Journal of Animal Science and Research
ISSN 2576-6457 | Open Access

CASE REPORT

Volume 2 - Issue 1 | DOI: http://dx.doi.org/10.16966/2576-6457.108

Reoccurrence of West Nile Virus Disease in Humans and Successive Entomological Investigation in Sardinia, Italy, 2017

Cappai Stefano[1]*, Rolesu Sandro[1], Coccollone Anna Maria[1], Loi Federica[1], Meloni Giorgio[2], Foxi Cipriano[2], Satta Giuseppe[2], Puggioni Giantonella[2], Campus Marco[3], Cappai Riccardo[4] and Coghe Ferdinando[4]

[1]*Istituto Zooprofilattico Sperimentale della Sardegna, Centro di Sorveglianza Epidemiologica, Via XX Settembre, Cagliari, Italy*

[2]*Istituto Zooprofilattico Sperimentale della Sardegna, dipartimento di Sanità Animale, Via Vienna, Sassari, Italy*

[3]*Azienda Tutela Salute Sardegna, Azienda Socio Sanitaria Locale Cagliari, Presidio Ospedaliero SS Trinità, Divisione Malattie Infettive e Tropicali, Via Is Mirrionis, Cagliari, Italy*

[4]*Azienda Ospedaliero Universitaria di Cagliari, Laboratorio Analisi Chimico Cliniche e Microbiologia, Presidio Ospedaliero Duilio Casula, SS 554 Monserrato, Italy*

***Corresponding author:** Cappai Stefano, Istituto Zooprofilattico Sperimentale della Sardegna, Centro di Sorveglianza Epidemiologica, Via XX Settembre, Cagliari, Italy, Tel: 0039-3284179963; **E-mail:** stefano.cappai@izs-sardegna.it

Received: 23 Oct, 2017 | **Accepted:** 05 Dec, 2017 | **Published:** 13 Dec, 2017

Citation: Stefano C, Sandro R, Maria CA, Federica L, Giorgio M, et al. (2017) Reoccurrence of West Nile Virus Disease in Humans and Successive Entomological Investigation in Sardinia, Italy, 2017. J Anim Sci Res 2(1): dx.doi.org/10.16966/2576-6457.108

Abstract

West Nile Virus (WNV) has been endemic to Sardinia region since 2014. Numerous cases of WNV infections were reported in Sardinia in 2011, which had already been on alert since 2008 because of the situation in the rest of Italy. In addition to cases involving wild birds and equidae, four human cases were reported, all of which were lethal. In July 2017, a new case of Neuroinvasive West Nile Virus Disease (WNVD) was diagnosed in a human patient by the detections of WNV antibodies in the patient's cerebrospinal fluid (CSF) and blood. Following confirmation of WNV-positivity, the Istituto Zooprofilattico Sperimentale (IZS) surveyed mosquitoes near the patient's residence by placing traps according to recommendations from the Italian entomological surveillance program. One pool of mosquitoes tested positive for WNV, making this the first case of detection of WNV in a patient's residence.

Keywords: West Nile human case; *Aedes caspius*; Entomological surveillance

Introduction

West Nile Virus (WNV) is a neurotropic pathogen belonging to the Japanese encephalitis antigenic complex of the family *Flaviviridae*, genus *Flavivirus* [1]. The life cycle of the WNV involves competent mosquitoes and reservoir hosts, represented by wild birds [2,3]. Horses and humans are considered "dead-end" hosts for WNV because of the low level of viraemia, usually not sufficient to infect mosquitoes, and the inability to complete the transmission cycle [4,5]. In Italy, the first evidence of virus circulation was reported in 1998 in horses in the region of Tuscany [6,7]. Since 2001, a national veterinary surveillance plan and human surveillance recommendation have been in place for the rapid detection of WNV circulation in horses, birds, mosquitoes, and humans. Seven years after the establishment of this surveillance system, during which no virus circulation was detected, WNV was detected in 2008 in Veneto and Emilia Romagna regions, with both WNV lineages, 1 and 2, being identified and even co-circulating in the same area. The first human cases of WNV disease (WNVD) were detected in the Po river area in September-October 2008, following an alert from the national veterinary surveillance plan of equine cases in the same area [8]. An overview of the WNVD cases in Sardinia is shown in table 1. In particular the disease first manifested on 13th September 2011, disappeared in the middle of December, then re-manifested in May 2012, as in clinical cases in horses. During this period, there were six human cases of WNVD. From previous studies, the reported mortality rates among hospitalized patients from recent epidemics being between 4 and 14% [9]. In Sardinia, during 2011, four WNVD human cases were occurred, two of them related with fatal outcome: the first case of WNVD-related mortality, in November

Table 1: WNV cases in Sardinia from 2011-2016.

Years	Horses	Birds	Mosquitoes	Humans
2011	39	14	1	4
2012	10	8	1	2
2013	1	3	1	0
2014	0	0	1	0
2015	0	4	1	0
2016	1	4	1	0

2001, was in an 83-year-old man hospitalized in July and the second, in September, in a 75-year-old man. In October, two more patients were diagnosed with WNVD, a 76-year and a 34-year-old mans, in very good health. All cases occurred in the province of Oristano, including 54 non-human outbreaks of WNV (39 horses, 14 birds, 1 mosquito); only one human case in Olbia province. In 2012, two human cases of WNVD were recorded in Oristano province. In July 2017, an elderly patient presented with symptoms of meningoencephalitis and fever and was eventually diagnosed with neuroinvasive WNVD. The present report presents this first human case of the disease, after its spread in 2012 in Sardinia, and the second case in 2017 in Italy.

Case Presentation

On 25th July 2017, a 54-year-old woman presented to a hospital in Oristano. She had a previous medical history of rheumatoid arthritis and allergic asthma. Dysarthria and aphasia were observed upon clinical examination. Routine biochemical and haematological examination, brain scanning, and neurological counseling were performed. The day after hospitalization, the results from encephalography were indicative of cephalic involvement, and a lumbar puncture was performed. The results of cerebrospinal fluid (CSF) examination were as follows: 48 mg/dL glucose (normal range: 40-70 mg/dL), 128 mg/dL protein (normal range: 15-45 mg/dL), and 127 mmol/L Cl (normal range: 118-132 mmol/L). In the serum sample, the glucose concentration was 108 mg/dL (normal range: 74-106 mg/dL) and total protein concentration was 6.7 g/dL (normal range 6.0-8.3 g/dL). The patient's general condition deteriorated and she was hospitalized at the Cagliari Hospital (body temperature 36°C; arterial pressure 110/70); WNV antibody testing of blood and CSF samples was performed. Laboratory investigations for a possible diagnosis were carried out at the Regional Reference Laboratory in the Azienda Ospedaliera Universitaria of Cagliari on 26th July 2017. Antibody testing was performed using the West Nile Virus IgM-IgG Captured DxSelectTM kit, according to the manufacturer's instructions (FOCUS DIAGNOSTIC, California). Immunoglobulin M (IgM) antibodies were detected in the CSF sample (index value 8.21) and serum sample (index value 2.06; index values: absent, <0.8; borderline, 0.8-1.1; present, >1.1). Immunoglobulin G (IgG) antibodies were not detected in either sample, with index values of 0.09 (index value: <0.8) and 0.11 (index value: <0.8), respectively. On 27th July 2017 (body temperature 36.6°C) the patient was started on treatment with ceftriaxone, omeprazole, enoxaparin, furosemide, and dexamethasone, with intravenous fluid therapy. As indicated in the National Surveillance Plan, the positive samples were sent to the National Reference Laboratory of the Infectious Disease Department of Istituto Superiore di Sanità (Rome) to confirm the test results [10]. Serum and CSF samples were tested by the WNV plaque reduction neutralization test (PRNT), with results of PRNT50 ≥ 1:10 in both samples. In addition, real-time PCR was performed using the same samples, with negative outcomes. This result was suggestive of a recent WNV infection. On the basis of these findings and the patient's clinical presentation, the patient was diagnosed with meningoencephalitis due to WNV on 27th July 2017. Until 30th July 2017, five days after the appearance of the first symptoms (AFS), the patient remained in a critical condition, presenting with hemiparesis, aphasia, a severely reduced consciousness level, and ascending muscle weakness. On the sixth day AFS, her mental status and general conditions ameliorated. On day 23 AFS, the patient's ambulation improved; her pharmacotherapy was stopped and her blood samples were found to be positive for IgG antibodies (Value 2.14; index values: positive ≥ 1.5; dubbio ≤ 1.50 and ≥ 1.30; negative<1). The patient was discharged after a prolonged hospitalization of 26 days.

Mosquito Collection

According to the National Surveillance Plan of WNVD, 36 mosquito traps for entomological surveillance activities are distributed in Sardinia, four of which are located in the Oristano province. The plan indicates that these activities must be strengthening when a human case is confirmed. Immediately upon the confirmation of WNV infection, additional insect trapping should be performed in order to investigate the presence of mosquitoes and identify the possible species involved in the disease spread. On the basis of this, three different models of mosquito traps were positioned in the garden of the patient's house on 31 July 2017. These included: a Centers for Disease Control and Prevention (CDC) Light Trap to collect adult female mosquitoes of various species from sunset to sunrise, a BG Sentinel trap (Biogents AG, Regensburg, Germany) to catch mosquitoes with diurnal activity, and a gravid mosquito trap (BioQuip Products, Rancho Dominguez, California, United States), which was selective mostly for gravid female mosquitoes [11]. All traps operated once for 48 hours. Mosquitoes were sexed and identified to the species level on the basis of morphological features according to identification keys [12]. Females were defined as "engorged" if they contained freshly ingested blood that gave their abdomen a bright red appearance; otherwise, they were considered "not engorged". Mosquitoes were pooled according to date, species, sex, and type of trap, with a maximum of 25 specimens per pool.

Results, Discussion and Conclusion

A total of 77 mosquitoes belonging to 6 species were collected: *Culex pipiens* (63 specimens), *Aedes caspius* (6), *Aedes albopictus* (3), *Culex theileri* (3), *Anopheles labranchiae* (1) and *Culiseta longiareolata* (1) [13,14]. Each mosquito pool was subsequently tested by quantitative real-time reverse-transcriptase polymerase chain reaction (qRT-PCR) for the simultaneous detection of WNV lineage 1 and 2 strains [15]. WNV was detected in one pool of *Oc. caspius* containing four non-engorged females (Table 2). RNA material has been send to CESME (reference centre of exotic disease) to lineage research,

Table 2: Trapping methods, pool compositions (species; numbers of mosquitoes; stages), and qRT-PCR results. NE=not engorged; I=engorged; F=female; M=male.

Trap	Species	Number	Stages	qRT-PCR
Gravid Trap	*Culex pipiens*	1	EF	Negative
BG Sentinel Trap	*Aedes albopictus*	1	EF	Negative
	Culex pipiens	2	M	Negative
	Culex pipiens	4	NE-F	Negative
CDC Light Trap	*Aedes albopictus*	2	M	Negative
	Anopheles labranchiae	1	NE-F	Negative
	Culex pipiens	20	M	Negative
	Culex pipiens	6	M	Negative
	Culex pipiens	20	NE-F	Negative
	Culex pipiens	6	NE-F	Negative
	Culex pipiens	4	EF	Negative
	Culex theileri	3	NE-F	Negative
	Culiseta longiareolata	1	NE-F	Negative
	Aedes caspius	2	M	Negative
	Aedes caspius	4	NE-F	Positive

but, unfortunately, the sample was not enough for diagnosis test. All other pools were negative for WNV. Although all species are generally not present at the same abundance, the number of trapped species in this case was comparable to that collected over the last four years [16,17]. The data confirmed that the most abundant species was *Cx. pipiens*, similar to that in the rest of Italy, and that *Aedes caspius* was the second most abundant insect vector in Sardinia. Although *Ae. capsius* have been reported to be moderately susceptible to WNV oral infection, the detection of WNV in such a small number of host-seeking females was unexpected and fortuitous because this species feeds almost exclusively on large mammals which typically are dead end hosts and have very low viremias [18,19]. According to the surveillance plan this species was found several times positive for Usutu virus but only two times for WNV, Emilia Romagna in 2008 and in the island of Sardinia for the first time in this humane out break [20].

This case is an excellent example of the importance to considering small focus zone in Sardinia as an Oristano province, for specific control measures against WND and also underscores the need to improve surveillance activities and monitoring in humid areas. In addition, the reinforcement of insect population density control activities are encouraged by the authors, in view of critic situation regarding blood donations in Sardinia and the real risk to WNV transmission through blood and organs, reduced by serological control in infected areal. This case report was prepared by the clinicians directly involved in the patient's care and entomological investigations and provides no identifiable personal information. The patient agreed to the publication of this paper.

Acknowledgement

The authors thank the local and regional health authorities for their collaboration.

References

1. Colpitts TM, Conway MJ, Montgomery RR, Fikrig E (2012) West Nile Virus: biology, transmission, and human infection. Clin Microbiol Rev 25: 635-648.
2. Hubalek Z, Halouzka J (1999) West Nile fever-a reemerging mosquito-borne viral disease in Europe. Emerg Infect Dis 5: 643-650.
3. Gubler DJ (2007) The continuing spread of West Nile virus in the western hemisphere. Clin Infect Dis 45: 1039-1046.
4. Komar N, Langevin S, Hinten S, Nemeth NM, Edwards E, et al. (2003) Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg Infect Dis 9: 311-322.
5. Bowen RA, Nemeth NM (2007) Experimental infections with West Nile virus. Curr Opin Infect Dis 20: 293-297.
6. Autorino GL, Battisti A, Deubel V, Ferrari G, Forletta R, et al. (2002) West Nile virus Epidemic in Horses, Tuscany Region, Italy. Emerg Infect Dis 8: 1372-1378.
7. Conte A, Candeloro L, Ippoliti C, Monaco F, De Massis F, et al. (2015) Spatio-Temporal Identification of Areas Suitable for West Nile Disease in the Mediterranean Basin and Central Europe. PLoS One 10: e0146024.
8. Barzon L, Pacenti M, Franchin E, Squarzon L, Lavezzo E, et al. (2013) The Complex Epidemiological scenario of West Nile Virus in Italy. Int J Environ Res Public Health 10: 4669-4689.
9. Campbell GL, Marfin AA, Lanciotti RS, Gubler DJ (2002) West Nile virus. Lancet Infect Dis 2: 519-529.
10. Ministry of public Health (2017) Piano nazionale integrato di sorveglianza e risposta ai virus West Nile e Usutu. DGPRE N 0016990-31/05/2017.
11. Bellini R, Veronesi R, Gentile G, Pandolfi N (2002) Optimization of carbon dioxide traps for mosquito monitoring in Italy. 68th Ann Meet Am Mosq Control Assoc, Denver, Colorado, 16-21.
12. Severini F, Toma L, Di Luca M, Romi R (2009) Le Zanzare Italiane: Generalita E Identificazione Degli Adulti (Diptera, Culicidae) Fragmenta entomologica, 41: 213-372.
13. Wilkerson RC, Linton YM, Fonseca DM, Schultz TR, Price DC et al. (2015) Making Mosquito Taxonomy Useful: A Stable Classification of Tribe Aedini that Balances Utility with Current Knowledge of Evolutionary Relationships. PLoS One 10: e0133602.
14. Gaffigan TV, Wilkerson RC, Pecor JE, Stoffer JA, Anderson T (2015) Systematic Catalog of Culicidae.
15. Eiden M, Vina Rodriguez A, Hoffmann B, Ziegler U, Groschup MH (2010) Two new real-time quantitative reverse transcription polymerase chain reaction assays with unique target sites for the specific and sensitive detection of lineages 1 and 2 West Nile virus strains. J Vet Diagn Invest 22: 748-753.
16. Puggioni G, Rossi R, Rocchigiani AM, Foxi C, Rolesu Sandro, et al. (2014) West nile disease in Sardegna nel 2013: risultati della sorveglianza entomologica. Abstract Book V Workshop Nazionale di Virologia Veterinaria Teramo, giugno 26-27.
17. Foxi C et al. (2016) West Nile disease, risultati della sorveglianza entomologica in Sardegna nel 2015. Abstract book Congresso nazionale di Entomologia, Padova, 2015.

18. Akhter R, Hayes CG, Baqar S, Reisen WK (1982) West Nile virus in Pakistan. III. Comparative vector capability of Culex tritaeniorhynchus and eight other species of mosquitoes. Trans R Soc Trop Med Hyg 76: 449-453.

19. Reisen WK, Boreham PF (1979) Host selection patterns of some Pakistan mosquitoes. Am J Trop Med Hyg 28: 408-421.

20. Mancini G, Montarsi F, Calzolari M, Capelli G, Dottori M, et al. (2017) Mosquito species involved in the circulation of West Nile and Usutu viruses in Italy. Vet Ital 53: 97-110.