

USUTU VIRUS IN THE IBERIAN PENINSULA: A SYSTEMATIC REVIEW

Filipa LOUREIRO^{1,2*} , Luís CARDOSO^{2,3} , João R. MESQUITA^{4,5,6} ,
Ana C. MATOS^{7,8} , Manuela MATOS⁹ , Ana Cláudia COELHO^{2,3} 

1 – University of Trás-os-Montes e Alto Douro (UTAD), Veterinary Teaching Hospital, Wildlife Rehabilitation Centre (CRAS), Vila Real, Portugal;

2 – UTAD, Animal and Veterinary Research Centre (CECAV), Associate Laboratory for Animal and Veterinary Sciences (AL4Animals), Vila Real, Portugal;

3 – UTAD, School of Agrarian and Veterinary Sciences (ECAV), Department of Veterinary Sciences, Vila Real, Portugal;

4 – Porto University, School of Medicine and Biomedical Sciences (ICBAS), Porto, Portugal;

5 – Instituto de Saúde Pública da Universidade do Porto, Epidemiology Research Unit (EPIUnit), Porto, Portugal;

6 – Laboratory for Integrative and Translational Research in Population Health (ITR), Porto, Portugal;

7 – Polytechnic Institute of Castelo Branco; Research Centre for Natural Resources, Environment and Society (CERNAS), Castelo Branco, Portugal;

8 – Polytechnic Institute of Castelo Branco, Quality of Life in the Rural World (Q-RURAL), Castelo Branco, Portugal;

9 – Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), UTAD, Vila Real, Portugal.

Received 22 August 2024; Accepted 02 December 2024

Published online: 29 January 2025

Copyright © 2025 Loureiro et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

How to cite: Filipa Loureiro, Luís Cardoso, João R. Mesquita, Ana C. Matos, Manuela Matos, Ana Cláudia Coelho. Usutu virus in the Iberian peninsula: a systematic review. *Veterinarski Glasnik*, 2025. 00: 1-19. <https://doi.org/10.2298/VETGL240822001L>

Abstract

Studies on Usutu virus (USUV) in Europe have been increasing through time, with USUV infection being identified in humans and animals. The emergence of USUV in Europe draws our attention to how an arbovirus is able to spread, adapt, and evolve in a unconventional environment.

*Corresponding author – e-mail: filipal@utad.pt

Understanding the epidemiological and evolutionary processes that contribute to the appearance, maintenance, and further spread of viral diseases is the key to develop and implement surveillance strategies to control them. The aim of the present systematic review was to compile and evaluate all the published data on USUV from studies performed in humans, animals, and environmental samples in the Iberian Peninsula.

The electronic databases Mendeley, PubMed, Scopus, and Web of Science were thoroughly searched, and research published up until July 6th 2024 were included. After full reading and application of PRISMA exclusion/inclusion criteria, a total of 20 eligible papers were considered.

Overall, the present review shows that USUV is circulating in humans, animals and in the environment in the Iberian Peninsula.

Key Words: Arbovirus, Flavivirus, Infection, USUV, Zoonotic.

INTRODUCTION

Mosquito-borne viruses are the cause of some of the biggest threats for human health worldwide (Ferrero et al., 2022). Due to anthropogenic causes, such as climate change and globalization, some of these arboviruses are being introduced and others are re-emerging in new regions (Johnson et al., 2018).

Usutu virus (USUV) is an emerging arbovirus of the family Flaviviridae, genus *Flavivirus*, and a member of the Japanese encephalitis serocomplex (Clé et al., 2019; Roesch et al., 2019). This pathogen has zoonotic potential and, despite most of the infections in humans being asymptomatic, there are reports of neurological disorders (Cadar and Simonin, 2023; Pecorari et al., 2009). Like other flaviviruses, USUV is spherical and enveloped, with 40-60 nm in diameter, with a single-stranded, positive-sense RNA genome (length of around 11,000 nucleotides). This genome encodes a single polyprotein that is subsequently cleaved into three structural proteins, the capsid (C), membrane (prM) and envelope (E), and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (Roesch et al., 2019; Bakonyi et al., 2004).

USUV was first identified in South Africa in 1959 from a *Culex neavei* mosquito (Williams et al., 1964), then spread into other African countries, and finally introduced into Europe in 2001 (Ashraf et al., 2015; Weissenböck et al. 2013; Weissenböck et al., 2002). USUV isolates are currently classified into eight lineages, clustered into the African (AF1, - 2, - 3) and the European (EU1, - 2, - 3, - 4, - 5) groups (Roesch et al., 2019). The virus is transmitted and maintained in the natural cycle by mosquitoes (mostly of the genus *Culex*) as vectors and birds as amplifying hosts and natural reservoirs. Some episodes of avian mortality have been registered in the last few years (Störk et al., 2021; Roesch et al., 2019). Mammals, like humans, horses, and rodents, can be accidentally infected, which makes them incidental hosts (Romeo et al., 2018; Barbic et al., 2013). Co-infections with other flaviviruses, like West Nile virus (WNV), are possible and probable, since they share the same vectors, namely *Culex* mosquitoes (Scaramozzino et al., 2021). The impact of this co-circulation should be further investigated.

There are no vaccines or specific treatment to USUV-caused disease, and the best way to avoid it is by preventing mosquito bites (ECDC, 2023).

The increasing prevalence of USUV in European countries has drawn the attention of the scientific community (Clé et al., 2019). Transmission dynamics and in-depth phylogenetic analysis of USUV in Europe have already been studied (Siljic et al., 2023; Engel et al., 2016). The current systematic review aimed to compile and evaluate all published data on USUV seroprevalence and detection studies of the agent itself in the Iberian Peninsula until July 2024.

MATERIALS AND METHODS

The present study consisted of a systematic review of the literature in order to answer the following research question “What are the reports of USUV infection in the Iberian Peninsula?, and to analyze the prevalence of this pathogen”.

Exhaustive searches were carried out in the electronic databases Mendeley, PubMed (PM), Scopus, and Web of Science (WOS), including studies published until July 6th 2024, with no restriction on the start date. The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) criteria were followed for the systematic review (Shamseer et al., 2015), and the studies included were published, indexed, and peer reviewed. Language barriers were not included.

The literary search used the following keywords (Usutu OR USUV) AND (Portugal OR Spain OR Iberian Peninsula). After reading the title and the abstract, papers that did not address the detection of USUV in Iberian Peninsula, and reviews and experimental studies were excluded from this systematic review. Articles with unclear information in the title and abstract were read in full, and then we selected only those with the target topic for inclusion in this study. The types of publications included original articles, short communications, and case reports that addressed serological or molecular detection of USUV in animals and humans in the Iberian Peninsula.

Two independent investigators (FL and ACC) screened the databases, and relevant information was extracted. All differences of opinion about including a particular article were resolved between the coauthors.

Duplicate papers ($n = 41$) were identified, and a thorough analysis of exclusion criteria was conducted, which resulted in the identification of 88 unrelated studies. After applying both inclusion and exclusion criteria, 20 potential papers were suitable for the systematic review.

RESULTS AND DISCUSSION

A total of 129 articles were found: 77 in the PM database, 50 in the WOS database, and one each in Mendeley and Scopus. Thirty-nine articles were repeated in PM and

WOS, from which one was repeated among those and Mendeley and Scopus. All articles that did not report USUV detection studies were excluded because they were considered to be outside the scope of this study. Reviews, experimental studies, and errata were also set aside. The last exclusion criterion applied was studies outside the Iberian Peninsula. In the end, 20 articles were included in this systematic review: six were present only in the PM search, 13 were repeated in PM and WOS, and one was present only in WOS (Figure 1).

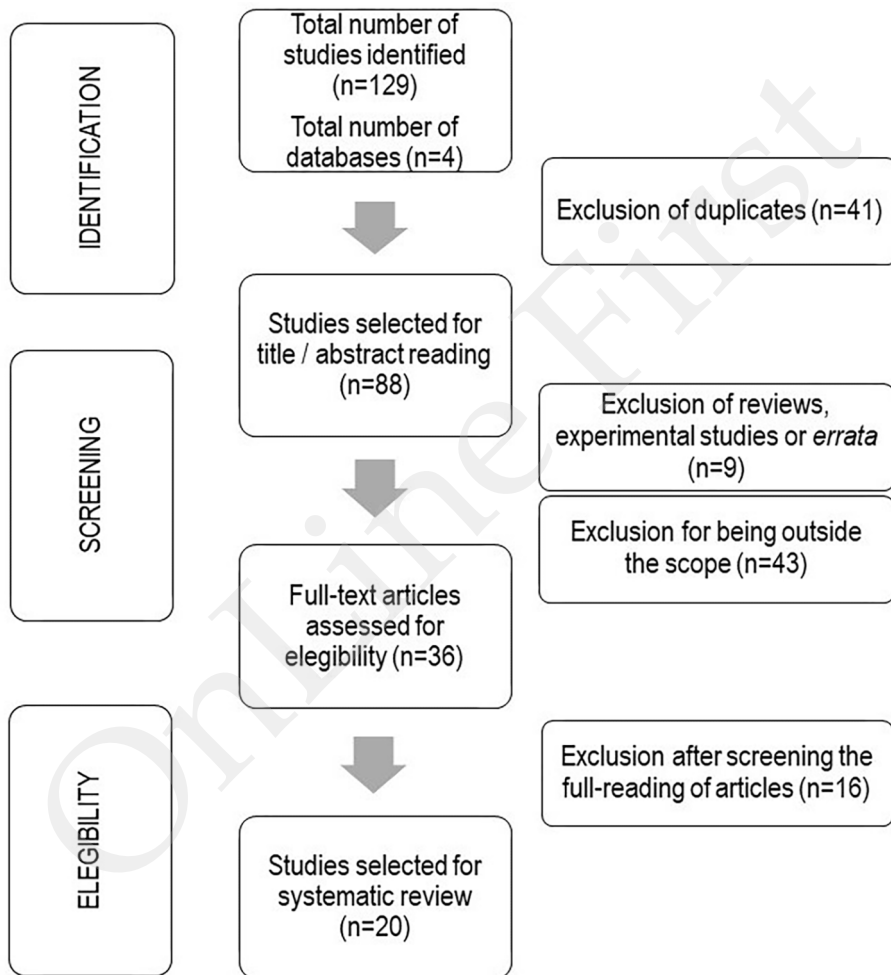


Figure 1. Flow diagram of the search, selection and inclusion process for articles in this systematic review based on PRISMA guidelines.

Our main goal was to access all the reported cases of USUV circulation in the Iberian Peninsula. The 20 studies included in this systematic review were conducted in Spain, and none in Portugal. In 2021-2022, animal USUV infection was not a notifiable disease at the European Union (EU) level. According to the European Centre for

Disease Prevention and Control (ECDC) technical report, Portugal did not carry out specific surveillance activities related to USUV, and no cases were reported from there in the referred period of time. On the other hand, USUV infections in animals (predominantly in birds) were described in the same report, but also in non-avian hosts. Spain was one of the 30 countries that implemented a USUV surveillance system in animals (ECDC, 2023).

The studies found report USUV circulation in the following Spanish autonomous communities: Andalusia, Balearic Islands, Castile y León, Catalonia, Extremadura, and Madrid (Figure 2). The west of the country seems to be the worst affected, and the fact that it is the area that borders Portugal should not be ignored. Spain, like other countries of the Mediterranean Basin, is vulnerable to climate change, with greater risk than before of heatwaves, droughts, and dry weather (UNEP, 2024). This region, as the rest of Europe, was not considered prone to arboviral diseases, but the presence of newly introduced competent mosquitoes has led to a growing number of imported cases (Mariconti et al., 2019). The ambient temperature influences the vector capacity, both in terms of the development of the vector and of the pathogen inside the vector. Within the tolerable temperature range of vectors and viruses, pathogen multiplication is faster the higher the temperature, so the virus transmission increases with a greater frequency of mosquito bites. Rising temperatures could also allow the transmission of arboviruses not only by known potential vectors, but also by native mosquito species not yet recognized as such (Beermann et al., 2023).

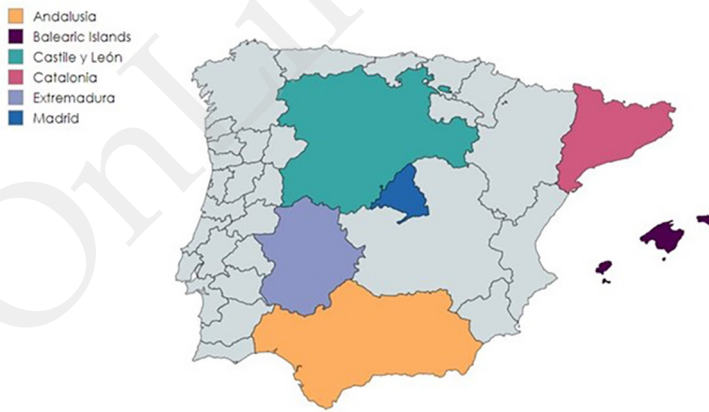


Figure 2. Map of the autonomous communities of Spain where USUV infections have been described : Andalusia, Balearic Islands, Castile y León, Catalonia, Extremadura, and Madrid (created by <https://www.mapchart.net/>).

The 20 articles selected were published between 2008 and 2023. No article was published on data in Portugal during this time. Five articles analyzed USUV virus in mosquitoes, 10 in birds, five in horses or other equids, two in zoo animals, one in

ruminants, one in dogs, and one in ticks (Table 1). Lesions caused by USUV infections have only been reported in birds (Agliani et al., 2023), but evidence of the circulation of antibodies against USUV has also been described in dogs and horses in other places outside the Iberian Peninsula (Laidoudi et al., 2023; Schwartz et al., 2020; Durand et al., 2016). One of the reasons for this is because both of these animals can be considered suitable sentinels for evidence of USUV (and WNV) circulation in any specified area. So far, USUV does not seem to be pathogenic for horses, but they can be incidental and dead-end hosts, as they do not develop the high levels of viremia needed to maintain the transmission cycle. There is evidence of a close relationship between the viral seroprevalence among populations of animals that share the same areas, which can be predictive of infections in humans (Laidoudi et al. 2023). Regarding zoo animals, their living environment allows contact with wildlife species (including mosquitoes), which are a potential source of infectious diseases, like arboviruses and other emerging pathogens (Kvapil et al., 2021). Therefore, zoos have been considered useful to the surveillance of zoonotic viruses (Bergmann et al., 2023; Constant et al. 2020). Zoological collections include a wide variety of animals, which can be scientifically interesting, allowing us to analyze susceptibility between species in the same place.

Among other mammals, USUV antibodies were detected in an Iberian wolf (*Canis lupus signatus*), a threatened carnivorous mammal species, and have also been detected in many endangered bird species (Bergmann et al., 2023; Constant et al., 2020). Serious outbreaks of bird mortality have been recorded in Europe, so it is vital to invest in the protection of valuable individual birds, in preventing new introductions into zoos or other collections, as well as in protected natural areas.

Wild birds play an important role as reservoir hosts and as carriers of zoonotic arboviruses and their vectors in Europe. It has been suggested that the phylogenetic structure of the virus is shaped by the geographic location and pattern of migratory flyways, which probably facilitates the rapid spread of the virus over long distances. Nevertheless, with globalization, the long-distance spread of the virus cannot be ruled out through the transport of USUV-infected mosquitoes by ships or planes (Engel et al., 2016).

Mosquitoes have been the subject of many studies to detect the virus. *Culex pipiens* is considered the main USUV vector in Europe (Čabanová et al., 2023). The distribution of *Culex perexiguus* in Europe has been accessed recently, and the species is remarkably widely present in the Iberian Peninsula (ECDC and EFSA, 2023). USUV has been detected in this mosquito in Spain (Vázquez et al., 2011). Following the pattern of other interactions between viruses and vectors, bear in mind that *C. perexiguus* could become an important vector of the virus for the rest of Europe.

Several diagnostic techniques were used in the selected articles (Table 1). Nine articles used molecular techniques (RT-PCR), and 16 used serological assays: 15 studied

enzyme-linked immunosorbent assays (ELISA), 16 used viral neutralization tests (VNT) and one used microsphere based multiplex immuno-assay (MIA).

Although serological tests continue to be necessary and important for identifying flaviviral infections that are not in the viremic phase, molecular methods can be useful for detecting USUV infections. RT-PCR assays have been developed to detect the agent and distinguish it from other arboviruses (Cavrini et al., 2011; Scaramozzino et al. 2001). The genome target region in all the studies analyzed was the NS5 gene. Non-structural (NS) proteins are typically expressed in infected host cells, contribute to viral pathogenesis, and are usually not enclosed in assembled progeny flavivirus particles (Dey et al., 2021).

The diagnosis of infections caused by Flavivirus is often very difficult due to the short period of viremia, the non-specific clinical signs induced, and the high cross-reactivity exhibited in the diagnostic tests (Čabanová et al., 2023; Gomes da Silva et al., 2023). Conventional ELISA presents moderate specificity. Neutralization tests are the most reliable serological assays, being plaque reduction neutralization test (PRNT), micro-neutralization test (MNT), and VNT, all of which are considered as gold standard techniques for quantifying and detecting the levels of neutralizing antibodies against different flaviviruses. MNT and VNT are commonly performed to confirm the results of conventional serological methods because they allow the detection of virus-specific antibodies, are cost effective, and are largely automated. The disadvantages are that the test is complex and requires BSL-3 facilities (Giri et al., 2024; Chan et al., 2022; Musso and Després, 2000).

Co-circulation between flaviviruses of the same group is analyzed in several of the selected articles: 15 articles evaluated simultaneously WNV, five reported on Bagaza virus (BAGV), five on Meaban virus (MBV), and three on tick-borne encephalitis virus (TBEV) (Table 1).

Co-circulation of USUV, WNV, and TBEV has been described in Central Europe and has been causing public health concerns (Čabanová et al., 2023). The diagnosis and monitoring of USUV is still not part of the routine for public health authorities in many countries, but the increasing trend of flaviviral infections might change this paradigm soon. Meanwhile, the epidemiological data is a valuable help to guide the diagnosis.

Regarding co-circulation of USUV and WNV, this is a reality that can be expected. Both of these viruses share the same vectors (*Culex* spp. mosquitoes) and migratory wild birds as the major amplifying host. The transmission dynamics of these pathogens are influenced by common biological and environmental factors, such as humidity, temperature, and density of vectors and reservoirs, among others (Engel et al., 2016).

Table 1. USUV detection in the Iberian Peninsula.

Country	Area of study	Sampling date	Population details	Sample type	Number USUV positive samples / total tested (%)	USUV RNA detection method	USUV genome target region	USUV genetic lineage	USUV serology	USUV viral strains used in VNT	Additional data	References
ES	Guadalquivir region – Southern ES	February 2008 – November 2009	Mosquitoes	Pools of mosquitoes	1/3471 (0.03%)	RT-PCR	NS5 gene	African isolates	NA	NA	First report of USUV in Culex peregrinus mosquitoes (the only species with positive samples).	Vázquez et al., 2011
ES	Extremadura – Southwestern ES	October 2017 – December 2019	Birds	Sera Organs (brain, heart, intestine, liver, lung, muscle, kidney, spleen, stomach, pancreas and the pulp of immature feathers)	ELISA + micro-VNT: 2/120 (1.67%)	RT-PCR	NA	NA	bELISA; micro-VNT	USUV SAAR-1776 (GenBank ID: AY453412)	The study highlights the widespread circulation of WNV in the region and its co-circulation with USUV. BAGV was also tested, but all the samples were negative.	Bravo-Barriga et al., 2021
ES	Badajoz, Cáceres, Huelva, Salamanca, Toledo – Western ES	2018-2019	Fquids	Sera Organs (blood, hair, serum, spleen, lung, brain, cerebellum, heart, liver and kidney)	ELISA + micro-VNT: 11/725 (1.52%)	RT-PCR	NA	USUV (SAAR strain)	bELISA; ELISA WNV-specific IgM; micro-VNT	USUV SAAR-1776 (GenBank ID: AY453412)	Demonstration of WNV and USUV co-circulation.	Guerrero-Carvajal et al., 2021
ES	Doñana National Park (Andalusia) – Southern ES	2005-2020	Horses	Sera	VNT: 31/259 (11.96%)	NA	NA	NA	bELISA; VNT	USUV SAAR-1776 (GenBank ID: AY453412)	Besides WNV, BAGV was also tested for 4 years of the study, but none of the sera tested positive for it.	Magallanes et al., 2023

Continuation of the table 1.

ES	Catalonia – Northeastern ES	2010-2019	Birds Horses	Sera	Birds VNT: 19/205 (9.27%); Horses VNT: 11/205 (5.37%)	NA	NA	NA	eELISA; micro-VNT	Birds: USUV SAAR-1776 (GenBank ID: AY453412); Horses: USUV strain France 2018 (GenBank ID: MT863562.1)	BAGV and WNV were also tested by VNT.	Napp et al., 2021
ES	Extremadura – Southwestern ES	December 2018; January – October 2019	Birds	Plasma	bELISA: 75/645 (11.6%); micro-VNT: 1/645 (0.16%)	NA	NA	NA	bELISA; micro-VNT	USUV SAAR-1776 (GenBank ID: AY453412)	WNV was also tested by confirmatory micro-VNT.	Marzal et al., 2022
ES	Andalusia – Southern ES	November-December 2011	Birds	Sera	Decoys: bELISA: 137/1052 (13.00%); VNT: 38/1052 (3.61%) Wild raptors: bELISA: 16/123 (13.00%); VNT: WNV and/or USUV 4/123 (3.25%)	NA	NA	NA	bELISA; VNT	USUV It12 strain (GenBank ID: KF055446.1)	First study on WNV and USUV infections in decoys and the first report of MBV infections in waterfowl and raptors.	Jurado-Larúa et al., 2016
ES	Doñana National Park (Andalusia) – Southern ES	March and November 2010	Mosquitoes	Pools of mosquitoes	9/2802 (0.32%)	RT-PCR	N5S gene	NA	NA	NA	Culex perexiguus was the only species of mosquitoes with positive results to USUV infection. The aim of the study was to investigate the relationship between USUV occurrence and host biodiversity.	Roiz et al., 2019

Continuation of the table 1.

ES	Andalusia – Southern ES and Ceuta – North Africa; 1(Extremadura – Southwestern ES)	May 2013 – June 2015; (2003)	Dogs	Sera	bELISA: 39/815 (4.8%); 2VNT: 2/815 (0.24%)	NA	NA	NA	bELISA; VNT	USUV It12 strain (Genbank ID: KF055446.1)	WNV and TBEV were also tested.	García-Bocanegra et al., 2018
ES	Across the country	2002-2019	Zoo animals	Sera	bELISA: 19/570 (3.3%); VNT: 5/570 (0.90%)	NA	NA	NA	bELISA; VNT	USUV (Fr18 strain)	WNV and TBEV were also tested, as well as MBV, but for this last agent there were no positive results.	Caballero-Gómez et al., 2020
ES	Mallorca Island	May-June and September-October 2011 and September-October 2012	Horses	Sera	2/172 (1.16%)	NA	NA	NA	MIA; PRNT90 or micro-VNT	USUV It12 strain (Genbank ID: KF055446.1)	WNV and TBEV were also tested.	Vanhonweggen et al., 2017
ES	Across the country	2013-2014	Ruminants	Sera	bELISA: 153/4693 (3.3%); micro-VNT: 4/140 (2.85%)	NA	NA	NA	bELISA; micro-VNT	USUV It12 strain (Genbank ID: KF055446.1)	Species tested: Cervus elaphus, Dama dama, Ovis aries musimon and Capreolus capreolus. Positive samples on bELISA were tested for neutralizing antibodies against WNV, USUV, and MBV.	García-Bocanegra et al., 2016

Continuation of the table 1.

ES	Huelva, Cádiz and Sevilla – Southern ES	July–October 2013	Birds	Sera	bELISA: 6/149 (4.03%); VNT: 1/149 (0.67%)	NA	NA	bELISA; VNT	USUV SAAR–AY453412; VNT 1776 (GenBank ID)	VNT using WNV and USUV were performed. The serum from a <i>Turdus merula</i> showed neutralization of both WNV and USUV.	Ferraguti et al., 2016
ES	Córdoba (Andalusia) – Southern ES	November 2013–May 2014	Birds Zoo animals	Sera	Birds: bELISA: 11/142 (7.75%); VNT: 3/142 (2.11%); Zoo animals: bELISA: 4/49 (8.16%); VNT: 1/49 (2.04%)	NA	NA	bELISA; VNT	USUV It12 strain (GenBank ID: KF055446.1)	Birds apart from the zoo samples were all <i>Columba livia</i> var. domestica. The prevalences of other pathogenic zoonotic agents besides flaviviruses (WNV, USUV, and MBY) were studied: avian influenza viruses, <i>Salmonella</i> spp. and <i>Toxoplasma gondii</i> . First report of USUV infection in ostriches.	Cano-Terriza et al., 2015
ES, FR, ALG, TN	Medes Islands and L'Escala – Northeastern ES	March and April 2009–2011	Birds Ticks Mosquitoes	Eggs (egg yolks) Sera Pools of ticks and mosquitoes	Eggs: eELISA: 109/193 (56.48%); VNT: 0/10 (0%); Sera: eELISA: 18/503 (3.58%); Ticks: PCR: 8/135 (5.93%); Mosquitoes: PCR: 0%	NA	fragment of the viral NS5 gene (143 bp)	eELISA; VNT	USUV SAAR–AY453412	Species tested: <i>Larus michahellis</i> . First report of MBY-like virus in the Mediterranean.	Arnal et al., 2014
ES	Southern ES	November 2012	Birds	Oral and cloacal swabs Sera Organs (heart, kidney, spleen, and brain)	2/2 (100%)	RT-PCR	10 NT-sequences (1,048 bp)	NA	NA	Species tested: <i>Turdus philomelos</i>	Höfle et al., 2013

Continuation of the table 1.

ES	Cádiz (Andalucía) – Southern ES	October 2011-February 2012	Birds	Sera Brain tissue	Partridges: VNT: 1/159 (0.63%) Pheasants: VNT: 0/13 (0%) PCR: 0/172 (0%)	RT-PCR	NA	NA	bELISA; micro-VNT	USUV SAAR-1776 (GenBank ID. AY453412)	Species tested: Alectonix rufa and Phasianus colchicus. Other flaviviruses tested: WNV and BAGV.	Llorente et al., 2013
ES	Madrid, Toledo, Segovia, Guadalajara, Cuenca and Avila – Central ES	2011-2013	Horses	Sera	NA	NA	NA	NA	bELISA; IgM capture ELISA; VNT	USUV SAAR-1776 (GenBank ID. AY453412)	WNV detection was the focus of the study.	Abad-Cobo et al., 2017
ES	Catalonia – Northeastern ES	May-October 2006	Mosquitoes	Pools of mosquitoes	1/456 (0.23%)	RT-PCR	NS5 gene	NA	NA	NA	NA	Busquets et al., 2008
ES	Extremadura – Southwestern ES	May-October 2020 February-December 2020; March-May 2021	Mosquitoes Birds	Pools of mosquitoes Sera	Mosquitoes: PCR: 2/438 (0.46%) Birds: bELISA: 103/1413 (7.29%) VNT: 17/1413 (1.20%)	RT-PCR	NS5 gene	NA	bELISA; micro-VNT	USUV SAAR-1776 (GenBank ID. AY453412)	Other flaviviruses tested: WNV and BAGV. Culex pipiens positive for USUV.	Bravo-Barriga et al., 2025

ALGR: Algeria; **CSF:** Cerebrospinal fluid; **ES:** Spain; **FR:** France; **NA:** not applicable; **NT:** nucleotide; **TN:** Tunisia
bELISA: blocking ELISA; **cELISA:** competitive ELISA; **Ig:** Immunoglobulins; **MIA:** microsphere based multiplex immuno-assay;
NAb: Neutralizing antibody; **NS:** Non-structural; **PRNT:** plaque reduction neutralization test; **RT-PCR:** Real-time polymerase chain reaction;
VNT: viral neutralization test;
BAGV: Bagaza virus; **JEV:** Japanese encephalitis virus; **LJV:** Louping ill virus; **MBV:** Meaban virus; **TBEV:** Tick-borne encephalitis virus;
¹ A retrospective analysis.

² Simultaneous occurrence of antibodies against WNV + USUV and WNV + USUV + TBEV.

CONCLUSION

The increased international movement of people promotes the spread of pathogens throughout different continents. The transformation of habitats and increase of inhabited areas, in turn, promote increased contact between wildlife, vectors, and humans. Flaviviruses are currently distributed worldwide and, due to their overlapping distributions, pose a major threat to global health. In the last decades, flaviviruses have showed an increasing occurrence and/or wider geographical distribution area. The JEV group, specifically, causes febrile illness and neurological syndromes, when patients develop symptoms. USUV has an enzootic cycle between birds and mosquitoes, and after it was first detected in Europe (Italy) in 1996, it spread to many European countries. Due to the gathering of articles with very different study populations and diagnostic techniques used, there is a lot of variability in the presented results, but it is undoubtedly shown that the virus is present in the Iberian Peninsula. Its impact for animal and public health ought to be better assessed in this region. An integrated surveillance system could improve the monitoring of its evolution in a One Health approach.

Authors' contributions

Conceptualization, FL and JRM; methodology, FL, LC and ACM; software, FL; validation, JRM; formal analysis, FL, JRM, LC and ACC; investigation, FL, ; resources, JRM; data curation, FL, JRM, LC, ACM, MM and ACC; writing—original draft preparation, FL; writing—review and editing, FL, JRM, LC, ACM, MM and ACC; visualization, FL; supervision, ACM, MM and ACC. All authors have read and agreed to the published version of the manuscript.

Competing interests

We declare that we have no known conflict of financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ORCID iDs


Filipa Loureiro  <https://orcid.org/0000-0002-5370-5333>

Luís Cardoso  <https://orcid.org/0000-0002-6145-7560>

João R. Mesquita  <https://orcid.org/0000-0001-8769-8103>

Ana C. Matos  <https://orcid.org/0000-0001-9709-862X>

Manuela Matos  <https://orcid.org/0000-0002-9584-6636>

Ana Cláudia Coelho  <https://orcid.org/0000-0002-7196-4179>

REFERENCES

- Abad-Cobo A., Llorente F., Barbero M.D.C., Cruz-López F., Forés P., Jiménez-Clavero M.Á. 2017. Serosurvey reveals exposure to West Nile virus in asymptomatic horse populations in central Spain prior to recent disease foci. *Transboundary & Emerging Diseases* 64(5):1387-1392. doi:10.1111/tbed.12510.

- Agliani G., Giglia G., Marshall E.M., Gröne A., Rockx B.H.G., van den Brand J.M.A. 2023. Pathological features of West Nile and Usutu virus natural infections in wild and domestic animals and in humans: A comparative review. *One Health* 16:100525. doi:10.1016/j.onehlt.2023.100525.
- Arnal A., Gómez-Díaz E., Cerdà-Cuellar M., Lecollinet S., Pearce-Duvet J., Busquets N., García-Bocanegra I., Pagès N., Vittecoq M., Hammouda A., Samraoui B., Garnier R., Ramos R., Selmi S., González-Solís J., Jourdain E., Boulinier T. 2014. Circulation of a Meaban-like virus in yellow-legged gulls and seabird ticks in the western Mediterranean basin. *PLoS One* 9(3):e89601. doi:10.1371/journal.pone.0089601.
- Ashraf U., Ye J., Ruan X., Wan S., Zhu B., Cao S. 2015. Usutu virus: an emerging flavivirus in Europe. *Viruses* 7(1):219-38. doi:10.3390/v7010219.
- Bakonyi T., Gould E.A., Kolodziejek J., Weissenböck H., Nowotny N. 2004. Complete genome analysis and molecular characterization of Usutu virus that emerged in Austria in 2001: comparison with the South African strain SAAR-1776 and other flaviviruses. *Virology* 328(2):301-310. doi:10.1016/j.virol.2004.08.005.
- Barbic L., Vilibic-Cavlek T., Listes E., Stevanovic V., Gjenero-Margan I., Ljubin-Sternak S., Pem-Novosel I., Listes I., Mlinaric-Galinovic G., Di Gennaro A., Savini G. 2013. Demonstration of Usutu virus antibodies in horses, Croatia. *Vector Borne and Zoonotic Diseases*. 13(10):772-4. doi:10.1089/vbz.2012.1236.
- Beermann S., Dobler G., Faber M., Frank C., Habedank B., Hagedorn P., Kampen H., Kuhn C., Nygren T., Schmidt-Chanasit J., Schmolz E., Stark K., Ulrich R.G., Weiss S., Wilking H. 2023. Impact of climate change on vector – and rodent-borne infectious diseases. *Journal of Health Monitoring* 8(Suppl 3):33-61. doi:10.25646/11401.
- Bergmann F., Schmoock-Wellhausen M., Fast C., Holicki C.M., Michel F., Wysocki P., Sadeghi B., Groschup M.H., Ziegler U. 2023. Longitudinal study of the occurrence of Usutu virus and West Nile virus infections in birds in a zoological garden in Northern Germany. *Pathogens* 12(6):753. doi:10.3390/pathogens12060753.
- Bravo-Barriga D., Aguilera-Sepúlveda P., Guerrero-Carvajal F., Llorente F., Reina D., Pérez-Martín J.E., Jiménez-Clavero M.Á., Frontera E. 2021. West Nile and Usutu virus infections in wild birds admitted to rehabilitation centres in Extremadura, western Spain, 2017-2019. *Veterinary Microbiology* 255:109020. doi:10.1016/j.vetmic.2021.109020.
- Bravo-Barriga D., Ferraguti M., Magallanes S., Aguilera-Sepúlveda P., Llorente F., Pérez-Ramírez E., Vázquez A., Guerrero-Carvajal F., Sánchez-Seco M.P., Jiménez-Clavero M.A., Mora-Rubio C., Marzal A., Frontera E., de Lope F. 2023. Identification of Usutu virus Africa 3 lineage in a survey of mosquitoes and birds from urban areas of Western Spain. *Transboundary & Emerging Diseases* 23 (1): 6893677. doi:10.1155/2023/6893677.
- Busquets N., Alba A., Allepuz A., Aranda C., Ignacio Nuñez J. 2008. Usutu virus sequences in *Culex pipiens* (*Diptera: Culicidae*), Spain. *Emerging Infectious Diseases* 14(5):861-3. doi:10.3201/cid1405.071577.
- Caballero-Gómez J., Cano-Terriza D., Lecollinet S., Carbonell M.D., Martínez-Valverde R., Martínez-Nevado E., García-Párraga D., Lowenski S., García-Bocanegra I. 2020. Evidence of exposure to zoonotic flaviviruses in zoo mammals in Spain and their potential role as sentinel species. *Veterinary Microbiology* 247:108763. doi:10.1016/j.vetmic.2020.108763.
- Čabanová V., Kerlik J., Kirschner P., Rosochová J., Klempa B., Sláviková M., Ličková M. 2023. Co-Circulation of West Nile, Usutu, and tick-borne encephalitis viruses in the same area: a great challenge for diagnostic and blood and organ safety. *Viruses* 15(2):366. doi:10.3390/v15020366.

- Cadar D., Simonin Y. 2022. Human Usutu virus infections in Europe: a new risk on horizon?. *Viruses* 15(1):77. doi:10.3390/v15010077.
- Cano-Terriza D., Guerra R., Lecollinet S., Cerdà-Cuellar M., Cabezón O., Almería S., García-Bocanegra I. 2015. Epidemiological survey of zoonotic pathogens in feral pigeons (*Columba livia* var. *domestica*) and sympatric zoo species in Southern Spain. *Comparative Immunology, Microbiology & Infectious Diseases* 43:22-7. doi:10.1016/j.cimid.2015.10.003.
- Cavrini F., Della Pepa M.E., Gaibani P., Pierro A.M., Rossini G., Landini M.P., Sambri V. 2011. A rapid and specific real-time RT-PCR assay to identify Usutu virus in human plasma, serum, and cerebrospinal fluid. *Journal of Clinical Virology* 50(3):221-3. doi:10.1016/j.jcv.2010.11.008.
- Chan K.R., Ismail A.A., Thergarajan G., Raju C.S., Yam H.C., Rishya M., Sekaran S.D. 2022. Serological cross-reactivity among common flaviviruses. *Frontiers in Cellular and Infection Microbiology* 12:975398. doi: 10.3389/fcimb.2022.975398.
- Clé M., Beck C., Salinas S., Lecollinet S., Gutierrez S., Van de Perre P., Baldet T., Foulongne V., Simonin Y. 2019. Usutu virus: a new threat? *Epidemiology and Infection* 147:e232. doi:10.1017/S0950268819001213.
- Constant O., Bollore K., Clé M., Barthelemy J., Foulongne V., Chenet B., Gomis D., Virolle L., Gutierrez S., Desmetz C., Moares R.A., Beck C., Lecollinet S., Salinas S., Simonin Y. 2020. Evidence of exposure to USUV and WNV in zoo animals in France. *Pathogens* 9(12):1005. doi:10.3390/pathogens9121005.
- Dey D., Poudyal S., Rehman A., Hasan S.S. 2021. Structural and biochemical insights into flavivirus proteins. *Virus Research* 296:198343. doi:10.1016/j.virusres.2021.198343.
- Durand B., Haskouri H., Lowenski S., Vachieri N., Beck C., Lecollinet S. 2016. Seroprevalence of West Nile and Usutu viruses in military working horses and dogs, Morocco, 2012: dog as an alternative WNV sentinel species? *Epidemiology and Infection* 144(9):1857-64. doi:10.1017/S095026881600011X.
- Engel D., Jöst H., Wink M., Börstler J., Bosch S., Garigliany M.M., Jöst A., Czajka C., Lühken R., Ziegler U., Groschup M.H., Pfeffer M., Becker N., Cadar D., Schmidt-Chanasit J. 2016. Reconstruction of the evolutionary history and dispersal of Usutu virus, a neglected emerging arbovirus in Europe and Africa. *mBio* 7(1):e01938-15. doi:10.1128/mBio.01938-15.
- European Centre for Disease Prevention and Control, European Food Safety Authority, Surveillance, prevention and control of West Nile virus and Usutu virus infections in the EU/EEA. Stockholm: 2023. doi:10.2903/sp.efsa.2023.EN-8242.
- European Centre for Disease Prevention and Control and European Food Safety Authority. Mosquito maps [internet]. Stockholm: ECDC; 2023. Available at: <https://ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/mosquito-maps>. Accessed 15.08.2024.
- Ferraguti M., La Puente J.M., Soriguer R., Llorente F., Jiménez-Clavero M.Á., Figuerola J. 2016. West Nile virus-neutralizing antibodies in wild birds from southern Spain. *Epidemiology and Infection* 144(9):1907-11. doi:10.1017/S0950268816000133.
- Ferrero D.S., Albertosa-González L., Mas A., Verdaguer N. 2022. Structure and function of the NS5 methyltransferase domain from Usutu virus. *Antiviral Research* 208:105460. doi:10.1016/j.antiviral.2022.105460.
- García-Bocanegra I., Paniagua J., Gutiérrez-Guzmán A.V., Lecollinet S., Boadella M., Arenas-Montes A., Cano-Terriza D., Lowenski S., Gortázar C., Höfle U. 2016. Spatio-temporal trends and risk factors affecting West Nile virus and related flavivirus exposure in Spanish wild ruminants. *BMC Veterinary Research* 12(1):249. doi:10.1186/s12917-016-0876-4.

- García-Bocanegra I., Jurado-Tarifa E., Cano-Terriza D., Martínez R., Pérez-Marín J.E., Lecollinet S. 2018. Exposure to West Nile virus and tick-borne encephalitis virus in dogs in Spain. *Transboundary & Emerging Diseases* 65(3):765-772. doi:10.1111/tbed.12801.
- Girll P., Euringer K., Coroian M., Mihalca A.D., Borde J.P., Dobler G. 2024. Comparison of five serological methods for the detection of West Nile virus antibodies. *Viruses* 16(5):788. doi: 10.3390/v16050788.
- Gomes da Silva P., Seixas Dos Reis J.A., Rodrigues M.N., da Silva Ardaya Q., Mesquita J.R. 2023. Serological cross-reactivity in zoonotic flaviviral infections of medical importance. *Antibodies (Basel)*12(1):18. doi:10.3390/antib12010018.
- Guerrero-Carvajal F., Bravo-Barriga D., Martín-Cuervo M., Aguilera-Sepúlveda P., Ferraguti M., Jiménez-Clavero M.Á., Llorente F., Alonso J.M., Frontera E. 2021. Serological evidence of co-circulation of West Nile and Usutu viruses in equids from western Spain. *Transboundary & Emerging Diseases* 68(3):1432-1444. doi:10.1111/tbed.13810.
- Höfle U., Gamino V., de Mera I.G., Mangold A.J., Ortíz J.A., de la Fuente J. 2013. Usutu virus in migratory song thrushes, Spain. *Emerging Infectious Diseases* 19(7):1173-5. doi:10.3201/eid1907.130199.
- Johnson N., Fernández de Marco M., Giovannini A., Ippoliti C., Danzetta M.L., Svartz G., Erster O., Groschup M.H., Ziegler U., Mirazimi A., Monteil V., Beck C., Gonzalez G., Lecollinet S., Attoui H., Moutailler S. 2018. Emerging mosquito-borne threats and the response from European and Eastern Mediterranean countries. *International Journal of Environmental Research and Public Health* 15(12):2775. doi:10.3390/ijerph15122775.
- Jurado-Tarifa E., Napp S., Lecollinet S., Arenas A., Beck C., Cerdà-Cuellar M., Fernández-Morente M., García-Bocanegra I. 2016. Monitoring of West Nile virus, Usutu virus and Meaban virus in waterfowl used as decoys and wild raptors in southern Spain. *Comparative Immunology, Microbiology & Infectious Diseases* 49:58-64. doi:10.1016/j.cimid.2016.10.001.
- Kvapil P., Račnik J., Kastelic M., Bártoová E., Korva M., Jelovšek M., Avšič-Županc T. 2021. A sentinel serological study in selected zoo animals to assess early detection of West Nile and Usutu virus circulation in Slovenia. *Viruses* 13(4):626. doi:10.3390/v13040626.
- Laidoudi Y., Durand G., Watier-Grillot S., Dessimoulie A-S., Labarde C., Normand T., Andréo V., Guérin P., Grard G., Davoust B. 2023. Evidence of antibodies against the West Nile virus and the Usutu virus in dogs and horses from the Southeast of France. *Transboundary & Emerging Diseases* 8779723. doi:10.1155/2023/8779723.
- Llorente F., Pérez-Ramírez E., Fernández-Pinero J., Soriguer R., Figuerola J., Jiménez-Clavero M.A. 2013. Flaviviruses in game birds, Southern Spain, 2011-2012. *Emerging Infectious Diseases* 19(6):1023-1025. doi:10.3201/eid1906.130122.
- Magallanes S., Llorente F., Ruiz-López M.J., Martínez-de la Puente J., Soriguer R., Calderon J., Jiménez-Clavero M.Á., Aguilera-Sepúlveda P., Figuerola J. 2023. Long-term serological surveillance for West Nile and Usutu virus in horses in south-West Spain. *One Health* 17:100578. doi:10.1016/j.onehlt.2023.100578.
- Mariconti M., Obadia T., Mousson L., Malacrida A., Gasperi G., Failloux A.B., Yen P.S. 2019. Estimating the risk of arbovirus transmission in Southern Europe using vector competence data. *Scientific Reports* 9(1):17852. doi:10.1038/s41598-019-54395-5.
- Marzal A., Ferraguti M., Muriel J., Magallanes S., Ortiz J.A., García-Longoria L., Bravo-Barriga D., Guerrero-Carvajal F., Aguilera-Sepúlveda P., Llorente F., de Lope F., Jiménez-Clavero M.Á., Frontera E. 2022. Circulation of zoonotic flaviviruses in wild passerine birds in Western Spain. *Veterinary Microbiology* 268:109399. doi:10.1016/j.vetmic.2022.109399.

- Musso D, Despres P. 2020. Serological diagnosis of flavivirus-associated human infections. *Diagnostics (Basel)* 10(5):302. doi: 10.3390/diagnostics10050302.
- Napp S, Llorente F, Beck C., Jose-Cunilleras E., Soler M., Pailler-García L., Amaral R., Aguilera-Sepúlveda P, Pifarré M., Molina-López R., Obón E., Nicolás O., Lecollinet S., Jiménez-Clavero M.Á., Busquets N. 2021. Widespread circulation of Flaviviruses in horses and birds in Northeastern Spain (Catalonia) between 2010 and 2019. *Viruses* 13(12):2404. doi:10.3390/v13122404.
- Pecorari M., Longo G., Gennari W., Grottole A., Sabbatini A., Tagliuzucchi S., Savini G., Monaco F, Simone M., Lelli R., Rumpianesi F. 2009. First human case of Usutu virus neuroinvasive infection, Italy, August-September 2009. *Euro Surveillance* 14(50):19446.
- Roesch F, Fajardo A., Moratorio G., Vignuzzi M. 2019. Usutu virus: an arbovirus on the rise. *Viruses* 11(7):640. doi:10.3390/v11070640.
- Roiz D., Vázquez A., Ruiz S., Tenorio A., Soriguer R., Figuerola J. 2019. Evidence that passerine birds act as amplifying hosts for Usutu virus circulation. *Ecohealth* 16(4):734-742. doi:10.1007/s10393-019-01441-3.
- Romeo C., Lecollinet S., Caballero J., Isla J., Luzzago C., Ferrari N., García-Bocanegra I. 2018. Are tree squirrels involved in the circulation of flaviviruses in Italy? *Transboundary & Emerging Diseases* 65(5):1372-1376. doi:10.1111/tbed.12874.
- Scaramozzino N., Crance J.M., Jouan A., DeBriel D.A., Stoll F., Garin D. 2001. Comparison of flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. *Journal of Clinical Microbiology* 39(5):1922-7. doi:10.1128/JCM.39.5.1922-1927.2001.
- Scaramozzino P., Carvelli A., Bruni G., Cappiello G., Censi F., Magliano A., Manna G., Ricci I., Rombolà P., Romiti F., Rosone F., Sala M.G., Scicluna M.T., Vaglio S., De Liberato C. 2021. West Nile and Usutu viruses co-circulation in central Italy: outcomes of the 2018 integrated surveillance. *Parasites & Vectors* 14(1):243. doi:10.1186/s13071-021-04736-z.
- Schwartz G., Tirosh-Levy S., Erester O., Shenhar R., Levy H., Bazanow B., Gelman B., Steinman A. 2020. Exposure of Horses in Israel to West Nile Virus and Usutu Virus. *Viruses* 12(10):1099. doi:10.3390/v12101099.
- Shamseer L., Moher D., Clarke M., Ghersi D., Liberati A., Petticrew M., Shekelle P., Stewart L.A.; PRISMA-P Group. 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ*. 350:g7647. doi:10.1136/bmj.g7647. Erratum in: 2016. *BMJ*. 354:i4086. doi:10.1136/bmj.i4086.
- Siljic M., Sehovic R., Jankovic M., Stamenkovic G., Loncar A., Todorovic M., Stanojevic M., Cirkovic V. 2023. Evolutionary dynamics of Usutu virus: Worldwide dispersal patterns and transmission dynamics in Europe. *Frontiers in Microbiology* 14:1145981. doi:10.3389/fmicb.2023.1145981.
- Störk T., de le Roi M., Haverkamp A.K., Jesse S.T., Peters M., Fast C., Gregor K.M., Könenkamp L., Steffen I., Ludlow M., Beineke A., Hansmann F, Wohlsein P, Osterhaus A.D.M.E., Baumgärtner W. 2021. Analysis of avian Usutu virus infections in Germany from 2011 to 2018 with focus on dsRNA detection to demonstrate viral infections. *Scientific Reports* 11(1):24191. doi:10.1038/s41598-021-03638-5.
- United Nations Environment Programme (UNEP). Available at: <https://www.unep.org/unepmap/resources/factsheets/climate-change>. Accessed 05.08.2024.
- Vanhomwegen J, Beck C., Desprès P, Figuerola A., García R., Lecollinet S., López-Roig M., Manuguerra J.C., Serra-Cobo J. 2017. Circulation of Zoonotic Arboviruses in Equine

- Populations of Mallorca Island (Spain). Vector Borne and Zoonotic Diseases 17(5):340-346. doi:10.1089/vbz.2016.2042.
- Vázquez A., Ruiz S., Herrero L., Moreno J., Molero F., Magallanes A., Sánchez-Seco M.P., Figuerola J., Tenorio A. 2011. West Nile and Usutu viruses in mosquitoes in Spain, 2008-2009. American Journal of Tropical Medicine and Hygiene 85(1):178-81. doi:10.4269/ajtmh.2011.11-0042.
- Weissenböck H., Bakonyi T., Rossi G., Mani P., Nowotny N. 2013. Usutu virus, Italy, 1996. Emerging Infectious Diseases. 19(2):274-7. doi:10.3201/eid1902.121191.
- Weissenböck H., Kolodziejek J., Url A., Lussy H., Rebel-Bauder B., Nowotny N. 2002. Emergence of Usutu virus, an African mosquito-borne flavivirus of the Japanese encephalitis virus group, central Europe. Emerging Infectious Diseases. 8(7):652-6. doi:10.3201/eid0807.020094
- Williams M.C., Simpson D.I., Haddow A.J., Knight E.M. 1964. The isolation of West Nile virus from man and of Usutu virus from the bird-biting mosquito *Mansonia aurites* (theobald) in the Entebbe area of Uganda. Annals of Tropical Medicine and Parasitology 58:367-74. doi:10.1080/00034983.1964.11686258.

USUTU VIRUS NA IBERIJSKOM POLUOSTRVU: SISTEMATIČNI PRIKAZ

Filipa Loureiro, Luís Cardoso, João R. Mesquita,
Ana C. Matos, Manuela Matos, Ana Cláudia Coelho

Studije o Usutu virusu (USUV) u Evropi postaju sve brojnije tokom vremena, pri čemu je infekcija USUV identifikovana kod ljudi i životinja. Pojava USUV u Evropi skreće pažnju na to kako se arbovirus može širiti, prilagođavati i evoluirati u nekonvencionalnom okruženju. Razumevanje epidemioloških i evolutivnih procesa koji doprinose pojavi, održavanju i daljem širenju virusnih bolesti ključno je za razvoj i sprovođenje strategija nadzora radi njihove kontrole.

Cilj ove sistematske publikacije bio je da se prikupe i procene svi objavljeni podaci o USUV-u iz studija sprovedenih na ljudima, životinjama i uzorcima iz okoline na Iberijskom poluostrvu. Elektronske baze podataka Mendeley, PubMed, Scopus i Web of Science temeljno su pretražene, a uključena su istraživanja objavljena zaključno sa 6. julom 2024. godine. Nakon potpunog pregleda i primene PRISMA kriterijuma za isključivanje/uključivanje, ukupno je razmatrano 20 relevantnih radova.

Ovaj rad pokazuje da USUV cirkuliše među ljudima, životinjama i u životnoj sredini na Iberijskom poluostrvu.

Ključne reči: Arbovirus, Flavivirus, infekcija, USUV, zoonoza