Seroprevalence of West Nile Virus in birds in European countries: a systematic review

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Abstract

West Nile fever is a mosquito-borne viral disease that can affect birds, humans and horses, causing asymptomatic infection, mild fever, meningitis, encephalitis, and death. The aim of this study was to perform a systematic review of West Nile virus (WNV) seroprevalence studies in birds in European countries between 2010 and 2023. Three electronic databases – PubMed, ScienceDirect and Scopus – were searched for relevant publications using predetermined keywords. A total of 4,872 papers were found, and 39 results included in the article, after removing duplicates and applying the eligibility criteria. Further monitoring and epidemiological studies of WNV in Europe is advised, considering the threat that this disease can pose to humans and animals.

Key words: *Bird; Europe; Seroprevalence; West Nile Virus*

Introduction

West Nile Virus (WNV) is a mosquito-borne RNA flavivirus identified for the first time in Uganda in 1937. It presents a zoonotic cycle of transmission, maintained by mosquitoes and wild avian species. Though other genera have been implicated in WNV transmission, *Culex* spp. are considered the main vectors in Europe and North America.

Humans, domestic animals (especially equines) and wild mammals can often be infected and act as incidental hosts. Nevertheless, mammals are not amplifying agents of the virus, and so they are unable to efficiently maintain the transmission cycle (Lourenço et al., 2022). Birds are the natural reservoir hosts for WNV. Many avian species develop transient high titre

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viraemia, allowing transmission of the virus to feeding mosquitoes (Campbell et al., 2002; Rappole and Hubálek, 2003).

Most human infections are asymptomatic, but when clinical signs are present, they are usually characteristic of a central neuroinvasive disease, and potentially fatal (Campbell et al., 2002). WNV infections have been notifiable at the European Union (EU) level since 2008, but only later became notifiable in some EU countries.

The occurrence of disease in humans and animals along with bird and mosquito surveillance for WNV activity shows that the reach of the virus has expanded to the whole American continent, as well as Europe (OIE, 2022). In recent decades, increasing epidemic activity of WNV has been observed in several European countries, including in the Mediterranean basin (Young et al., 2017).

The role of migratory birds in the spread of the virus into new places is widely described (Cito et al., 2013), simultaneously with the dispersion of vectors around the world (Rappole and Hubálek, 2003). More recently, it has been suggested that local movements of long-range migratory and resident birds play the main role in the spread of WNV in Europe. On the contrary, migratory birds returning from their overwintering territories in Africa play a more discreet position, since the virus is already endemic in Europe (Seidowski et al., 2010; Young et al., 2017).

Birds that recover from a WNV infection usually develop long-term immunity, and the detection of WNV antibodies in resident bird populations is indicative for local virus circulation (Nemeth et al., 2008; Kwan et al., 2012; Young et al., 2017). Resting sites for wild birds that combine a high population density and the presence of vectors are considered high-risk zones for introduction of the virus (Seidowski et al., 2010).

Seroprevalence-based studies in wild or sentinel birds can be useful and extremely important in terms of active surveillance, enabling the competent authorities to take appropriate measures to protect animals and people in a One Health context. Furthermore, many bird species are endangered and further updates on the health status of their populations can be important for species conservation and wildlife medicine.

The aim of this study was to conduct a systematic review of the WNV seroprevalence studies in birds between 2010 and 2022 in European countries.

Materials and methods

A systematic review of the scientific literature was conducted to answer the research question: *What do we know about WNV seroprevalence in birds in Europe?*

This review was performed according to the methodological recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA statement) (Moher et al., 2015).

Search Strategy

A systematic search procedure was performed in the PubMed, ScienceDirect and Scopus databases to identify all published studies responding to the "West Nile" AND virus AND bird*" keywords. The search was performed from 20 February 2022 to 20 March 2023. The search terms were applied to all fields in the three databases. No language restrictions were applied at this stage. Collected data were saved and organised in an Excel file (Microsoft™ 365).

Eligibility Screening

Inclusion criteria were first related to the literature search. Only studies published from 2010 onwards were considered and the language of the

full articles was set as English, French, Portuguese or Spanish. Reviews, case reports, clinical retrospective studies, experimental studies and epidemiological surveillance reports werenot considered. All studies for which the title and/or abstract were not relevant within the scope of our search or did not fulfil the above criteria were excluded (Figure 1).

Study selection and data extraction

Duplicates were eliminated by the main author (FL) based on the title and/or DOI results. Abstracts were then screened by two authors (FL and ACC) to identify those relevant to the present study. Once the title and abstract could not be rejected with certainty, the full text was read.

In the second stage, the results from the first screening step were analysed in a stricter fashion by the same authors, ensuring adherence to the eligibility criteria.

After assessment, data were classified into three categories: (1) general data related to the study, (2) data related to the diagnostic techniques, and (3) data related

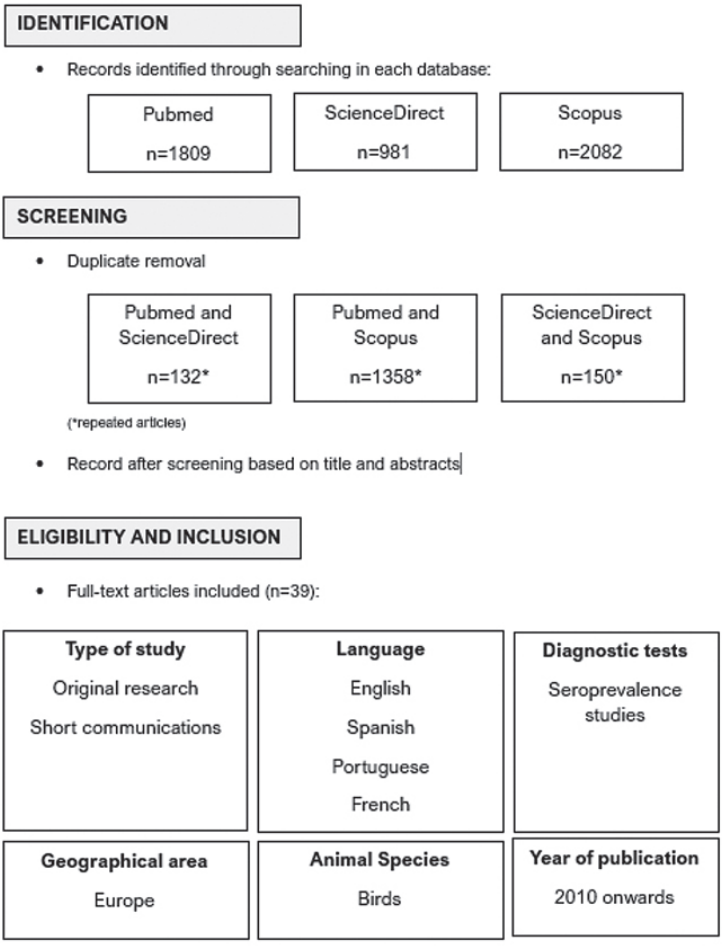

Figure 1. Flow diagram of article selection for the present systematic review.

to the animals. A new Excel file was created, summarising the selected information.

Results

Search Results and Study Selection

From the initial database, 39 potentially relevant publications were identified. Of these, some appeared in multiple databases, *i.e.*, 37 were found in PubMed, 31 in Scopus and four in ScienceDirect, and four of the articles were found in all three databases, two only in Scopus and three only in PubMed. All publications that satisfied the inclusion criteria were in English (Table 1).

Of the 15 European countries represented (Austria, Cyprus, France, Germany, Greece, Italy, Kosovo, Netherlands, Poland, Portugal, Romania, Serbia, Slovakia, Spain, and Sweden), the majority of studies were conducted in Spain (*n*=9) (Figure 2).

Most studies (*n*=25) used enzyme-linked immunosorbent assay (ELISA) as the first diagnostic test, and in a large proportion of these (*n*=19), some or all positive samples were confirmed by virus neutralization test (VNT). Other studies performed other serological tests as the first option, like VNT (*n*=7), immunofluorescence assay (IFA) (*n*=3), plaque reduction neutralisation test (PRNT) (*n*=3) and serum neutralisation test (SNT) (*n*=2). Reverse transcription polymerase chain reaction (RT-PCR) was used as an additional test in 13 studies. Of these, ten studied examined tissues/organs other than serum or blood for analysis.

In 19 of the 39 studies samples were exclusively tested for WNV. In the other 20, other flaviviruses were investigated: USUTU (USUV) in 15, Bagaza (BAGV) in 1, USUV and BAGV in 2, USUV and Tick-borne encephalitis virus (TBEV) in 1 and

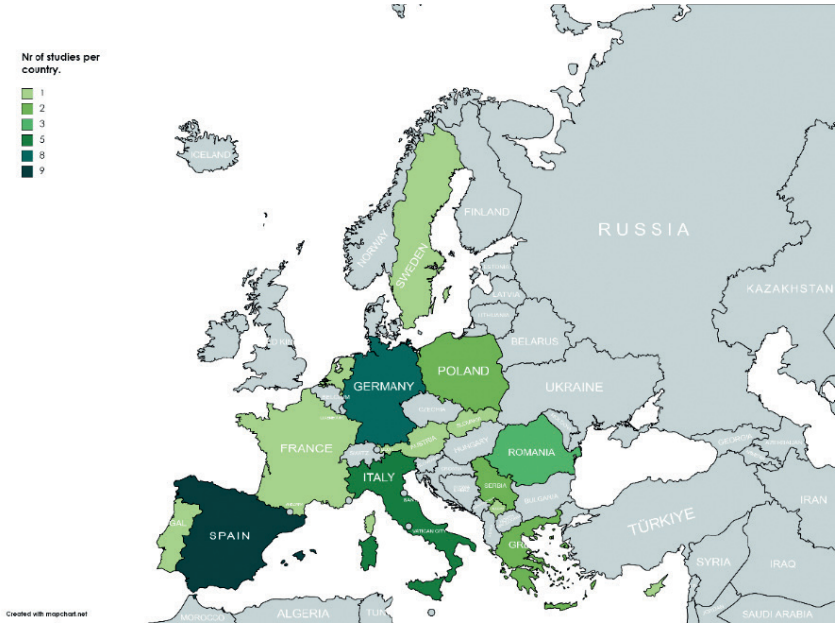

Figure 2. Map showing European countries for which data were included in the systematic review. Depth of shading indicating number of studies performed in each country (map created using https://mapchart. net/europe.html).

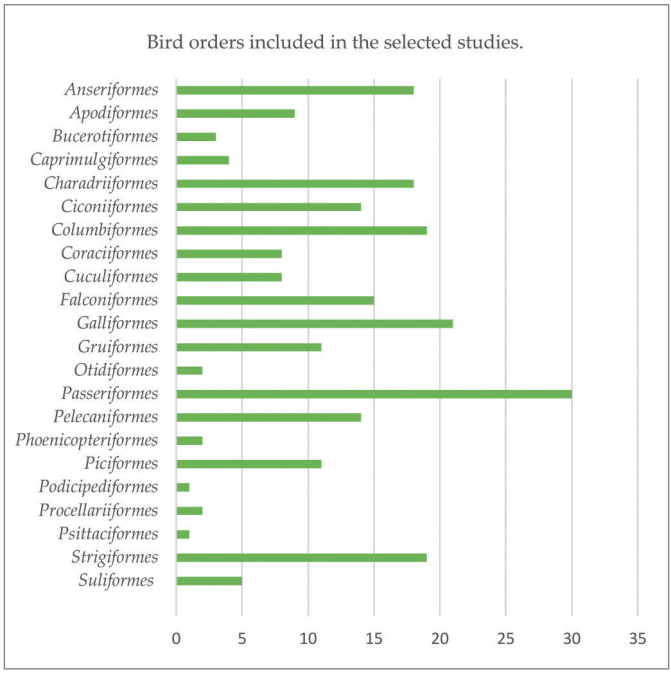

Figure 3. Number of times each order was analysed in the selected studies.

Japanese encephalitis virus (JEV) complex in 1.

The great majority of studies (*n*=34) evaluated samples from various species; only five studies were based on only one species.

In total, samples from 23 different avian orders were included (*Accipitriformes, Anseriformes, Apodiformes, Bucerotiformes, Caprimulgiformes, Charadriiformes, Ciconiiformes, Columbiformes, Coraciiformes, Cuculiformes, Falconiformes, Galliformes, Gruiformes, Otidiformes, Passeriformes, Pelecaniformes, Phoenicopteriformes, Piciformes, Podicipediformes, Procellariiformes, Psittaciformes, Strigiformes, Suliformes*). From these, birds of ten orders were related to wetlands or aquatic zones.

The order represented in the largest number of studies was *Passeriformes* (*n*=30), followed by *Galliformes* (*n*=21), and *Strigiformes* and *Columbiformes* (*n*=19) (Figure 3).

The study showing the highest seroprevalence was conducted in Italy, where 49.1% of the population tested (VNT) revealed the presence of WNV antibodies (Monaco et al., 2010). The second highest seroprevalence detected was 43.7% (ELISA), from which only 66% were confirmed by VNT (Wodak et al., 2011).

Börstler et al. (2016) analysed the presence of WNV antibodies in egg yolk, rather than in serum.

Table 1. Characteristics of studies included in the systematic review

Publication	Country	Orders	Screening test	*n tested*	Seroprevalence (%)	Sample collection period
1. Mancuso et al. (2022)	Italy	*Bucerotiformes, Caprimulgiformes, Charadriiformes, Columbiformes, Coraciiformes, Galliformes, Gruiformes, Passeriformes, Strigiformes,*	SNT, RT-PCR	1241	5.80	2012-2014
2. Santos et al. (2022)	Germany	*Accipitriformes, Anseriformes, Columbiformes, Galliformes, Strigiformes*	VNT, RT-PCR	67	8.95	2018-2019
3. Ziegler et al. (2022)	Germany	*Accipitriformes, Anseriformes, Apodiformes, Charadriiformes, Ciconiiformes, Columbiformes, Falconiformes, Gruiformes, Passeriformes, Pelecaniformes, Piciformes, Strigiformes, others*	ELISA, VNT, RT-PCR	2281	14.77-16.15	2019-2020
4. Bravo-Barriga et al. (2021)	Spain	*Accipitriformes, Ciconiiformes, Falconiformes, Otidiformes, Passeriformes, Pelecaniformes, Strigiformes, others*	ELISA, VNT	384	18.23	2017-2019
5. Napp et al. (2021)	Spain	*Accipitriformes, Anseriformes, Charadriiformes, Ciconiiformes, Columbiformes, Falconiformes, Galliformes, Passeriformes, Pelecaniformes, Strigiformes*	ELISA, MNT	3791	10.00	2010-2019
6. Pallari et al. (2021)	Cyprus	*Charadriiformes, Galliformes, Passeriformes, Piciformes, Strigiformes*	ELISA	836	1.32	2015-2020
7. Rexhepi et al. (2021)	Kosovo	*Galliformes*	ELISA, PRNT, VNT, RT-PCR	50	2.00	2019
8. Michel et al. (2019)	Germany	*Accipitriformes, Anseriformes, Apodiformes, Charadriiformes, Ciconiiformes, Columbiformes, Coraciiformes, Cuculiformes, Falconiformes, Galliformes, Gruiformes, Passeriformes, Pelecaniformes, Piciformes, Podicipediformes, Procellariiformes, Psittaciformes, Strigiformes, Suliformes*	VNT, RT-PCR	1533	2017A: *n*=171;2.92 2018A: *n*=304;2.30 2017B: *n*=76;2.63 2018B:*n*=92;6.52 2017C: *n*=492;3.05 2018C: *n*=398;3.27	2017-2018
9. Napp et al. (2019)	Spain	*Passeriformes*	ELISA, SNT	215	2017: *n*=89;3.37 2018: *n*=91;14.29 2019: *n*=35;17.14	2017-2019

10. Vasić et al. (2019)	Romania	*Columbiformes, Coraciiformes, Passeriformes, Piciiformes*	ELISA, VNT	68	11.80	2016
11. Bazanów et al. (2018)	Poland	*Accipitriformes*	ELISA	14	35.70	2012-2013
12. Csank et al. (2018)	Slovakia	*Accipitriformes, Ciconiiformes, Falconiformes, Passeriformes*	ELISA, VNT	109	9.20	2012-2014
13. Lim et al. (2018)	the Netherlands	*Galliformes, Passeriformes*	ELISA	265	10.19	2014-2015
14. Michel et al. (2018)	Germany	*Accipitriformes, Anseriformes, Apodiformes, Charadriiformes, Ciconiiformes, Columbiformes, Falconiformes, Gruiformes, Passeriformes, Pelecaniformes, Piciformes, Strigiformes, Suliformes*	RT-PCR, VNT	1962	2014: *n*=248; 8.87 2015: *n*=821; 2.44 2016: *n*=756; 2.12	2014-2016
15. Petrović et al. (2018)	Serbia	*Galliformes*	ELISA	3809	5.75	2014
16. Börstler et al. (2016)	Germany	*Galliformes*	ELISA	1990	0.00	2013
17. Ferraguti et al. (2016)	Spain	*Columbiformes, Coraciiformes, Cuculiformes, Passeriformes, Pelecaniformes*	ELISA, VNT	149	4,03 (ELISA positive); 4.70 (ELISA doubtful); only 1.34 confirmed by VNT	2013
18. Pastiu et al. (2016)	Romania	*Anseriformes, Charadriiformes, Columbiformes, Coraciiformes, Cuculiformes, Falconiformes, Galliformes, Gruiformes, Passeriformes, Pelecaniformes, Piciformes, Strigiformes,*	ELISA	159 (W)+ 21 (D)	32.1 (W) + 19.1 (D)	2011-2012; 2014
19. Llopis et al. (2015)	Italy	*Accipitriformes, Anseriformes, Apodiformes, Caprimulgiformes, Charadriiformes, Ciconiiformes, Columbiformes, Coraciiformes, Falconiformes, Galliformes, Passeriformes, Pelecaniformes, Piciformes, Strigiformes*	WNV neutraliz-ing-antibodies	233	4.29	2012-2013
20. Niczyporuk et al. (2015)	Poland	*Accipitriformes, Anseriformes, Apodiformes, Ciconiiformes, Columbiformes, Galliformes, Passeriformes*	ELISA, VNT	474	13.29	2010-2014

Publication	Country	Orders	Screening test	*n tested*	Seroprevalence (%)	Sample collection period
21. Ziegler et al. (2015)	Germany	*Accipitriformes, Anseriformes, Apodiformes, Charadriiformes, Ciconiiformes, Columbiformes, Cuculiformes, Falconiformes, Gruiformes, Passeriformes, Pelecaniformes, Piciformes, Strigiformes, Suliformes*	VNT, RT-PCR	902	4.99	2011-2013
22. Alba et al. (2014)	Spain	*Accipitriformes, Apodiformes, Bucerotiformes, Caprimulgiformes, Charadriiformes, Ciconiiformes, Cuculiformes, Falconiformes, Galliformes, Gruiformes, Passeriformes, Phoenicopteriformes, Piciformes, Procellariiformes, Strigiformes, Suliformes*	ELISA, VNT	1086	3.78	2007-2009
23. Oslobanu et al. (2014)	Romania	*Anseriformes, Bucerotiformes, Charadriiformes, Columbiformes, Galliformes, Passeriformes, Piciformes, Suliformes,*	PRNT	713	5.20	2006-2008
24. Llorente et al. (2013)	Spain	*Galliformes*	ELISA, VNT, RT-PCR	172	29 ELISA (23 confirmed by VNT)	2011-2012
25. Petrović et al. (2013)	Serbia	*Accipitriformes, Anseriformes, Apodiformes, Charadriiformes, Ciconiiformes, Columbiformes, Coraciiformes, Cuculiformes, Galliformes, Gruiformes, Passeriformes, Pelecaniformes,Pocidipediformes, Strigiformes,*	ELISA, PRNT, RT-PCR	133	8.00	2012
26. Vittecoq et al. (2013)	France	*Passeriformes*	PNT	65	10.77	2009-2010
27. Lelli et al. (2012)	Italy	*Charadriiformes, Coraciiformes, Strigiformes, Passeriformes, Galliformes, Caprimulgiformes, Cuculiformes, Pelecaniformes, Phoenicopteriformes*	VNT	1405	0.21	2006-2008
28. Valiakos et al. (2012)	Greece	*Anseriformes, Columbiformes, Passeriformes,*	IFA, VNT	295	23.70	2009-2011
29. Valiakos et al. (2012)	Greece	*Columbiformes (C), Passeriformes (P)*	IFA, VNT	113 (P) + 85 (C)	20.4 (P); 7.1 (C)	2011

30. Ziegler et al. (2012)	Germany	*Anseriformes, Apodiformes, Charadriiformes, Ciconiiformes, Columbiformes, Cuculiformes, Falconiformes, Gruiformes, Passeriformes, Piciformes, Strigiformes*	ELISA, VNT, RT-PCR	364	4.67	2009-2011
31. Barros et al. (2011)	Portugal	*(Not specified)*	ELISA, VNT	116	19.80	2004-2020
32. Busani et al. (2011)	Italy	*Passeriformes*	ELISA, SNT, RT-PCR	621	1.77	2008-2009
33. García-Bocanegra et al. (2011)	Spain	*Accipitriformes, Gruiformes, Otidiformes, Pelecaniformes, Strigiformes*	ELISA, VNT	201	1.00	2006-2009
34. Jourdain et al. (2011)	Sweden	*Anseriformes, Charadriiformes, Falconiformes, Passeriformes, others*	ELISA, VNT	1935	2.4 ELISA (0.10 confirmed by VNT)	2005-2006
35. López et al. (2011)	Spain	*Accipitriformes, Anseriformes, Charadriiformes, Ciconiiformes, Falconiformes, Galliformes, Gruiformes, Passeriformes, Pelecaniformes, Strigiformes*	VNT, RT-PCR	227	2.20	2004-2006
36. Wodak et al. (2011)	Austria	*Accipitriformes, Anseriformes, Columbiformes, Galliformes, Passeriformes, Strigiformes*	ELISA, VNT	71	43,70	2008-2009
37. Gangoso et al. (2010)	Spain	*Falconiformes*	VNT	81	4,90	2006
38. Monaco et al. (2010)	Italy	*Accipitriformes, Anseriformes, Charadriiformes, Columbiformes, Galliformes, Passeriformes, Pelecaniformes, Strigiformes*	PRNT, VNT	163	49,10	2008
39. Seidowski et al. (2010)	Germany	*Accipitriformes, Anseriformes, Charadriiformes, Ciconiiformes, Columbiformes, Falconiformes, Galliformes, Passeriformes*	IFA, VNT, RT-PCR	2736	0,84	2005-2009

Note: IFA = indirect fluorescent antibody; MNT = micro-neutralization test; PRNT = plaque reduction neutralization test; RT-PCR = reverse transcription polymerase chain reaction; SNT = serum neutralization test; VNT = virus neutralization test.

Discussion

This systematic review sought to compile relevant studies in WNV seroprevalence in birds in Europe. In total, 39 studies from 2010 to 2022 were reviewed.

The first outbreak of WNV in Europe occurred in 1996 in Romania (Tsai et al., 1998), and since 1998, WNV has been reported in other European countries. WNV is currently the most widespread virus among the flaviviruses, on all continents, and has been reported in Western, Eastern, Southern and Northern Europe (Figure 2).

The differences founded in antibody prevalence may be due to the different detection methods used, the range of host species considered, the geographic area and the epidemic period studied.

Some studies used different methods to present their seroprevalence results (by year, by region, in total), which made it difficult to compare information. The ELISA, VNT and PRNT methods are most commonly used for identifying antibody against WNV in avian serum. For confirmation of clinical cases, IgM capture ELISA is the preferred option available for the detection of immune response, right after RT-PCR for the identification of the agent. In some assays, serological cross-reactivity with related flaviviruses, such as St. Louis encephalitis virus, USUV virus, Japanese encephalitis virus, or TBE virus may occur. PRNT is the most specific among WNV serological tests; serum antibody titres against other flaviviruses from the same group can be tested in parallel (OIE, 2022).

ELISA has several advantages: it is rapid, sensitive, simple and is easily accessible on the market. For that reason, it is a frequent choice for conducting seroepidemiology studies. However, this type of test does not distinguish between antibodies against different flaviviruses, which can lead to false positive results. Examples of these cross-reactions were found in some of the selected studies, e.g., the overall seroprevalence for WNV in gamebirds detected by epitope-blocking ELISA was 29%, but the prevalence of neutralising antibodies measured by VNT was only 23% (Llorente et al., 2013). WNV neutralisation test is less sensitive than ELISA, which means that serum samples positive to ELISA may have antibodies against WNV at levels undetectable by the neutralisation assay (Jourdain et al., 2011). However, neutralising antibodies against other flaviviruses (BAGV and USUV) were also found (Llorente et al., 2013). In another case in Spain, of six ELISA positive results, only one was confirmed by VNT (Ferraguti et al., 2016). In order to increase the accuracy of pathogen seroprevalence estimates, it is highly recommended that all positive and doubtful results obtained from the ELISA kits can be confirmed by VNT or another test.

The target population was also quite distinct among the studies, with an extraordinarily wide range of species tested. Order was the criterion chosen for analysis. Wetlands and areas near water are often stopover points for many migratory birds, particularly at certain times of the year that coincides with the greatest abundance of mosquitoes. This can influence the infection rates of birds with aquatic habits. Waders, for instance, are even more exposed to mosquito bites, because of their long bare legs (Hubálek et al., 2008; Fereidouni et al., 2011). The Passeriformes order was included in most of the studies here reported, mostly represented by Corvidae family. The role of corvids and birds of prey in the ecology of WNV has been widely documented, including their high susceptibility to

infection and transmission (Angelini et al., 2010; Napp et al., 2011). It would be interesting to evaluate the seroprevalence among long-distance migrants, short-distance migrants and resident bird species, though the lack of homogeneity in the sampling of the selected studies would not give consistent results.

The season and time of year when samples are collected may also have an influence on the prevalence of antibodies detected. The European Centre for Disease Prevention and Control (ECDC) in the EU/EEA and EU-neighbouring countries considers the WNV transmission season to be from June to November. As already reported, after a WNV infection birds usually develop long-term immunity, maintaining circulating antibodies. Age would be another variable to confront with season, because until autumn, juvenile migratory birds have not yet migrated to Africa. Unfortunately, time of the year was not mentioned in the majority of the studies, and accordingly we did not analyse the correlations between months and seroprevalence.

The emergence of viruses in distinct territories, which was previously restricted to Africa, represents a public health threat. Given the important eco-epidemiological role of birds in the transmission of WNV and other flaviviruses, it would be advisable to improve and reinforce surveillance and control programmes. Since most transmitted diseases have zoonotic potential, medical and veterinary staff must work closely together in clinical, public health and research settings, to achieve greater success in prevention and control strategies. Standardised seroprevalence studies are vital to gain insights into the current epidemiological situation of WNV in Europe and to monitor future variations.

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Seroprevalencija virusa Zapadnog Nila u ptica u europskim državama: sistematski pregled

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Groznica Zapadnog Nila je virusna bolest koju prenose komarci, a koja pogađa ptice, konje i ljude prouzročeći asimptomatsku infekciju, blagu groznicu, meningitis, encefalitis i smrt. Cilj je ove studije bio obaviti sistematski pregled za analizu studija seroprevalencije virusa Zapadnog Nila u ptica u europskim državama između 2010. i 2023. godine. Pretražene su tri elektroničke baze podataka – PubMed, ScienceDirect i Scopus – za relevantne publikacije uporabom unaprijed određenih ključnih riječi. Prvotno je pronađeno ukupno 4.872 rada, a 39 rezultata je na kraju uključeno u članak, nakon uklanjanja duplikata i primjene kriterija prihvatljivosti. Savjetuje se daljnji nadzor i epidemiološke studije virusa Zapadnog Nila u Europi, s obzirom na prijetnju koju ova bolest može predstavljati za ljude i životinje.

Ključne riječi: *ptica, Europa, seroprevalencija, virus Zapadnog Nila*