochthonous human case of West Nile Neuroinvasive Disease (WNND) in Sicily (Southern Italy).

**Methods:** Serological (anti-WNV IgM and IgG ELISA, anti-WNV neutralizing antibodies) and molecular tests were conducted on blood, liquor and urine of a 38-year-old man with encephalitis and meningitis. Overall, 2704 adult culicids from 160 mosquito catches were morphologically identified. Female mosquitoes were analysed in pools for WNV RNA detection. Serological (anti-WNV IgM and IgG ELISA) and molecular analyses for WNV were carried out in 11 horses, 271 chickens and two dogs sampled in farms around the man's residence.

**Results and conclusions:** WNND was confirmed by serological analysis on patient's liquor and serum. Collected mosquito species included *Culex pipiens* (93.56%, CI<sub>95%</sub> 92.64%–94.49%), *Aedes albopictus* (5.25%, CI<sub>95%</sub> 4.41%–6.09%), *Culex hortensis* (0.59%, CI<sub>95%</sub> 0.30%–0.88%), *Culiseta longiareolata* (0.55%, CI<sub>95%</sub> 0.27%–0.83%) and *Anopheles maculipennis* s.l. (0.04%, CI<sub>95%</sub> –0.04% to 0.11%). Mosquito pools were negative for WNV RNA. Two dogs (100%) and two horses (18.18%, CI<sub>95%</sub> –4.61 to 40.97%) resulted positive for anti-WNV specific antibodies.

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The 'One Health' approach allowed to report the first human neuroinvasive WND in Sicily and to confirm the local circulation of WNV in animals of the same area where the clinical case occurred, defining the autochthonous origin of the infection.

#### KEYWORDS

human case, Integrated Surveillance System, Italy, mosquito, West Nile Virus, 'One Health'

## 1 | INTRODUCTION

Multiple ecological factors influence the epidemiology of arboviral zoonoses and thus an integrated and robust surveillance system is required for an early identification of the infection and implementation of proper control measures.

### Impacts

- 'One Health' approach for West Nile Disease surveillance in Italy.
- Description of the first neuroinvasive human case of West Nile disease in Sicily.
- Detection of West Nile Virus antibodies in dogs and horses in the surrounding area.
- Presence of the main West Nile Virus vector *Culex pipiens* in the investigated area.

West Nile Virus (WNV) belongs to *Flaviviridae* family, *Flavivirus* genus, which also includes other human-pathogenic viruses such as Zika, Dengue, Yellow fever, Usutu and Japanese encephalitis viruses (Petersen et al., 2013).

WNV maintenance in nature occurs through a bird-mosquito cycle. In particular, WNV mostly infects *Culex* mosquitoes that are potentially able to transmit the virus to every vertebrate on which they feed (Andreadis, 2012; Engler et al., 2013; Lustig et al., 2015; Mancini et al., 2017).

Birds are amplifying hosts (Malkinson et al., 2002); of them, certain species display signs of the disease, other species, such as crows and jays, may also die (Gamino & Hofle, 2013). Mammals, mainly horses and humans, are dead-end hosts (Colpitts et al., 2012). In addition to bites from infected mosquitoes, transmission can also occur through the transfusion/transplant of contaminated substances of human origin (SoHO) (Velati et al., 2017).

In humans, West Nile disease (WND) often occurs asymptotically or with a moderate febrile disease (Campbell et al., 2002). Less than 1% of patients have significant neurological symptoms, which can be classified into three major syndromes: meningitis, encephalitis and poliomyelitis (acute flaccid paralysis) (Petersen et al., 2013). The disorder is normally subclinical in horses although they may often display neurological symptoms (Kulasekera et al., 2001).

Africa is most likely the origin of all WNV lineages and genotypes (May et al., 2011), from which WNV has spread to other regions, as well as Europe, mainly through migratory birds (Calistri, Giovannini, Hubalek, et al., 2010; Mackenzie et al., 2004). In Israel, a major cross-

roads for bird migration between Africa and Eurasia, studies have showed four distinct genotypes within two WNV lineages that have circulated in recent years (Lustig et al., 2015).

Probably, birds mediate long distance WNV dispersal, while mosquitoes are responsible of short distance virus spreading (Liu et al., 2006; Maidana & Yang, 2009). For these reasons, in case of viral circulation the entomological investigation is usually limited to an area within a radius of about 200 m around the virus detection site.

The first West Nile Neuroinvasive Disease (WNND) outbreak in Italy occurred in Tuscany during the late summer in 1998, when 14 horses showed neurological disorders (Autorino et al., 2002), while no human cases of encephalitis had been recorded (Rizzo et al., 2012). In Sicily, the first outbreak of animal WNV infection occurred in August 2010: neurological symptoms were observed in five horses in Trapani. Subsequent investigations activated according to the surveillance plan, confirmed 46 foci in that area, with seven horses showing neurological symptoms. Retrospective serological analysis of sera collected from dogs in Trapani surrounding area proved that the infection was present in the area at least since the end of January (Calistri, Monaco, et al., 2010). Two other horses suffering from neurological disabilities were observed near Messina and Palermo in October 2011 (unpublished data).

Several human cases have been reported in Italy since 2008, following the re-introduction of WNV in Italy (Rizzo et al., 2016). Moreover, until 2011, only WNV lineage 1 has been identified in the whole country (Calistri, Giovannini, Savini, et al., 2010), while a co-circulation with lineage 2 has been reported afterwards (Savini et al., 2008, 2012). A possible introduction of lineage 2 from Central and/or Eastern Europe has suggested, possibly through migratory birds (Barzon et al., 2015; Ravagnan et al., 2015). The Italian National WNV veterinary surveillance plan started in 2001 and included an entomological surveillance as well as a serological surveillance of horses and sentinel poultry. The Ministry of Health (Italian Ministry of Health, Circular No. 400.3/3.2/4234) issued recommendations for human surveillance in 2002.

Following the revision of the WNV national veterinary surveillance plan (Italian Ministry of Health, 2008) and of the WNND human surveillance recommendations (Italian Ministry of Health, 2010), the Italian Ministry of Health adopted a multidisciplinary approach in 2016, with the National Integrated WNV Surveillance Plan (Italian Ministry of Health, Circular no. 10/08/2016).

The plan provides for an integrated medical, veterinarian and entomological surveillance and it represents a unique model in Europe. Recently, WNV and Usutu virus (USUV) surveillances have

been included in the National Plan for Prevention, Surveillance and Response to Arbovirus 2020–2025.

This study describes the application of the integrated surveillance system for WNV activated during 2016 in a rural area of Trapani province, western Sicily, following the first WNND human case in Sicily, according to the guidelines defined in the 'Integrated National Plan and response to the West Nile virus for 2016'.

Exchanges of data among the different involved disciplines are described and the results obtained from the surveillance are reported.

## 2 | MATERIALS AND METHODS

### 2.1 | WNV human surveillance

#### 2.1.1 | Clinical symptoms

A 38-year-old patient from Trapani with fever  $\geq 38.5^{\circ}\text{C}$  and neurological symptoms (encephalitis, meningitis and clear liquor) arrived at the emergency department on the 1 September 2016 and was hospitalised. The patient recorded the first clinical symptoms on 23 August and reported that he had spent his holidays in Santo Domingo during the first two weeks of the month.

#### 2.1.2 | Laboratory diagnosis

According to the 'Integrated National Plan and Response to the West Nile Virus for 2016', a WNND case description includes a person with fever and neurological symptoms and at least one of the following laboratory conditions: anti-WNV IgM specific antibodies in blood/serum ('probable case'); viral isolation in blood/serum, urine and/or cerebrospinal fluid (CSF); anti-WNV IgM positive in CSF; positive polymerase chain reaction (PCR) in blood, urine and/or CSF; high titer of anti-WNV IgM and anti-WNV IgG in serum confirmed by neutralisation test ('confirmed case').

To evaluate the clinical case described here, the patient's serum and liquor were sampled. In particular, two blood samples were taken on 5 and 9 September, respectively. As indicated by the Italian Ministry of Health, blood samples, liquor and urine were also sent to the National Reference Center.

CSF and serum were tested by enzyme-linked immunosorbent assay (ELISA) using viral antigens according to the WHO recommendations for anti-WNV IgM and IgG tests (West Nile Virus IgG DxSelect, West Nile Virus IgM Capture DxSelect; Focus Diagnostics). Blood and urine were screened by PCR for viral RNA detection (Fortuna et al., 2017; Vilibic-Caviek et al., 2014). The presence of anti-WNV antibodies was confirmed by plaque reduction neutralisation test (PRNT) for anti-WNV neutralizing antibody (Fortuna et al., 2017) performed by the National Reference Center. The assay was performed in six-well tissue culture plates with subconfluent VERO cell monolayers (approximately 70% confluence). Infectivity titration of each viral strain was performed by plaque assay using VERO cells. Patient serum was diluted

1:10 in serum-free maintenance medium, heat-inactivated and titrated in duplicate in twofold dilution steps. Equal volumes (100  $\mu\text{l}$ ) of a viral dilution containing approximately 100 plaque forming units (PFU) and serum dilutions were mixed and incubated at  $4^{\circ}\text{C}$  overnight. Subsequently, VERO cells plates were infected with 200  $\mu\text{l}$ /well of virus-serum mixtures in duplicate. After 1 h incubation at  $+37^{\circ}\text{C}$  and 5%  $\text{CO}_2$ , the inocula were aspirated and the wells were overlaid with a mixture of one part of 2% Gum Tragacanth and one part of supplemented medium ( $2 \times \text{MEM}$ , 2.5% inactivated FCS and 2% 1 M HEPES). The plates were incubated at  $+37^{\circ}\text{C}$  and 5%  $\text{CO}_2$  for 7 days, and then were stained with 1.5% crystal violet. Neutralizing antibody titers were calculated as the reciprocal of the serum dilution that gave a 50% or 80% reduction of the number of plaques (PRNT50/PRNT80), as compared to the virus control. PRNT80  $\geq 10$  were considered positive, while PRNT50 = 10 were considered as border line.

### 2.2 | WNV entomological surveillance

#### 2.2.1 | Sampling sites

According to the entomological surveillance plan of the Health Department of Sicily and the 'Integrated National Plan and response to the West Nile virus for 2016', an entomological investigation started. A 'Type B risk level' area was identified for the simultaneous presence of both the vector and the human clinical case.

The affected area, characterised by a semi-rural environment, was geo-referenced and the potential larval outbreaks and internal shelters were identified. Entomological traps were positioned in six collection sites (Sites A–F) (Figure 1). The man house, situated in a suburban area, corresponded to Site A. The other sites were located also at a distance greater than 200 m, near humid areas, potential larval breeding sites, such as cisterns and gardens (Table 1). The nearby wetlands were also monitored as an ideal habitat for migratory birds. These wetlands are highly at risk of WNV diffusion and may be the probable origin of the infection spreading. Traps were placed not only outside but also within the houses, given the endophilic behaviour of *Cx. pipiens*.

#### 2.2.2 | Mosquito collection and identification

In order to maximise mosquito collection efficiency, different traps based on different capture mechanisms were used, such as BG Sentinel traps baited with BG-Lure (Biogents), CDC-light traps (John W. Hock) and Gravid traps (BioQuip). The first trap works with a chemo-attractant to capture adult mosquitoes that are active both at night and during the day (e.g. *Aedes* genus). The second one is a  $\text{CO}_2$ -releasing light trap, particularly suitable for collecting adults belonging to species with crepuscular and nocturnal activity, that is *Culex* genus.

The gravid traps operate by mimicking an efficient egg-laying site and are useful for catching gravid mosquito females that have completed and digested at least one blood meal and thus can harbour the virus in the salivary glands.



**FIGURE 1** Sites of entomological surveillance monitored in the study

**TABLE 1** Entomological surveillance collection sites, with indication of geographical coordinates, altitude, main environmental characteristics and of positioned traps

	Latitude	Longitude	Altitude	Description	Number of traps (kind)
Site A	37.867093	12.493394	13	Clinical case habitation	3 (CDC, BG Sentinel, Gravid traps)
Site B	37.870616	12.496388	17	400 m from site A, private house, presence of a cistern	2 (CDC, Gravid traps)
Site C	37.856701	12.477844	1	Neighbouring wetland site, natural larval foci	1 (CDC)
Site D	37.86278	12.484889	2	Neighbouring wetland site, natural larval foci	2 (BG Sentinel, Gravid traps)
Site E	37.87424	12.87034	8	Neighbouring wetland site, natural larval foci	2 (CDC, Gravid traps)
Site F	37.874277	12.50105	26	Hen-house	2 (CDC, Gravid traps)

Sampling was performed for 25 days (from 23 September 2016 to 19 October 2016) and each trap worked and was examined uninterruptedly every 1–3 days. All the collected specimens were maintained at  $-80^{\circ}\text{C}$ . Mosquitoes were counted and identified based on their morphological characteristics using a stereo microscope with reflected light (Severini et al., 2009).

### 2.2.3 | Laboratory diagnosis

Field-collected mosquitoes were divided in pools according to the species, the collection site and sampling date. Pools, consisting of 1–50 individuals, were homogenised in phosphate-buffered saline (PBS) pH 7.2 for viral RNA extraction by a commercial kit (QIAamp

Viral RNA Mini Kit–Hilden–Germany QIAGEN), following the manufacturer's instructions.

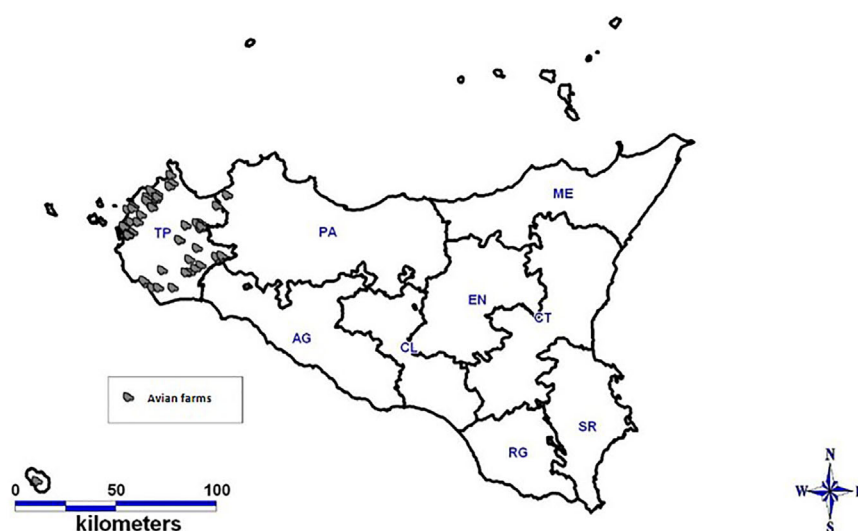
A non-competitive exogenous WNV Armoured RNA (HNY1999; Asuragen, Inc.) was used as an internal positive control (IPC) for the extraction.

Testing of mosquito pools for WNV was performed using multiplex real-time reverse transcription-PCR (RT-PCR), which included the IPC.

The multiplex real-time RT-PCR was carried out using a Quantitect Probe RT-PCR Kit – QIAGEN, for the simultaneous detection of WNV lineages 1 (L1) and 2 (L2). The assay combines a primer pair common to both WNV L1 and L2 and two WNV lineage-specific TaqMan-MGB probes (Del Amo et al., 2013; Jimenez-Clavero et al., 2006). The real-time RT-PCR targeting the NS5-2 region of WN-HNY99 was performed according to Eisler et al. (2004) (Table 2).

**TABLE 2** Primers and probes used for the detection of West Nile Virus (WNV) by real-time reverse transcription polymerase chain reaction (RT-PCR), Name, Sequences and labelling

Name	Sequence 5'-3'	Reference
WNV primers WN-LCV-F1	5'-GTGATCCATGTAAGCCCTCAGAA-3'	Jimenez-Clavero et al. (2006); Del Amo et al. (2013)
WN-LCV-R1	5'-GTCTGACATTGGGCTTTGAAGTTA-3'	
Probes WN-LCV-S1	5'-FAM-AGGACCCCACATGTT-3'-MGB	
WN-LCV-S2	5'-VIC-AGGACCCCACGTGCT-3'-MGB	
WN-NY99 primers NS5-2F NS5-2R	5'-GAA GAG ACC TGC GGC TCA TG-3' 5'-CGG TAG GGA CCC AAT TCA CA-3'	Eisler et al. (2004)
Probe NS5-2 probe	5'-NED-CCA ACG CCA TTT GCT CCG CTG-3'-MGB	

**FIGURE 2** Sites of avian farms investigated in the study

## 2.3 | Animal surveillance

### 2.3.1 | Collection sites and animal sampling

Blood samples were collected from 11 horses from 10 horse farms, located in an area distant up to 20 km from the house of the human clinical case, from 271 domestic chickens (*Gallus gallus*), from 62 avian farms in the nearby rural area and from two dogs living next to the patient's house (Figures 2 and 3).

Sampling was carried out from 29 September to 6 December 2016. Blood samples were obtained by pricking the jugular (horses and dogs) or brachial veins (chickens) into vacuum tubes with the clot activator and stored at +4°C.

### 2.3.2 | Laboratory diagnosis

Animal sera were harvested after clotting at room temperature and centrifugation at 3000 g for 10 min at +4°C and stored at -20°C until analysis. A commercial ELISA kit (ID Screen West Nile Competition Multi-species - ID-Vet), with a sensitivity and a specificity > 95%, was used to detect anti-WNV pr-E IgG antibodies. In addition, to detect recent WNV infection, a commercially available Equine IgM capture

ELISA was used (West Nile Virus IgM Antibody ELISA kit; IDEXX Laboratories, Inc.). Tests were performed according to the manufacturer's recommendations. The Optical Density of each well was measured at a wavelength of 450 nm in an ELISA reader.

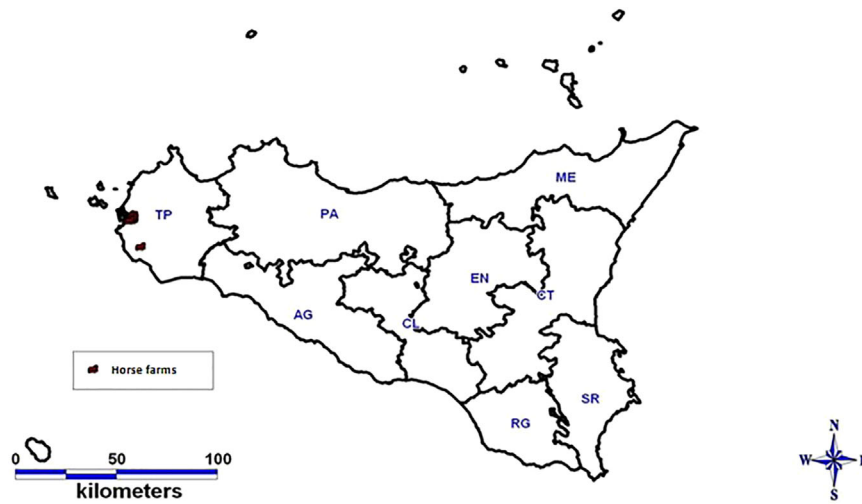
Viral RNA was extracted from blood samples as described above and tested for WNV L1 and L2 RNA presence by real-time RT-PCR (Del Amo et al., 2013; Jimenez-Clavero et al., 2006).

## 3 | RESULTS

### 3.1 | WNN case definition

Both the serum and liquor of the patient resulted positive for anti-WNV IgM. IgG antibodies were absent at the first blood sampling, while they were detected at the second one. The National Reference Center confirmed the positivity of IgM and, thus, the diagnosis of a clinical case of neuroinvasive WND. The neutralisation test confirmed the positivity at the second sampling.

All the examined samples (blood, CSF and urine) resulted negative at molecular analyses. The Italian Ministry of Health considered the reported case as the first autochthonous case of WNV in the region. From that date, Sicily has been included into the regions with viral



**FIGURE 3** Sites of horse farms investigated in the study

**TABLE 3** Results of mosquito identification with indication of mosquito species collected in each collection site

	Number of catches	Number of culicids	<i>Culex pipiens</i>	<i>Culex hortensis</i>	<i>Aedes albopictus</i>	<i>Culiseta longiareolata</i>	<i>Anopheles maculipennis s.l.</i>
Site A	42	507	420	0	80	7	0
Site B	25	333	290	0	40	2	1
Site C	17	6	5	0	1	0	0
Site D	34	1728	1692	16	17	3	0
Site E	28	32	30	0	2	0	0
Site F	14	98	93	0	2	3	0
Total	160	2704	2530	16	142	15	1
(%)			93.56%	0.59%	5.25%	0.55%	0.04%

circulation in humans by the Italian Ministry of Health (Istituto Superiore di Sanità, Report no. 6, 2016).

### 3.2 | Entomological surveillance

Overall, 2704 adult culicids were collected from 160 catches. *Cx. pipiens* was the most representative species (93.56%,  $CI_{95\%}$  92.64%–94.49%), followed by *Aedes albopictus* (5.25%,  $CI_{95\%}$  4.41%–6.09%). *Culex hortensis* (0.6%,  $CI_{95\%}$  0.30%–0.88%), *Culiseta longiareolata* (0.55%, 0.27%–0.83%) and a specimen of *Anopheles maculipennis s.l.* (0.04%,  $CI_{95\%}$  –0.04% to 0.11%) were the other identified species. Species distribution per collection site is listed in Table 3.

Female mosquitoes (no. 2624) were divided into 175 pools and analysed for WNV presence. The pools contained *Cx. pipiens* (no. 2488), *Cx. hortensis* (no. 16), *Ae. albopictus* (no. 105), *Cs. longiareolata* (no. 15). All the mosquito pools were negative for WNV RNA (Table 4).

### 3.3 | Animal surveillance

Both the dogs (100%) resulted positive at the ELISA for anti-WNV IgG, while, among the animals sampled in the farms, two horses (18.18%,

$CI_{95\%}$  –4.61% to 40.97%) were positive for anti-WNV IgM. No chicken tested positive by ELISA. There were no positive samples at molecular tests (Table 5).

## 4 | DISCUSSION

An increase in notification of West Nile infections has been observed in Europe and the Mediterranean Basin during the last decade (Conte et al., 2015). The first large human outbreak of WND in Europe was reported in Romania in 1996, with 393 confirmed cases (Tsai et al., 1998), since then the number of WND cases reported in humans increased significantly.

However, the epidemiological situation of WNV in Europe is heterogeneous: Some European countries record human and animal outbreaks each year, while others have never reported autochthonous cases (EFSA and ECDC, 2015). Human WNV cases in Europe were high in 2012–2013 (935 and 785 cases, respectively) and in 2018 (1670 cases), interspersed by years of lower activity (Barrett, 2018).

In Italy, WNV has caused severe illnesses in humans. Cases occur sporadically and the epidemiology varies according to the lineage of the virus and the affected geographic area (Rizzo et al., 2016). Since the beginning of the 2020 transmission season, EU Member States

**TABLE 4** Female pools examined for West Nile Virus (WNV) presence

	Number of pools	Number of culicids	<i>Culex pipiens</i>	<i>Culex hortensis</i>	<i>Aedes albopictus</i>	<i>Culiseta longiareolata</i>	WNV presence
Site A	59	484	416	0	61	7	0
Site B	35	316	289	0	25	2	0
Site C	5	5	4	0	1	0	0
Site D	54	1686	1653	16	14	3	0
Site E	12	31	29	0	2	0	0
Site F	10	102	97	0	2	3	0
Total	175	2624	2488	16	105	15	0

**TABLE 5** Animal surveillance results

Species	Number of samples	Anti-WNV IgM positive	Anti-WNV IgG positive	WNV RNA positive
Horse	11	2 (18.18%)	0	0 (0%)
Chicken	271	0 (0%)	0	0 (0%)
Dog	2	0	2 (100%)	0 (0%)
Total	284	2 (0.70%)	2 (0.70%)	0 (0%)

have reported 316 human cases of WNV infection, including 37 deaths: Greece (143, 23 deaths), Spain (77, 7 deaths), Italy (66, 5 deaths), Germany (13), Romania (6, 1 death), the Netherlands (7), Hungary (3) and Bulgaria (1, 1 death) (ECDC, 2020).

The continued detection of foci of WNV throughout Europe and the Mediterranean Basin, which represents a constant threat for public health, might imply the presence of endemic areas in these regions. The increase of WNV rate could also be related to the greater detection efforts by veterinary and public health authorities.

WNV is mainly transmitted to humans and animals through the mosquitoes-bird enzootic cycle, and it is influenced by climatic and environmental factors such as temperature, seasons and fluctuations in water level (Paz & Semenza, 2013). For these reasons, WNV requires a collaborative multidisciplinary and integrated methodology, as promoted by the 'One Health' approach that recognises the close correlation among environment, human and animal health (Lerner & Berg, 2015). The National Integrated WNV Surveillance Plan has been demonstrated a key point to timely implement control measures against the spread of this infection and the risk of human transmission by detecting viral circulation early and triggering both vector-control and SoHO safety measures.

As reported above, the first WNV outbreak in Sicily occurred in August 2010 (Calistri, Monaco, et al., 2010), followed by the identification of other two clinical cases in October 2011. All these infections concerned horses, while no human cases of encephalitis have been recorded in the Sicily until this report.

In 2011, a serological monitoring was performed on samples from cattle and horses, not vaccinated for WNV, and from dogs resident within 5 and 10 km from the urban area of 7 Sicilian cities: 27 horses and two dogs from Palermo resulted positive. The presence of anti-WNV IgG in horses was related to the extensive viral circulation in the years 2010 and 2011. The survey confirmed the usefulness of serological monitoring on dogs to highlight virus circulation, as it was

observed in Trapani in 2010, well before the onset of symptoms in equine population.

During 2013, WNV circulation was detected in six regions (including Sicily), of which three reported human cases (Veneto, Emilia Romagna and Lombardy). In particular 50 new cases of WNV in equines were confirmed by animal surveillance, two of which characterised by clinical signs, in Sicily (<http://sorveglianza.izs.it/emergenze/westnile/emergenzeen.html>).

In 2015 virus circulation was detected in animals in the southern Italian regions of Apulia (one horse in Lecce province) and Sicily (two chickens in Catania province), where no human cases were reported.

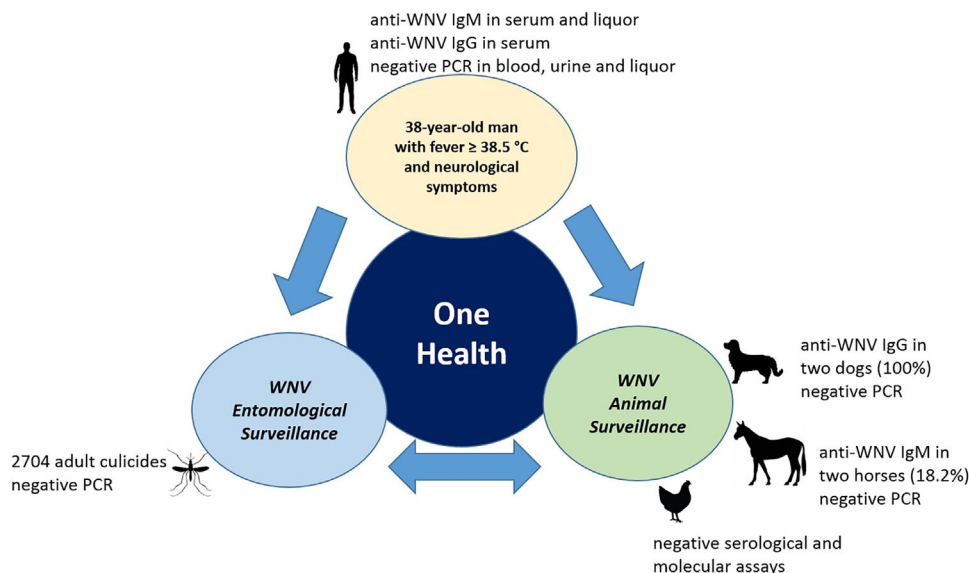
The integrated WNV Surveillance Plan provides data to detect the introduction and monitoring the circulation of the WNV on the whole national territory, which is intensified on the basis of seasonality and local epidemiology. The integrated Plan allowed the identification of WNV belonging to lineage 1 in wild birds in Campania region, in 2020, after several years, indicating the importance of a continuous surveillance in order to detect new lineages.

In this study, the first recorded human case of WNV in Sicily is described and the components of the activated integrated surveillance system are presented (Figure 4).

The patient showed clinical signs and anti-WNV specific antibodies in serum samples. The detection of specific anti-WNV IgM in CSF allowed defining the event as a confirmed WNV clinical case, according to the 'Integrated National Plan and response to the West Nile virus for 2016'.

Measures for infection prevention and control were promptly put in place near the man's house. Veterinary and medical surveillance were connected and worked as joint actions.

The patient did not travel abroad during the incubation period and for that reason the Ministry notified this case as the first autochthonous report.



**FIGURE 4** Components of the 'One Health' approach applied in West Nile disease surveillance

Besides, the identification of WNV seropositive dogs and horses provided further confirmation of virus circulation in that area in the same period.

Interestingly, this study confirmed the sentinel role of dogs for early detection and monitoring of virus circulation in urban and suburban areas. Even if they do not have a relevant role in the epidemiology of WNV, dogs can be incidentally involved in virus maintenance, supporting previous results obtained in a retrospective study on dog serum samples (Purpari et al., 2012).

The investigated area showed habitats suitable for the main WNV vector, *Cx. pipiens*, such as basements, abandoned buildings, chicken coops, stables or animal shelters (Severini et al., 2009). Indeed, *Cx. pipiens* was the most common species of sampled mosquitoes, even if the WNV RNA was not detected in the collected specimens.

In addition, the area nearby the patient's house is a wildlife-protected area characterised by salt flats where several migratory birds rest during migration. It represents a suitable habitat for mosquito larvae and provides a variety of hosts, which mature insects can feed on. For these reasons, it represents a potentially at-risk area for WNV infection.

To date, the most powerful instrument to prevent virus spread is the contact reduction between human and animal hosts and vector mosquitoes through control activities against these vectors.

Equine vaccination may be suggested in areas characterised by environmental and climatic conditions particularly suitable for mosquitoes, such as the one described here.

## 5 | CONCLUSION

The 'One Health' approach in this experience defined the first human WNND in Sicily detected WNV antibodies in dogs and horses and

showed the presence of the main WNV vector *Cx. pipiens* in the investigated area.

This Italian experience is a good example of cooperation among various public health fields (human and veterinary medicine, entomology) and allowed to define the autochthonous origin of the reported WNND infection.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

*Conceptualisation, data curation, investigation, methodology, writing-original draft and writing-review & editing:* Giusi Macaluso. *Data curation, investigation, methodology, writing-original draft and writing-review & editing:* Francesca Gucciardi. *Conceptualisation, project administration, resources, supervision, writing-original draft and writing-review & editing:* Annalisa Guercio. *Conceptualisation, data curation, methodology, writing-original draft and writing-review & editing:* Valeria Blanda. *Investigation:* Francesco La Russa. *Conceptualisation, supervision and writing-review & editing:* Alessandra Torina. *Investigation:* Francesco Mira. *Data curation, methodology and writing-review & editing:* Santina Di Bella. *Investigation:* Antonio Lastra. *Investigation:* Ilenia Giacchino. *Investigation:* Calogero Castronovo. *Data curation, investigation and methodology:* Giustina Vitale. *Conceptualisation, data curation, methodology, project administration, resources, supervision, writing-original draft and writing-review & editing:* Giuseppa Purpari.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.



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