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REVIEW ARTICLE

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Systematic review and meta-analysis of seroprevalence studies of West Nile virus in equids in Europe between 2001 and 2018

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Abstract

There is some evidence that West Nile virus (WNV), which causes encephalomyelitis in equids, is an emerging disease in Europe. The aim of this study was to perform a systematic review and meta-analysis to analyse seroprevalence studies of WNV in equids in European countries between 2001 and 2018. Two electronic databases, PubMed and Scopus, were searched for relevant publications published from 2001 to 2018 using predetermined keywords. A total of 1,484 papers were initially found. After applying the eligibility criteria, 39 papers were finally included in the systematic review. Analysis of 28,089 equids from 16 European countries revealed a pooled seroprevalence of 8% (95% CI 5%–12%, $p < .001$, $I^2 = 99.3\%$) in Europe. The pooled seroprevalence was slightly higher in Mediterranean basin countries than other countries and when calculated for samples collected between 2001 and 2009 compared to 2010 to 2018. Differences in study design (e.g. sampling associated with recent outbreaks of WNV) contributed to a high degree of variability among studies. Further studies with harmonized study design and reporting of the results are recommended to better estimate and monitor European seroprevalence of WNV in equids.

KEYWORDS

equids, Europe, horses, Mediterranean basin, seroprevalence, WNV

1 | INTRODUCTION

West Nile virus (WNV) is a mosquito-borne zoonotic virus from the genus *Flavivirus* and family *Flaviviridae* (Anon, 2017). Its transmission cycle involves birds and mosquitoes, especially from the *Culex* species, which act as vectors of the virus. Several vertebrate species can be infected by the virus, but mammals, particularly humans and equids, are considered 'dead-end' hosts as they do not usually develop sufficiently high levels of viraemia for transmission to blood-feeding mosquitoes (Komar, 2000). The virus was first isolated from a human being in 1937 in the West Nile region of Uganda (Smithburn et al., 1940). Since the first reported case of WNV in horses in Egypt in 1963 (Schmidt & Mansoury, 1963), the disease has expanded in range causing significant human and animal health issues. An example of these are the outbreaks reported in EU member countries since the start of the 2020 transmission season, and as of 10 September 2020, 173 human cases of WNV infection and 15 deaths (Greece, Spain, Italy and Romania) and 60 outbreaks among equids (Spain, Italy, France, Portugal and Germany) have been reported through the European Animal Disease Notification System (ADNS).

Several genetic lineages of the virus have been found, but isolates from lineages 1 and 2 have mainly been responsible for the disease in humans and equids in European countries, with lineage 1 predominant until the mid-2000s (Ciccozzi et al., 2013; Long, 2014). As a neurotropic virus causing encephalomyelitis, clinical signs in horses include ataxia, paralysis of the limbs, prolonged recumbency, muscle fasciculations and abnormal mentation (Long, 2014). The mortality rate in horses has been estimated at 35%–45% (Long, 2014). However, studies suggest that only

around 10% of infected horses present neurological signs (Gardner et al., 2007). For diagnosis, laboratory testing is necessary to confirm the infection as the neurological signs are not pathognomonic for the disease. Treatment is mostly supportive as there are no known effective antiviral medications (Long, 2014). An equine WNV vaccine was first licensed in the United States in 2003, and further types of WNV vaccine have since been approved for use in horses, but an equine WNV vaccine was not licensed for use in Europe until 2009. Due to the low viral titres in horses, *ante-mortem* PCR-based detection of viral RNA is unreliable (Kleiboeker et al., 2004). Therefore, suspected cases of WNV infection are usually confirmed by IgM capture ELISA and/or measuring seroconversion using a plaque reduction neutralization test (PRNT). Equine WNV-specific IgM antibodies are usually detectable from around 8 days post-infection (so most horses with encephalitis test positive at the time that clinical signs are first observed) and remain detectable for up to 3 months (Beck et al., 2017). Neutralizing (IgG) antibodies are detectable in equine serum by 2 weeks post-infection and can persist for more than 1 year. The OIE Terrestrial Manual (OIE, 2018) suggests that IgG indirect and competitive ELISAs, virus neutralization test (VNT) or PRNT are suitable methods for determining prevalence of infection. However, as ELISA methods are less specific, where related flaviviruses co-circulate with WNV, positive results obtained should be confirmed by PRNT or VNT and testing against other flaviviruses in parallel. Other flaviviruses detected in Europe include Bagaza virus (BAGV), louping ill virus (LIV), tick-borne encephalitis virus (TBEV) and Usutu virus (USUV) (Llorente et al., 2015; Long, 2014).

The aim of this study was to conduct a systematic review and meta-analysis to analyse seroprevalence studies of WNV in equids in Europe from the year 2001 to 2018 inclusive, to compare the prevalence in countries of the Mediterranean basin with other European countries and to evaluate the prevalence of two periods: from 2001 to 2009 and from 2010 to 2018.

2 | MATERIALS AND METHODS

2.1 | Search strategy

A systematic search strategy was performed in the databases PubMed and Scopus to identify all published studies reporting the prevalence of WNV in equids in Europe from 1 January 2001 to 20 March 2019 (the date the search was performed). The following keywords and Boolean operators ('AND' and 'OR') were used: (prevalence OR incidence OR frequency OR occurrence OR detection OR identification OR isolation OR characterization OR investigation) AND (WNV OR WNV OR Flavivirus) AND (horse OR equine OR equid OR donkey OR mule OR foal). In Scopus, the search terms were applied to the title, abstract and the keywords. In PubMed, the search terms were applied to all fields. No language restrictions were applied. Retrieved searches were entered into a Microsoft Excel (2018) file.

The reference lists of the selected publications were reviewed manually to identify all potential studies that could have been missed in the two databases.

2.2 | Eligibility criteria

Inclusion criteria were divided into two categories: inclusion criteria related to the literature search and inclusion criteria inherent to the studies. First, the studies had to be published between 1 January 2001 and 20 March 2019 and the full text had to be available in English, Spanish or French. In addition, studies had to be prospective or retrospective serosurveys with animal-level prevalence and animals of the genus *Equus* (excluding zebra) reported, carried out in a European country and have performed a VNT and/or PRNT to confirm the specificity of antibodies detected by ELISA.

Studies were excluded if the titles and abstracts were not relevant to the subject of interest, did not fulfil the above eligibility criteria, had data missing or duplicated data published in another included study.

2.3 | Study selection and data extraction

In the first screening of all searched studies, duplicates were eliminated. The titles and abstracts of all retrieved studies were then independently screened by two authors (MBCM and MB) to identify potentially relevant studies. When the study could not be assessed from the title and abstract, the full text was screened. The full text of the studies retained after the first screening were further scanned independently and in a standardized manner by two authors (MBCM and MB) applying the eligibility criteria.

After the eligibility assessment process, data were extracted independently by two authors (MBCM and MB) and classified in three categories: general data related to the study, data related to the diagnostic techniques and data related to the animals. Any disagreements that arose between the authors were resolved through discussion with a third author (JMD). All the extracted data were summarized in a Microsoft Excel (2018) file.

The general data related to the study were as follows: title, first author's name, name of the journal, year of publication, database where the study was identified (PubMed or Scopus), type of study (i.e. prospective or retrospective serosurvey), language, country and region, sampling protocol (e.g. convenience or random sample), year and season of testing. The data related to the diagnostic techniques were as follows: initial serological test to detect the presence of antibody (immunoglobulin G), type of confirmatory test, strain of the confirmatory test and additional serological tests performed. The data related to the animals included number of equids, mean age, sex, breed, vaccination and clinical signs.

The total number of equids tested and the number testing positive specifically for WNV antibodies (and without a reported history of vaccination) were also extracted independently by two authors

(JMD and OTO) and any disagreement confirmed by a third author (MB).

2.4 | Data analysis and presentation

Statistical meta-analysis of the proportion of WNV antibody-positive animals was conducted and a forest plot generated using *metaprop* in Stata 16 (Nyaga et al., 2014). Subgroup meta-analysis was done for the Mediterranean and non-Mediterranean countries of Europe to investigate whether greater prevalence was reported in Mediterranean countries where the climate is more favourable for mosquitoes. As well, analysis of two balanced 9-year-period subgroups (2001–2009 and 2010–2018) was performed. This allowed the impact of the introduction of a WNV vaccine on seroprevalence studies to be determined. Estimates from individual studies were transformed using the Freeman–Tukey double-arcsine transformation to stabilize the variance. Heterogeneity was assessed using the I-squared statistic (I^2). A funnel plot to assess publication bias was generated and outliers identified using R (R Core Team, 2014).

2.5 | Maintenance of study standard

This study has been performed in accordance with guidelines for meta-analysis of observational studies (MOOSE statement) and preferred reporting items for systematic reviews and meta-analyses (PRISMA statement) (Moher et al., 2015; Stroup et al., 2000).

3 | RESULTS

3.1 | Search results and study selection

From the initial database search, 1,484 potentially relevant publications were identified of which 663 were found in Scopus and 821 in PubMed. After removing the duplicates, the title and abstract of 950 studies were screened. Of the 104 studies that remained, 65 studies were excluded for reasons listed in Figure 1. The lack of a confirmatory test to measure neutralizing antibodies was one of the main reasons for exclusion (n = 13). The other exclusion factors were as follows: review articles, type of study, language other than English, French or Spanish, insufficient data, full text not available, duplicated data, type of study and year of study.

Finally, a total of 39 publications satisfied the inclusion criteria and were included in the systematic review, of which 38 were in English and one in French. Of the 39 publications, 3 were found in Scopus, 8 in PubMed and 28 in both databases. No additional studies that satisfied the inclusion criteria were found in the reference lists of selected studies. Table 1 presents the studies included in the systematic review.

3.2 | Study characteristics

Of the 39 studies included in the systematic review, the majority (n = 36) were prospective serosurveys; 3 studies were retrospective. In 14 studies (35.9%), it was stated that the equine serum samples were taken randomly. It was assumed that in the other studies, convenience samples were obtained. In total, 28,089 equids were tested, of which 375 were donkeys or mules. The prevalence was described only in horses in 34 studies, only in donkeys and mules in one study (García-Bocanegra, Arenas-Montes, Jaén-Téllez, et al., 2012) and in both horses and donkeys in 3 studies (Bosiljka et al., 2013; Ozkul et al., 2013; Raleigh et al., 2012) The mean number of equids sampled in each study was 720 with a wide range (68 to 5,178).

Of the 16 European countries in which studies were conducted, 7 (Albania, Croatia, Spain, France, Italy, Portugal and Turkey) are part of the Mediterranean basin (Figure 2). The highest number of studies was found for Spain (n = 9), followed by France and Serbia (n = 4). Prevalence data were available for both date ranges in the following ten countries: Croatia, Czech Republic, France, Germany, Ireland, Poland, Portugal, Serbia, Spain and Turkey (34 studies). Twelve of the studies were carried out in the first date range (2001–2009) and 19 in the second period (2010–2018). In one study (Raleigh et al., 2012), prevalence data were separated in the two periods of time. There were five studies that started in the first period and finished in the second period and two for which the year(s) of sampling was not specified.

In the majority of studies (n = 24), samples were first screened by ELISA and some or all of the positive-testing samples were confirmed by testing for WNV-specific neutralizing antibodies. In 10 studies, a neutralization test was performed without prior screening by ELISA, and in one study, both ELISA and VNT were used to screen the samples. In three studies, the initial screening test was either agar gel immunodiffusion (AGID), immunofluorescence antibody test (IFAT) or multiplex immuno-assay (MIA). Additional tests performed included western blot and haemagglutination inhibition (HI) test.

The virus strain used for the VNT or PRNT was described in 24 studies. The strain 'Eg101' was used in 12 studies, 'New York (NY99)' in 7 studies and 'Israel 1998 (IS98-ST1)' in one study. These three strains belong to genetic lineage 1. Only five studies used genetic lineage 2 strains: 'Austrian' (n = 3), 'Hungary 578/2010' (n = 1) or unspecified (n = 1). Of the five studies that used genetic lineage 2 strains, one study also used a strain from genetic lineage 1. The remaining studies (n = 15) did not specify the strain used.

In 18 of the 39 studies, samples were additionally screened for neutralizing antibodies to other flaviviruses; TBEV only in 2, USUV only in 6, USUV and TBEV in 9 and Bagaza virus in 1. Positive titres were detected against TBEV or USUV in five and seven studies, respectively. In four studies, some samples had similar titres against both WNV and USUV.

Of the 39 selected studies, only 3 described minimal demographic data (age, sex and breed) and 8 reported whether or not any of the tested equids were vaccinated or showed any clinical signs.

More than half of the studies (n = 23) reported the season of year when the animals were sampled; the majority of the studies were carried out in autumn (September to November).

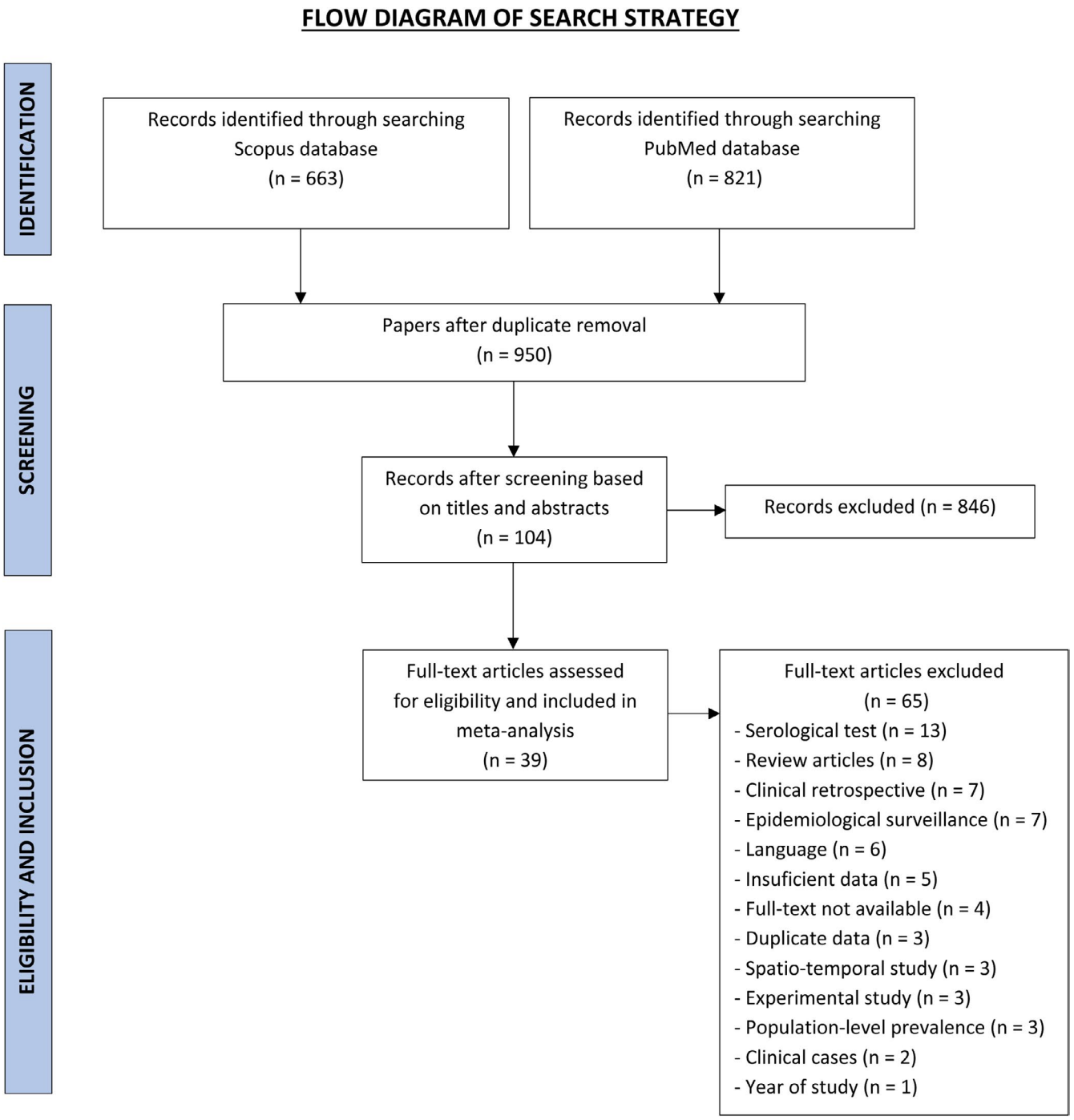

FIGURE 1 Flow diagram of article selection for West Nile prevalence in equids in Europe [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | Meta-analysis of WNV seroprevalence

The pooled seroprevalence was 8% (95% CI 5%–12%) with substantial heterogeneity (I^2 = 99.3%) (Figure 3). Pooled seroprevalence was slightly higher in Mediterranean (9%, 95% CI 5%–14%) than non-Mediterranean countries (7%, 95% CI 3%–14%) and in the first sampling period (8%, 95% CI 2%–17%) than in the second sampling period (7%, 95% CI 4%–10%) (Table 2). A funnel plot (Figure 4) did not identify significant publication bias. However, two studies (Calistri et al., 2010; Petrović et al., 2014) were identified as outliers in the studentized residual test.

4 | DISCUSSION

This systematic review sought to highlight important trends in WNV seroprevalence in equids in Europe; prevalence data of WNV reported in 39 studies of equids in Europe were analysed from the

TABLE 1 Characteristics of studies included in the systematic review

Publication	Country[a]	No. positive	No. tested	Seroprevalence (%)	Year	Screening test	Tests for other flaviviruses
1. Abad-Cobo et al. (2017)	Spain (ES)	5	369	1.36	2011–2013	ELISA	USUV
2. Alba et al. (2014)	Spain (ES)	0	178	0	2011	ELISA	
3. Bakonyi et al. (2013)	Hungary (HU)	79	276	28.62	2009	IFAT	
4. Barbić et al. (2012)	Croatia (HR)	72	2098	3.43	2010–2011	ELISA	TBEV + USUV
5. Barbić et al. (2013)	Croatia (HR)	48	1,380	3.48	2011	ELISA	TBEV + USUV
6. Barros et al. (2011)	Portugal (PT)	40	1,313	3.05	2004–2010	ELISA	
7. Barros et al. (2017)	Portugal (PT)	18	989	1.82	2011–2016	ELISA	
8. Bażanów et al. (2018)	Poland (PL)	62	411	15.09	2012–2013	VNT	USUV
9. Berxholi et al. (2013)	Albania (AL)	37	167	22.16	*N.S.*	ELISA & VNT	TBEV
10. Bosiljka et al. (2013)	Serbia (RS)	45	1199[b]	3.75	2008–2012	AGID	
11. Busani et al. (2011)	Italy (IT)	348	2,528	13.77	2008 & 2009	ELISA	TBEV + USUV
12. Busquets et al. (2019)	Spain (ES)	9	138	6.52	2017 & 2018	ELISA	BAGV
13. Cabre et al. (2005)	France (FR)	0	94	0	2003	ELISA	
14. Calistri et al. (2010)	Italy (IT)	794	2030	39.11	2008	PRNT	
15. Csank et al. (2018)	Slovakia (SK)	10	145	6.90	2013	ELISA	TBEV + USUV
16. Durand et al. (2005)	France (FR)	304	906	33.55	2003	ELISA	
17. Ergunay et al. (2014)	Turkey (TR)	48	389	12.34	2011–2013	PRNT	
18. García-Bocanegra, et al. (2012)	Spain (ES)	36	510	7.06	2010	ELISA	
19. García-Bocanegra, et al. (2012)	Spain (ES)	12	109	11.01	2010–2011	ELISA	
20. García-Bocanegra, et al. (2012)	Spain (ES)	12	165[c]	7.27	2011	ELISA	
21. Hubálek et al. (2008)	Poland (PL)	0	78	0	2006	PRNT	USUV
22. Hubálek et al. (2013)	Czechia (CZ) & Slovakia (SK)	19	395	4.81	2008–2011	PRNT	TBEV + USUV
23. Jiménez-Clavero et al. (2007)	Spain (ES)	13	157	8.28	2005	VNT	USUV
24. Jiménez-Clavero et al. (2010)	Spain (ES)	0	68[f]	0	2008	VNT	USUV
25. Lupulovic et al. (2011)	Serbia (RS)	42	349	12.03	2009–2010	ELISA	USUV
26. Madić et al. (2007)	Croatia (HR)	4	980	0.41	2010–2011	ELISA	
27. Maquart et al. (2017)	France (FR)	9	96	9.38	2014	ELISA	USUV
28. Medić et al. (2014)	Serbia (RS)	72	252	28.57	2007–2011	ELISA	
29. Monaco et al. (2010)	Italy (IT)	271	770	35.19	2008	VNT	TBEV + USUV
30. Ozkul et al. (2006)	Turkey (TR)	36	299[d]	12.04	*N.S.*	PRNT	
31. Ozkul et al. (2013)	Turkey (TR)	57	180	31.67	2011	PRNT	
32. Petrović et al. (2014)	Serbia (RS)	64	130	49.23	2012	ELISA	
33. Pradier et al. (2014)	France (FR)	143	1,159	12.34	2007–2008	ELISA	
34. Raleigh et al. (2012)	Ireland (IE)	0	490[e]	0	2005–2006 ($n = 90$) & 2010 ($n = 400$)	ELISA	
35. Vanhomwegen et al. (2017)	Spain (ES)	11	172	6.40	2011–2012	MIA	TBEV + USUV
36. Weissenböck et al. (2003)	Austria (AT)	0	350	0	2001	PRNT	
37. Ziegler et al. (2012)	Germany (DE)	1	1,282	0.08	2007–2009	ELISA	TBEV + USUV

(Continues)

TABLE 1 (Continued)

Publication	Country[a]	No. positive	No. tested	Seroprevalence (%)	Year	Screening test	Tests for other flaviviruses
38. Ziegler, et al. (2013)	Germany (DE)	2	5,178	0.04	2010–2012	ELISA	TBEV + USUV
39. Ziegler, et al. (2013)	Ukraine (UA)	42	310	13.55	2010–2011	ELISA	TBEV

Abbreviations: AGID, agar gel immunodiffusion; BAGV, Bagaza virus; ELISA, enzyme-linked immunosorbent assay; IFAT, immunofluorescence antibody test; MIA, multiplex immuno-assay; *N.S.*, not specified; PRNT, plaque reduction test; TBEV, tick-borne encephalitis virus; USUV, Usutu virus; VNT, virus neutralization test.

[a]Two-letter ISO country code.

[b]1133 horses and 66 donkeys.

[c]82 donkeys and 83 mules.

[d]259 horses and 40 mules.

[e]386 horses and 104 donkeys.

[f]Only results from samples collected in 2008 were included in the meta-analysis.

FIGURE 2 Map showing European countries for which data were included in the systematic review. Created using https://mapchart.net/europe.html with different colour shading used for Mediterranean and non-Mediterranean countries and depth of shading indicating number of studies performed in each country [Colour figure can be viewed at wileyonlinelibrary.com]

year 2001 to the year 2018. The pooled seroprevalence obtained was 8% (95% CI 5%–12%). However, few studies reported using random sampling methods; therefore, caution must be applied when generalizing the seroprevalence estimates to the target population. The substantial heterogeneity (I^2 = 99.3%) meant that meaningful conclusions could not be drawn about differences in seroprevalence between Mediterranean and non-Mediterranean countries or the two periods evaluated.

There was no evidence that small studies with small effect sizes were missing. However, the two studies that were identified as outliers (Calistri et al., 2010; Petrović et al., 2014) were also the two studies with the highest seroprevalence: 39% and 49%, respectively.

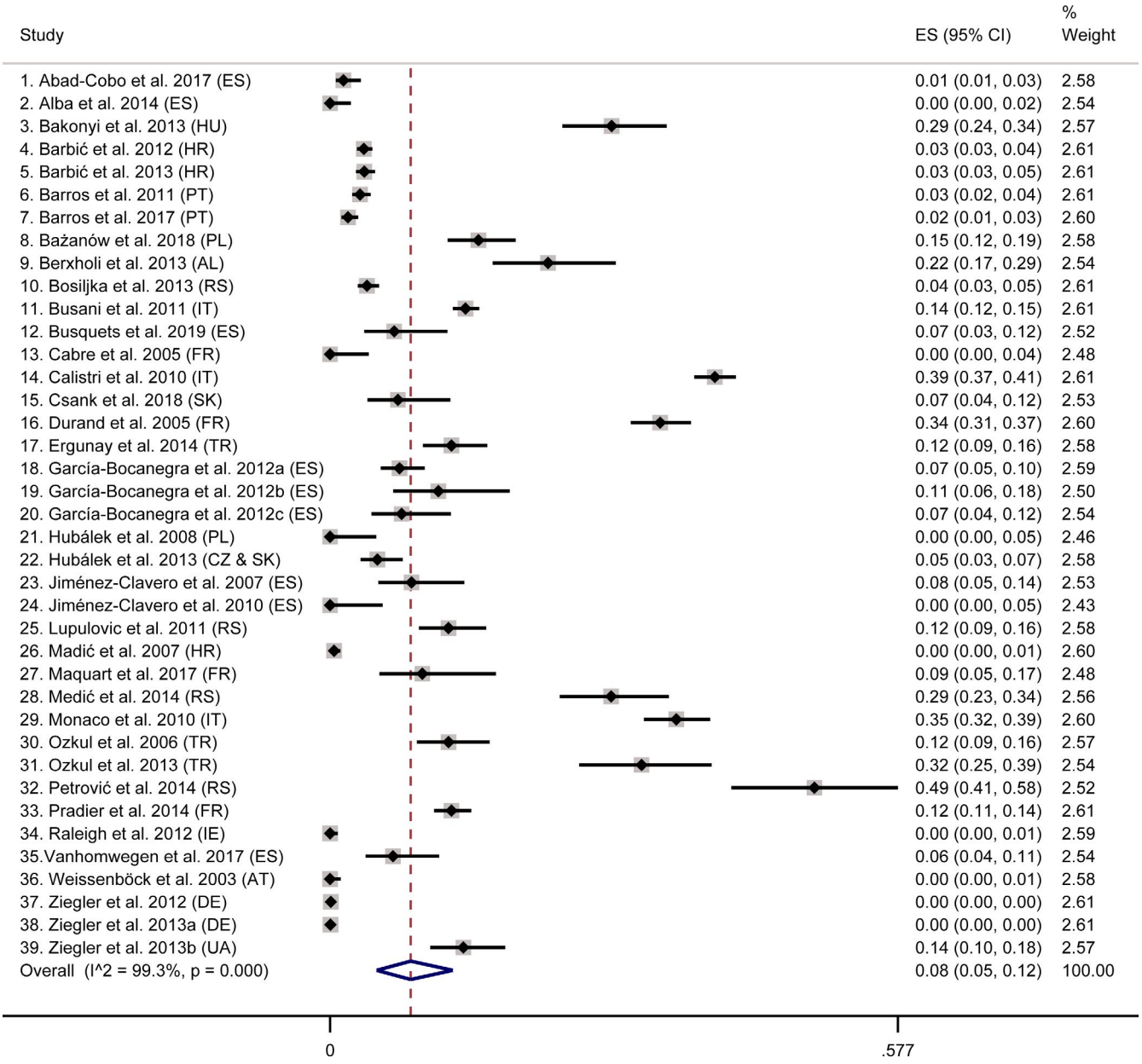

FIGURE 3 Forest plot showing the pooled estimated seroprevalence (ES) of West Nile virus among equids in Europe. Horizontal lines represent 95% confidence intervals (CIs). Each square box denotes the seroprevalence rate point estimate, and the area is proportional to the weight of the study [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Pooled seroprevalence of WNV in Europe

		No. positive	No. tested	% (95% CI)	D.F.	I^2 (%)
Region	Mediterranean	2,327	17,244	9 (5–14)	24	99.1
	Non-Mediterranean	438	10,845	7 (3–14)	13	99.0
Sampling period	2000–2009	1953	9,788	8 (2–17)	12	99.4
	2010–2018	521	14,327	7 (4–10)	19	98.3
Overall		2,765	28,089	8 (5–12)	38	99.3

The study by Calistri et al. (2010) was associated with investigation of an outbreak of WNV. Similarly, in the study by Petrović et al. (2014), samples were collected from horses in November and December 2012 after the first human outbreak of WNV reported in Serbia, which started in August 2012. Other studies with high seroprevalence were also associated with recent outbreaks.

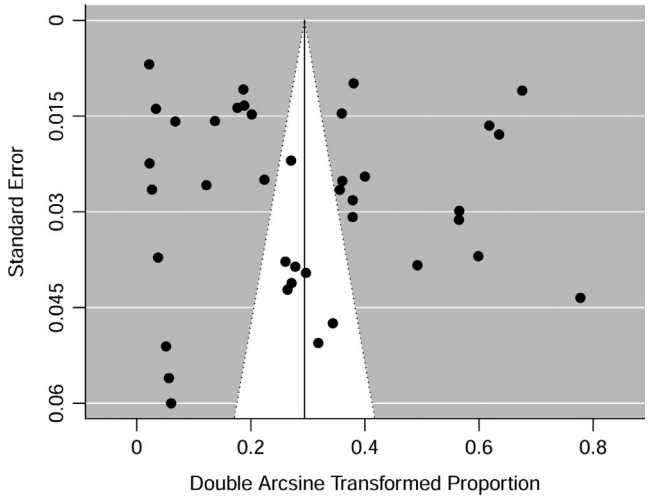

FIGURE 4 Funnel plot of standard error by Freeman–Tukey double-arcsine-transformed proportion for all studies ($n = 39$)

The quality of data reporting varied between studies, for example, only three studies provided information on animal characteristics such as age, sex and breed, each of which could influence risk of exposure to and/or susceptibility to WNV infection, for example, older animals are more likely to have been exposed to virus. Reporting these demographic data is important for comparisons to be made between the outcomes of different studies. Recruitment criteria are important in understanding disease transmission in mobile animal populations such as horses where animals may have been exposed to the virus somewhere other than the study location. Furthermore, vaccination status became important after an equine WNV vaccine was first licensed in Europe in 2009. For example, Ziegler et al. (2012) found four samples positive for WNV antibodies in a study conducted in Germany, but three of these were from vaccinated horses (and were therefore removed from the seroprevalence estimation in this study) and one was from a horse from Hungary. However, vaccination status was only reported in 8 studies although 19 were conducted on samples collected after 2009.

ELISA is often the assay of choice for conducting seroepidemiological studies because it is simple, sensitive, rapid and often commercially available. However, due to extensive cross-reactivity between antibodies raised against different flaviviruses, the ELISA can yield false-positive results where different flaviviruses co-circulate (Beck et al., 2013). Therefore, this systematic review only included studies that used virus/plaque reduction neutralization tests to confirm positive samples; however, 21 out of 39 studies did not test other flaviviruses in parallel, which could have introduced errors in their prevalence estimation. The issue of cross-reactivity in ELISA was illustrated in some of the studies, for example Berxholi et al. (2013) found that two of seven samples that were positive in ELISA but negative in WNV VNT were positive for TBEV antibodies. Similarly, Ziegler, et al. (2013) found that four samples that were positive by ELISA but negative by WNV VNT were positive for TBEV (but not for USUV). One of the included studies (Lupulovic et al., 2011) was the first to report neutralizing antibodies to USUV in horses; however, as the PRNT titres were 120 and 90 for WNV and USUV, respectively, they were not able to conclude whether this represented cross-reactive antibodies or prior exposure to both viruses. Calistri et al. (2010) mention that USUV was circulating in Italy in the year before samples were obtained in their study, but they did not test for USUV antibodies. In most cases, neutralization tests were positive for one virus only or titres were markedly higher (e.g. at least twofold) for one virus. However, neutralization tests were not always discriminatory, particularly where VNT titres were low (Jiménez-Clavero et al., 2007). Furthermore, Vanhomwegen et al. (2017) concluded that of 21 samples that were positive for flavivirus antibodies, 11 were specifically positive for WNV, 2 for USUV and 1 for TBEV, while 8 were positive for an unidentified flavivirus (1 of which they reported as positive for both WNV and an unidentified flavivirus). Ziegler, et al. (2013) reported four samples that were positive by WNV ELISA but VNT negative with WNV and USUV. Although this could be due to the lower sensitivity of VNT compared to ELISA (Beck et al., 2017). Furthermore, a high prevalence of tick-borne encephalitis virus (TBEV) has been reported in other studies in some European countries (Rushton et al., 2013). Therefore, there is evidence of non-WNV flaviviruses circulating in Europe, which should be taken into account when performing a serosurvey study.

Although most studies did not specify if seropositivity was caused by lineage 1 or 2 WNV strains, this is probably not critical, because it is not possible to tell by which virus lineage the immune response was elicited as the two strains differ only in three amino acids within domain III of the E protein, against which most neutralizing antibodies are directed (Berxholi et al., 2013).

Most studies were performed in autumn, probably after the beginning of the transmission season and occurrence of outbreaks in the summer. This should not affect the results of the meta-analysis other than the already mentioned bias caused by association of a serosurvey study after regional outbreaks, which could falsely increase the prevalence estimation.

Despite the fact that WNV transmission and outbreaks in equids occur in EU countries, the limitations detected in this study precluded the evaluation of an increase in seroprevalence over time. An analysis of the number of reported outbreaks could be useful to determine the re-emerging status of this virus; however, this was not the scope of this study.

Horses have been suggested as useful sentinels for WNV surveillance. However, the true seroprevalence of WNV in European equids remains uncertain due to variation in study design and reporting, and difficulty discriminating between cross-reactive antibodies. Standardized seroprevalence studies are critical to better understand the current epidemiological status of WNV in Europe and to monitor future changes.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No

ethical approval was required as this is a review article with no original research data.

CONFLICT OF INTEREST

No conflict of interest to declare by the authors.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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REFERENCES

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

Alba, A., Allepuz, A., Napp, S., Soler, M., Selga, I., Aranda, C., & Busquets, N. (2014). Ecological surveillance for West Nile in Catalonia (Spain), learning from a five-year period of follow-up. *Zoonoses Public Health*, *61*(3), 181–191. https://doi.org/10.1111/zph.12048

Anon (2017). Chapter 29 - Flaviviridae. In N. J. MacLachlan, & E. J. Dubovi (Eds.), *Fenner's Veterinary Virology*, 5th ed. (pp. 525–545). Academic Press.

Bakonyi, T., Ferenczi, E., Erdélyi, K., Kutasi, O., Csörgő, T., Seidel, B., & Nowotny, N. (2013). Explosive spread of a neuroinvasive lineage 2 West Nile virus in Central Europe, 2008/2009. *Veterinary Microbiology*, *165*(1–2), 61–70. https://doi.org/10.1016/j.vetmic.2013.03.005

Barbić, L., Listeš, E., Katić, S., Stevanović, V., Madić, J., Starešina, V., & Savini, G. (2012). Spreading of West Nile virus infection in Croatia. *Veterinary Microbiology*, *159*(3–4), 504–508. https://doi.org/10.1016/j.vetmic.2012.04.038

Barbić, L., Vilibić-Čavlek, T., Listeš, E., Stevanović, V., Gjenero-Margan, I., Ljubin-Sternak, S., & Savini, G. (2013). Demonstration of Usutu virus antibodies in horses. *Croatia. Vector Borne Zoonotic Dis*, *13*(10), 772–774. https://doi.org/10.1089/vbz.2012.1236

Barros, S. C., Ramos, F., Fagulha, T., Duarte, M., Henriques, A. M., Waap, H., & Fevereiro, M. (2017). West Nile virus in horses during the summer and autumn seasons of 2015 and 2016, Portugal. *Veterinary Microbiology*, *212*, 75–79. https://doi.org/10.1016/j.vetmic.2017.11.008

Barros, S. C., Ramos, F., Fagulha, T., Duarte, M., Henriques, M., Luís, T., & Fevereiro, M. (2011). Serological evidence of West Nile virus circulation in Portugal. *Veterinary Microbiology*, *152*(3–4), 407–410. https://doi.org/10.1016/j.vetmic.2011.05.013

Bażanów, B., Jansen van Vuren, P., Szymański, P., Stygar, D., Fracka, A., Twardón, J., & Pawęska, J. T. (2018). A survey on West Nile and Usutu viruses in horses and birds in Poland. *Viruses*, *10*(2), https://doi.org/10.3390/v10020087

Beck, C., Jiménez-Clavero, M. A., Leblond, A., Durand, B., Nowotny, N., Leparc-Goffart, I., & Lecollinet, S. (2013). Flaviviruses in Europe: Complex circulation patterns and their consequences for the diagnosis and control of West Nile disease. *Int J Environ Res Public Health*, *10*(11), 6049–6083. https://doi.org/10.3390/ijerph10116049

Beck, C., Lowenski, S., Durand, B., Bahuon, C., Zientara, S., & Lecollinet, S. (2017). Improved reliability of serological tools for the diagnosis of West Nile fever in horses within Europe. *PLoS Neglected Tropical Diseases*, *11*(9), e0005936. https://doi.org/10.1371/journal.pntd.0005936

Berxholi, K., Ziegler, U., Rexhepi, A., Schmidt, K., Mertens, M., Korro, K., & Groschup, M. H. (2013). Indigenous West Nile virus infections in horses in Albania. *Transbound Emerg Dis*, *60*(Suppl 2), 45–50. https://doi.org/10.1111/tbed.12141

Bosiljka, D., Vasić, A., Rogožarski, D., Vojinović, D., Elezović-Radovanović, M., Manić, M., & Gligić, A. (2013). Seroepizootiological-epidemiological investigation and mapping of West Nile infection in the republic of Serbia. *Acta Veterinaria*, *63*(5–6), 569–579. https://doi.org/10.2298/AVB1306569D

Busani, L., Capelli, G., Cecchinato, M., Lorenzetto, M., Savini, G., Terregino, C., & Marangon, S. (2011). West Nile virus circulation in Veneto region in 2008–2009. *Epidemiology and Infection*, *139*(6), 818–825. https://doi.org/10.1017/S0950268810001871

Busquets, N., Laranjo-González, M., Soler, M., Nicolás, O., Rivas, R., Talavera, S., & Napp, S. (2019). Detection of West Nile virus lineage 2 in North-Eastern Spain (Catalonia). *Transboundary and Emerging Diseases*, *66*(2), 617–621. https://doi.org/10.1111/tbed.13086

Cabre, O., Durand, J. P., Prangé, A., Gomez, J., Maurizi, L., Tolou, H., & Davoust, B. (2005). West Nile virus infection: Serological investigation among horses in France and in Africa. *Médecine Tropicale : Revue Du Corps De Santé Colonial*, *65*(5), 439–443.

Calistri, P., Giovannini, A., Savini, G., Monaco, F., Bonfanti, L., Ceolin, C., & Lelli, R. (2010). West Nile virus transmission in 2008 in north-eastern Italy. *Zoonoses and Public Health*, *57*(3), 211–219. https://doi.org/10.1111/j.1863-2378.2009.01303.x

Ciccozzi, M., Peletto, S., Cella, E., Giovanetti, M., Lai, A., Gabanelli, E., & Zehender, G. (2013). Epidemiological history and phylogeography of West Nile virus lineage 2. *Infect Genet Evol*, *17*, 46–50. https://doi.org/10.1016/j.meegid.2013.03.034

Csank, T., Drzewnioková, P., Korytár, L., Major, P., Gyuranecz, M., Pistl, J., & Bakonyi, T. (2018). A Serosurvey of Flavivirus Infection in Horses and Birds in Slovakia. *Vector Borne Zoonotic Dis*, *18*(4), 206–213. https://doi.org/10.1089/vbz.2017.2216

Durand, B., Dauphin, G., Zeller, H., Labie, J., Schuffenecker, I., Murri, S., & Zientara, S. (2005). Serosurvey for West Nile virus in horses in southern France. *The Veterinary Record*, *157*(22), 711–713. https://doi.org/10.1136/vr.157.22.711

Ergunay, K., Gunay, F., Erisoz Kasap, O., Oter, K., Gargari, S., Karaoglu, T., & Ozkul, A. (2014). Serological, molecular and entomological surveillance demonstrates widespread circulation of West Nile virus in Turkey. *PLoS Neglected Tropical Diseases*, *8*(7), e3028. https://doi.org/10.1371/journal.pntd.0003028

García-Bocanegra, I., Arenas-Montes, A., Jaén-Téllez, J. A., Napp, S., Fernández-Morente, M., & Arenas, A. (2012). Use of sentinel serosurveillance of mules and donkeys in the monitoring of West Nile virus infection. *The Veterinary Journal*, *194*(2), 262–264. https://doi.org/10.1016/j.tvjl.2012.04.017

García-Bocanegra, I., Arenas-Montes, A., Napp, S., Jaén-Téllez, J. A., Fernández-Morente, M., Fernández-Molera, V., & Arenas, A. (2012). Seroprevalence and risk factors associated to West Nile virus in horses from Andalusia. *Southern Spain. Vet Microbiol*, *160*(3–4), 341–346. https://doi.org/10.1016/j.vetmic.2012.06.027

García-Bocanegra, I., Jaén-Téllez, J. A., Napp, S., Arenas-Montes, A., Fernández-Morente, M., Fernández-Molera, V., & Arenas, A. (2012). Monitoring of the West Nile virus epidemic in Spain between 2010 and 2011. *Transbound Emerg Dis*, *59*(5), 448–455. https://doi.org/10.1111/j.1865-1682.2011.01298.x

Gardner, I. A., Wong, S. J., Ferraro, G. L., Balasuriya, U. B., Hullinger, P. J., Wilson, W. D., & MacLachlan, N. J. (2007). Incidence and effects of West Nile virus infection in vaccinated and unvaccinated horses in California. *Veterinary Research*, *38*(1), 109–116. https://doi.org/10.1051/vetres:2006045

Hubálek, Z., Ludvíková, E., Jahn, P., Treml, F., Rudolf, I., Svobodová, P., & Staššíková, Z. (2013). West Nile Virus equine serosurvey in the Czech and Slovak republics. *Vector Borne Zoonotic Dis*, *13*(10), 733–738. https://doi.org/10.1089/vbz.2012.1159

Hubálek, Z., Wegner, E., Halouzka, J., Tryjanowski, P., Jerzak, L., Šikutová, S., & Wlodarczyk, R. (2008). Serologic survey of potential vertebrate hosts for West Nile virus in Poland. *Viral Immunology*, *21*(2), 247–253. https://doi.org/10.1089/vim.2007.0111

Jiménez-Clavero, M. A., Gómez-Tejedor, C., Rojo, G., Soriguer, R., & Figuerola, J. (2007). Serosurvey of West Nile virus in equids and bovids in Spain. *The Veterinary Record*, *161*(6), 212. https://doi.org/10.1136/vr.161.6.212

Jiménez-Clavero, M. A., Llorente, F., Sotelo, E., Soriguer, R., Gómez-Tejedor, C., & Figuerola, J. (2010). West Nile virus serosurveillance in horses in Donana, Spain, 2005 to 2008. *The Veterinary Record*, *167*(10), 379–380. https://doi.org/10.1136/vr.c3155

Kleiboeker, S. B., Loiacono, C. M., Rottinghaus, A., Pue, H. L., & Johnson, G. C. (2004). Diagnosis of West Nile virus infection in horses. *Journal of Veterinary Diagnostic Investigation*, *16*(1), 2–10. https://doi.org/10.1177/104063870401600102

Komar, N. (2000). West Nile viral encephalitis. *Rev. Sci. Tech. off. Int. Epiz.*, *19*, 166–176. https://doi.org/10.20506/rst.19.1.1201

Llorente, F., Pérez-Ramírez, E., Fernández-Pinero, J., Elizalde, M., Figuerola, J., Soriguer, R. C., & Jiménez-Clavero, M. Á. (2015). Bagaza virus is pathogenic and transmitted by direct contact in experimentally infected partridges, but is not infectious in house sparrows and adult mice. *Veterinary Research*, *46*(1), 93. https://doi.org/10.1186/s13567-015-0233-9

Long, M. T. (2014). West Nile virus and equine encephalitis viruses: New perspectives. *The Veterinary Clinics of North America. Equine Practice*, *30*(3), 523–542. https://doi.org/10.1016/j.cveq.2014.08.009

Lupulovic, D., Martín-Acebes, M. A., Lazic, S., Alonso-Padilla, J., Blázquez, A. B., Escribano-Romero, E., & Saiz, J. C. (2011). First serological evidence of West Nile virus activity in horses in Serbia. *Vector Borne Zoonotic Dis*, *11*(9), 1303–1305. https://doi.org/10.1089/vbz.2010.0249

Madić, J., Savini, G., Di Gennaro, A., Monaco, F., Jukić, B., Kovač, S., & Listeš, E. (2007). Serological evidence for West Nile virus infection in horses in Croatia. *The Veterinary Record*, *160*(22), 772–773. https://doi.org/10.1136/vr.160.22.772

Maquart, M., Dahmani, M., Marié, J. L., Gravier, P., Leparc-Goffart, I., & Davoust, B. (2017). First serological evidence of West Nile virus in horses and dogs from Corsica Island. *France. Vector Borne Zoonotic Dis*, *17*(4), 275–277. https://doi.org/10.1089/vbz.2016.2024

Medić, S., van den Hoven, R., Petrović, T., Lupulović, D., & Nowotny, N. (2014). Serological evidence of West Nile virus infection in the horse population of northern Serbia. *J Infect Dev Ctries*, *8*(7), 914–918. https://doi.org/10.3855/jidc.3885

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., & Stewart, L. A. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews*, *4*(1), 1. https://doi.org/10.1186/2046-4053-4-1

Monaco, F., Lelli, R., Teodori, L., Pinoni, C., Di Gennaro, A., Polci, A., & Savini, G. (2010). Re-emergence of West Nile virus in Italy. *Zoonoses Public Health*, *57*(7–8), 476–486. https://doi.org/10.1111/j.1863-2378.2009.01245.x

Nyaga, V. N., Arbyn, M., & Aerts, M. (2014). Metaprop: A Stata command to perform meta-analysis of binomial data. *Archives of Public Health*, *72*(1), 39. https://doi.org/10.1186/2049-3258-72-39

OIE (2018). *West Nile fever. In* OIE Terrestrial Manual, 8th ed. (pp. 697–710). OIE.

Ozkul, A., Ergunay, K., Koysuren, A., Alkan, F., Arsava, E. M., Tezcan, S., & Us, D. (2013). Concurrent occurrence of human and equine West Nile virus infections in Central Anatolia, Turkey: The first evidence for circulation of lineage 1 viruses. *International Journal of Infectious Diseases*, *17*(7), e546–551. https://doi.org/10.1016/j.ijid.2013.02.005

Ozkul, A., Yildirim, Y., Pinar, D., Akcali, A., Yilmaz, V., & Colak, D. (2006). Serological evidence of West Nile Virus (WNV) in mammalian species in Turkey. *Epidemiology and Infection*, *134*(4), 826–829. https://doi.org/10.1017/S0950268805005492

Petrović, T., Lazić, S., Lupulović, D., Lazić, G., Bugarski, D., Vidanović, D., & Petrić, D. (2014). Serological study on WNV presence in horses in Vojvodina after the human outbreak in Serbia in 2012. *Archives of Biological Sciences*, *66*, 473–481. https://doi.org/10.2298/ABS1402473P

Pradier, S., Sandoz, A., Paul, M. C., Lefebvre, G., Tran, A., Maingault, J., & Leblond, A. (2014). Importance of wetlands management for West Nile Virus circulation risk, Camargue, Southern France. *Int J Environ Res Public Health*, *11*(8), 7740–7754. https://doi.org/10.3390/ijerph110807740

R Core Team (2014). *R: A language and environment for statistical computing*. Retrieved from http://www.R-project.org/.

Raleigh, P. J., Sammin, D. J., Connell, J., Markey, B. K., & O'Connor, M. (2012). Surveillance for antibodies to West Nile virus in Ireland. *The Veterinary Record*, *170*(7), 180. https://doi.org/10.1136/vr.100333

Rushton, J. O., Lecollinet, S., Hubálek, Z., Svobodová, P., Lussy, H., & Nowotny, N. (2013). Tick-borne Encephalitis virus in horses, Austria, 2011. *Emerg Infect Dis*, *19*(4), 635–637. https://doi.org/10.3201/eid1904.121450

Schmidt, J. R., & Mansoury, H. K. E. (1963). Natural and experimental infection of Egyptian Equines with West Nile Virus. *Annals of Tropical Medicine & Parasitology*, *57*(4), 415–427. https://doi.org/10.1080/00034983.1963.11686194

Smithburn, K. C., Hughes, T. P., Burke, A. V., & Paul, J. H. (1940). A neurotropic virus isolated from the blood of a native of Uganda. *American Journal of Tropical Medicine and Hygiene*, *20*, 471–492.

Stroup, D. F., Berlin, J. A., Morton, S. C., Olkin, I., Williamson, G. D., Rennie, D., & Thacker, S. B. (2000). Meta-analysis of observational studies in epidemiology: A proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA*, *283*(15), 2008–2012. https://doi.org/10.1001/jama.283.15.2008

Vanhomwegen, J., Beck, C., Desprès, P., Figuerola, A., García, R., Lecollinet, S., & Serra-Cobo, J. (2017). Circulation of zoonotic arboviruses in equine populations of Mallorca Island (Spain). *Vector Borne Zoonotic Dis*, *17*(5), 340–346. https://doi.org/10.1089/vbz.2016.2042

Weissenböck, H., Hubálek, Z., Halouzka, J., Pichlmair, A., Maderner, A., Fragner, K., & Nowotny, N. (2003). Screening for West Nile virus infections of susceptible animal species in Austria. *Epidemiology and Infection*, *131*(2), 1023–1027. https://doi.org/10.1017/s0950268803001031

Ziegler, U., Angenvoort, J., Klaus, C., Nagel-Kohl, U., Sauerwald, C., Thalheim, S., & Groschup, M. H. (2013). Use of competition ELISA for monitoring of West Nile virus infections in horses in Germany. *Int J Environ Res Public Health*, *10*(8), 3112–3120. https://doi.org/10.3390/ijerph10083112

Ziegler, U., Seidowski, D., Angenvoort, J., Eiden, M., Müller, K., Nowotny, N., & Groschup, M. H. (2012). Monitoring of West Nile virus infections in Germany. *Zoonoses Public Health*, *59*(Suppl 2), 95–101. https://doi.org/10.1111/zph.12015

Ziegler, U., Skrypnyk, A., Keller, M., Staubach, C., Bezymennyi, M., Damiani, A. M., & Groschup, M. H. (2013). West Nile virus antibody prevalence in horses of Ukraine. *Viruses*, *5*(10), 2469–2482. https://doi.org/10.3390/v5102469

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