Received: 16 January 2024 | Revised: 10 July 2024 | Accepted: 12 July 2024

DOI: 10.1111/zph.13171

ORIGINAL ARTICLE

WILEY

Orthoflavivirus surveillance in the Netherlands: Insights from a serosurvey in horses & dogs and a questionnaire among horse owners

Kiki Streng[1] | Renate W. Hakze-van der Honing[2] | Heather Graham[2] | Sophie van Oort[2] | Pauline A. de Best[3,4] | Ayat Abourashed[3,5] | Wim H. M. van der Poel[1,2]

[1]Quantitative Veterinary Epidemiology, Wageningen University and Research, Wageningen, The Netherlands

[2]Wageningen Bioveterinary Research, Wageningen University and Research, Lelystad, The Netherlands

[3]Viroscience, Erasmus MC, Rotterdam, The Netherlands

[4]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

[5]Centre for Advances Studies of Blanes, Blanes, Spain

Correspondence
Kiki Streng, Quantitative Veterinary Epidemiology, Wageningen University and Research, Wageningen, The Netherlands.
Email: kiki.streng@wur.nl

Abstract

Aims: Zoonotic arboviruses (*ar*thropod-*bo*rne) of the *Orthoflavivirus* genus, such as West Nile virus (WNV), Usutu virus (USUV) and Tick-borne encephalitis virus (TBEV), are emerging in Northwestern Europe and pose a threat to both human and animal health. In the Netherlands, passive symptomatic surveillance (notification of clinical cases) in horses is one of the main pillars for the early detection of WNV. For such passive surveillance to work properly, horse owners and veterinarians need to recognize symptoms and report suspected cases to the authorities. Currently, little is known about the seroprevalence of orthoflaviviruses in domestic animals in the Netherlands. Therefore, this study aims at identifying the seroprevalence of WNV and USUV in horses and dogs in the Netherlands. Additionally, this study seeks to evaluate the knowledge and perceptions of Dutch horse owners towards mosquito-borne viruses.

Methods and Results: A cross-sectional serosurvey in horses and dogs was conducted between May 2021 and May 2022. Serum samples were screened using an ELISA and doubtful and positive samples were confirmed by Virus Neutralization Tests for WNV, USUV and TBEV. A validated questionnaire, the MosquitoWise survey, was used to assess the knowledge and perceptions of Dutch horse owners towards mosquito-borne viruses between July and October 2022. The serosurvey revealed a low seroprevalence for WNV in horses and no WNV-positive dogs were found. Similarly, a low USUV seroprevalence was found in dogs. The MosquitoWise survey revealed a high knowledge level for horse owners and high awareness of WNV vaccination but a more limited intent to vaccinate.

Conclusions: The low seroprevalences of WNV and USUV indicate many dogs and horses remain susceptible, offering opportunities for trend analysis and surveillance. However, despite multiple recent detections of WNV, USUV, and TBEV in humans, the role of dogs and horses in early detection of human cases is debatable. High awareness among horse owners and the absence of detected equine WNV cases highlight this uncertainty. Continued surveillance is crucial for detecting increased virus circulation and protecting both animal and human health.

KEYWORDS
dogs, health belief model, horses, survey, Usutu virus, West Nile virus

1 | INTRODUCTION

Zoonotic arboviruses (*ar*thropod-*bo*rne) of the *Orthoflavivirus* genus are emerging in Northwestern Europe and pose a threat to human and animal health. Three orthoflaviviruses have been detected in the Netherlands so far: Usutu virus (USUV), West Nile virus (WNV) and Tick-borne encephalitis virus (TBEV) (Beck et al., 2013). The viruses are maintained in a cycle of enzootic circulation between mosquitoes and birds in the case of USUV and WNV and between ticks and mammals in the case of TBEV (Beck et al., 2013). Spill-over to dead-end hosts can occur through bites of infected vectors. Although infection often goes unnoticed in animals, clinical disease has been reported in birds (USUV and WNV), horses (WNV and sporadically for USUV and TBEV) and dogs (WNV and TBEV) (Hubálek et al., 2014). In humans, WNV and TBEV infections generally cause mild, flu-like symptoms but can develop into severe neurological disease in a small percentage of cases. In contrast, USUV infection rarely leads to severe human disease (Beck et al., 2013).

Mosquito-borne orthoflaviviruses have been present in Europe for over two decades. USUV was first detected in Austria in 2001 and, retrospectively, found in Italy in samples from 1996 (Weissenböck et al., 2013). WNV is one of the most widespread arboviruses in the world and has been present in Europe since 1996. Both USUV and WNV have now been detected in many parts of Europe. However, no clinical cases have been detected in the United Kingdom (UK) and Scandinavia (ECDC, 2023; Simonin, 2024). Bagaza virus (BAGV), an orthoflavivirus from the Ntaya serocomplex, appears to be restricted to Spain and Portugal (Falcão et al., 2023). Tick-borne orthoflaviviruses TBEV and Louping ill virus (LIV) have been present in Europe for almost a century. LIV detection is primarily limited to the British Isles, but cases have also been detected in Norway and Spain (Jeffries et al., 2014). TBEV is widespread in Europe, including the UK (Van Heuverswyn et al., 2023). Over the past decade, orthoflaviviruses have been moving towards the Netherlands, resulting in the first detection of USUV in 2016 (Zaaijer et al., 2019). USUV caused large bird die-offs in the Netherlands during an outbreak and has been detected in birds and mosquitoes every year since its detection (Oude Munnink et al., 2020; Rijks et al., 2016). Since 2016, 12 human cases of TBEV have been identified, and the virus has also been detected in ticks and wildlife (Dekker et al., 2019). In 2020, WNV was first detected in the Netherlands in birds and mosquitoes (Sikkema et al., 2020). Since then, eight human cases have been identified in two regions (RIVM, 2020; Vlaskamp et al., 2020). The virus was detected again in a Grey Heron (*Ardea cinerea*) in a new region in 2022 (RIVM, 2022).

Impacts

- This study identified the first serological detections of orthoflavivirus infections in horses and dogs in the Netherlands.
- Baseline WNV and USUV seroprevalence is low in horses and dogs in the Netherlands.
- Horse owner awareness and knowledge of mosquito-borne viruses is high compared to the general Dutch population.

Within the *Orthoflavivirus* genus, there are multiple serocomplexes that consist of antigenically related viruses (Calisher et al., 1989; Rathore & St. John, 2020). USUV and WNV are part of the Japanese encephalitis (JE) serocomplex, while TBEV and LIV are part of the TBE serocomplex (Calisher et al., 1989; Cavalleri et al., 2022). Antibodies within a serocomplex may show significant cross-reactive responses in serologic analyses. Moreover, cross-reactions even occur between viruses from the JE and the Ntaya serocomplex (Llorente et al., 2019). Cross-reaction hampers the diagnosis of clinical cases, as well as, the estimation of seroprevalence in a population. As many orthoflaviviruses co-circulate in parts of Europe, co-infection, or sequential infection of multiple viruses in one host, may occur. This further complicates the diagnosis and correct identification of the infecting virus(es). For seroprevalence studies, the general approach is to use a screening method with a sensitive but less specific test (Beck et al., 2013, 2017; WOAH, 2018). Positive samples are then confirmed using virus neutralization tests (VNTs) or similar neutralization tests (Beck et al., 2017; WOAH, 2018). To conclude which virus had been the infecting virus, titres between cross-reactive viruses are compared and a fourfold difference or greater is regarded to indicate the infecting virus (WOAH, 2018).

Early-detection and surveillance strategies are regularly used to protect human and animal health from emerging arboviruses. Usual surveillance strategies include vector, animal and human surveillance or a combination of these (Beck et al., 2013). In the Netherlands, passive symptomatic surveillance in horses is one of the pillars of early detection of WNV, in addition to human, bird and vector surveillance (Braks & van den Kerkhof, 2021). For the symptomatic surveillance to work accurately, horse owners and veterinarians need to recognize symptoms and report suspected cases to the authorities (Chapman et al., 2018). As mammals do not often display overt clinical signs of disease, serological investigations are often used in surveillance programmes to investigate past virus circulation or spread. Commonly used mammal species for serological investigations are horses, dogs and wildlife, such as deer and wild boar (Adjadj et al., 2022; Escribano-Romero et al., 2015; García-Bocanegra et al., 2018; Garcia-Vozmediano et al., 2022). However, horses can be vaccinated against WNV. While vaccination protects against disease, it also interferes with serological diagnostics. This interference is mainly seen for IgG detection, but IgM detection after recent vaccination has also been described (Cavalleri et al., 2022; Joó et al., 2017; Monaco et al., 2019). As such, the use of horses for surveillance is limited in areas where a large proportion of the population has been vaccinated (Beck et al., 2013).

To date, few studies have investigated the seroprevalence of the abovementioned viruses in the Netherlands (Jahfari et al., 2017; Wijburg et al., 2022; Zaaijer et al., 2019). However, cross-sectional or longitudinal serosurveys may provide useful information on risk regions and risk factors, which may guide future surveillance or intervention strategies. The Netherlands has a large equine community with an estimated number of 450,000 horses and 400,000 active riders (KNHS, 2016). In addition, around 1.8 million dogs are kept as pets (Dibevo, 2022). Thus, these widely distributed species may be useful in arboviral surveillance. Additionally, no studies have been performed to investigate perceptions and preventive behaviours of horse owners towards mosquito-borne viruses (MBVs) in the Netherlands. This is crucial because these perceptions and behaviours are key in the current symptomatic surveillance strategy.

In this study, we set out to determine baseline seroprevalences for USUV and WNV in horses (*Equus caballus*) and dogs (*Canis lupus familiaris*). Additionally, we investigated horse owner perceptions and preventive behaviours towards MBVs, with a special focus on WNV, by using an online questionnaire. By combining these two methods, we aimed to get a better understanding of the potential role of domestic animals for orthoflavivirus surveillance in the Netherlands.

2 | MATERIALS AND METHODS

A cross-sectional serosurvey was used to estimate seroprevalences of WNV and USUV in horses and dogs in the Netherlands. Additionally, horse owners and caretakers' (hereafter called horse owners) perceptions and preventive behaviours towards MBVs were assessed by using an adapted version of the MosquitoWise survey (Abourashed et al., 2024).

2.1 | Serosurvey

2.1.1 | Study design and sampling

The required sample size was 381 samples per species (CI 95%, error 1%), with an estimated seroprevalence of 1% and an estimated population of 450,000 horses and 1.8 million dogs (Sergeant, 2018). Veterinary practices were recruited through advertisements in e-mail, digital newsletters, social media (Facebook and LinkedIn), the Netherlands Journal of Veterinary Science and personal communication. All veterinary practices treating horses, dogs or both were eligible to participate in the study.

Animals were selected by the participating veterinarians. Animals were only enrolled if they were over 1-year old, had never been abroad and were never vaccinated against WNV (due to its interference with the diagnostic tests). Informed consent was acquired from owners prior to including their animal(s) in the study. A maximum of five animals per holding were included. Sampling took place from 1 May 2021 until 1 May 2022.

Serum samples were collected by jugular or cephalic venepuncture. A questionnaire was filled out for each animal (in Dutch, available in Appendix S2). At the laboratory, sera were stored at −20°C until testing.

2.1.2 | Serological assays

Laboratory analyses were performed at the Dutch national reference laboratory for notifiable diseases in animals in Lelystad. All samples were initially screened for orthoflavivirus antibodies by a commercial WNV enzyme-linked immunosorbent assay (ELISA) (ID Screen®, West Nile Competition Multi-species, IDvet Innovative Diagnostics, France). This ELISA detects anti-E protein antibodies of both USUV and WNV and is known to show cross-reactions to other orthoflaviviruses such as TBEV (Beck et al., 2017). In addition to the standard manufacturer protocol, a dilution series of the positive control was tested (1:8 and 1:16). The signal to noise (S/N%) was calculated by using the mean optical density (OD) value of the two negative controls. Horses sampled from the 1st of May until the 2nd of November 2021 (with sufficient amount of serum left) or with doubtful and positive competition ELISA results were also tested for WNV IgM antibodies by a commercial ELISA (ID Screen®, West Nile IgM Capture, IDvet Innovative Diagnostics, France). This was done to test for recent infections.

Doubtful and positive horse and dog samples were subsequently tested by Virus Neutralization Tests (VNTs). Sera were tested in duplicate in VNTs for WNV, USUV and TBEV, according to a previously described method (WOAH, 2018). In short, sera were inactivated for 30 min at 56°C. Three-fold serial dilutions were made ranging from 1:10 to 1:2430 for WNV and USUV and from 1:20 to 1:4860 for TBEV. Subsequently, 50ul containing about 100 TCID50 of either WNV (GenBank accession no. HQ537483), USUV (GenBank accession no. OP007489) or TBEV (GenBank accession no. M77799) was added to each well and incubated at 37°C in 5% CO_2 for 1.5 h. The virus titre was confirmed by performing back titrations alongside the VNTs. Positive (experimentally infected horse for WNV, field infected bird for USUV, vaccinated human for TBEV) and negative (Dutch horses pooled sample, 2007) controls were included. Finally, 50uL of virus susceptible cells were added, meaning 15,000 cells/well Vero-CCL81 cells for WNV and USUV or 7500 cells/well BHK-21 cells for TBEV. Plates were then incubated for four (TBEV) or six

(WNV and USUV) days at 37°C in 5% CO_2. Readout was performed by scoring each well for the degree of cytopathic effect (CPE). Titres of each replicate were calculated using the reciprocal dilution at which 50% or more CPE was present. The titre for each sample was calculated as the average of titres of both duplicates after logarithmic transformation. Titres of ≥10 (USUV and WNV) or ≥20 (TBEV) were considered positive as determined by testing reference samples of naturally and experimentally infected equines and/or birds and negative equine samples. Additionally, samples just below ELISA cut-off (S/N% values 50–60) were tested in all VNTs, and none of those had a neutralizing titre. Infection with a specific virus was confirmed if the VNT titre for the specific virus was at least four-fold higher compared to the other virus(es). In case the titre difference was smaller, results were classified as undetermined orthoflavivirus exposure. To double-check if animals with positive samples complied with all inclusion and exclusion criteria, veterinarians of ELISA-positive animals were contacted and asked to confirm vaccination and travel history.

2.2 | MosquitoWise survey

The MosquitoWise survey was developed for a European-wide audience and is based on an adapted Health Believe Model (HBM) (Abourashed et al., 2024). The survey was designed to measure health belief and intent to show preventive behaviour against MBVs. Additionally, other questions to obtain information on demographics and sources of information were included. For this study, seven additional questions were added and are shown in Appendix S3. These questions were shown only to Dutch participants who answered "Yes" to the question "Do you own or take care of at least one horse for a minimum of one day per week?" Horse owners were recruited through advertorials in newsletters from Dutch equine organizations (KNHS, KWPN and FNRS), social media posts (LinkedIn, Twitter and Facebook) and an online equine forum (Bokt.nl). The recruitment period ran from July 2022 until October 2022, after which the questionnaire was closed.

2.3 | Data analysis

MosquitoWise answers were extracted from the LimeSurvey platform in November 2022. Data from both the seroprevalence study and MosquitoWise survey were stored in Microsoft Excel (version 2208). Postal codes were converted into two-digit postal codes to ensure pseudonymization of owner data. Results were analysed with R by using RStudio, version 2023.03.1+446. The observed seroprevalence was calculated as the percentage of VNT-confirmed samples for a specific virus out of the total sample size for that species. MosquitoWise knowledge scores were determined by awarding one point to each correct knowledge question answer and then calculating the total number of points (min. 0, max. 9 points). Participants were then divided into minimum (0–2 points), intermediate (3–6 points) and high knowledge (7–9 points). Reverse barrier scoring was used in calculation of the HBM sum score and in the barriers construct score. A higher barrier construct score, thus, indicates lower barriers for using prevention measures were experienced by respondents.

3 | RESULTS

3.1 | Serosurvey

In total, 61 veterinary practices agreed to participate in the study. Eventually, 47 practices submitted one or more serum samples during the study period. They collected 628 sera: 258 from dogs and 370 from horses (see Table 2). Samples were collected in all 12 provinces of the Netherlands. Most samples originated from Utrecht (24%), South-Holland (19%) and North-Brabant (19%). All data obtained in the serosurvey is shown in Appendix S1.

3.1.1 | Serology

Seven dogs (7/258) and 18 horses (18/370) had doubtful or positive ELISA results. These 18 horses all tested negative for IgM antibodies. All competition ELISA-negative horses sampled from 1st of May–2nd of November 2021 (n = 166) also tested negative for IgM antibodies, except for one horse with a borderline doubtful result (S/P 36%). Seven ELISA-positive animals (six horses and one dog) were excluded from further analyses. Four horses appeared to have been vaccinated, two had been abroad and there was insufficient serum present to perform VNTs for one dog. More information on the excluded animals can be found in Appendix S4. Table 1 shows the results of all ELISA-positive sera tested in VNTs. A spatial overview of the results of the serosurvey is shown in Figure 1. The map shows that the seropositive animals were located near areas where USUV, WNV and TBEV (includes virus and serological detections) had been detected in mosquitoes, wildlife or humans from 2016 until May 2022 (RIVM, 2023; Oude Munnink et al., 2020; Sikkema et al., 2020).

Based on these results, an observed seroprevalence of 0.27% (1/364, 95% CI [0.00–0.81]) was found for WNV in horses. One dog was USUV positive, resulting in an observed seroprevalence of 0.39% (1/257, 95% CI [0.00–1.15]).

3.1.2 | Serosurvey questionnaire

Table 2 shows the owner responses to the serosurvey questionnaire. Most dogs (79%) were housed in urban areas, while most horses (75%) were housed in agricultural areas. The amount of time spent outside differed systematically between both species. While most dogs daily spent 0–6 h outside, the majority of horses spent >6 h outside. Interestingly, a higher percentage of dogs received insect

TABLE 1 Results of ELISA-positive sera tested in VNTs.

Animal ID	Species	Age[a]	WNV	USUV	TBEV	Conclusion
4	Horse	14	<10	<10	<20	Negative
67	Horse	13	<10	<10	<20	Negative
118	Horse	5	<10	<10	<20	Negative
122	Horse	10	30	<10	<20	WNV infection
133	Horse	5	<10	<10	<20	Negative
179	Horse	16	5.7	<10	<20	Negative
194	Horse	24	<10	<10	<20	Negative
208	Horse	13	<10	<10	<20	Negative
249	Horse	17	<10	<10	11.5	Negative
258	Dog	10	<10	<10	<20	Negative
328	Horse	18	<10	<10	<20	Negative
343	Dog	6	<10	<10	<20	Negative
366	Horse	11	<10	<10	<20	Negative
447	Dog	7	5.7	<10	<20	Negative
488	Dog	10	10	10	<20	Undetermined orthoflavivirus
525	Dog	7	<10	<10	<20	Negative
593	Dog	14	<10	17.3	<20	USUV infection
602	Horse	12	Cell toxicity		<20	Not interpretable

Abbreviations: TBEV, Tick-Borne Encephalitis virus; USUV, Usutu virus; WNV, West Nile virus.
[a]Age in years.

repellents, yet more dogs than horses had one or more ticks removed during the 12 months before sampling. Due to the small number of detected WNV and USUV infections in this study, we could not perform any further analyses on risk factors based on answers from the owner questionnaire.

3.2 | MosquitoWise survey targeted at horse owners

3.2.1 | Demographics

The horse owner-targeted survey was accessed 535 times during the sampling period. A total of 235 responses were excluded due to incompleteness ($n = 216$), not owning or taking care of a horse ($n = 18$) or living outside of the Netherlands ($n = 1$). Full responses were obtained from 300 horse owners. Of the respondents, 279 (93%) were female and 14 (4.7%) were male.

Ages of respondents ranged from 18 to 86 years with a median age of 41, which is lower than the median age of the Dutch population (42.4) (CBS, 2022b). Most respondents completed post-secondary education or higher (89%) and were employed when filling out the survey (79%). A large proportion (27%) of respondents were working as healthcare practitioners or technical and healthcare supporters at the time of the study, whereas the overall Dutch employment in healthcare is 16% (CBS, 2022a). The majority of the survey's respondents lived in rural areas (68%), compared to only about 30% of the total Dutch population (Steenbekkers et al., 2017).

3.2.2 | Knowledge and HBM scores

Knowledge scores ranged from 0 to 9, with a median of 6 and mean of 5.96. Twelve horse owners (4%) had a minimum knowledge level, 154 (51.33%) had an intermediate knowledge level and the remaining 134 (44.67%) had a high knowledge level. Scores for separate constructs and the total HBM score are shown in Table 3. The high 'cues to action' construct score indicates that cues have a positive effect on the horse owners' overall intent to use preventive measures. The perceived severity scores indicate that horse owners are aware that infections with MBVs can lead to (severe) disease in humans. The overall mean HBM score was 29.41. This suggests that horse owners have a moderate to moderately high level of intention to use prevention measures (to protect themselves) based on the HBM framework.

3.2.3 | Horse owner specific questions

Most horse owners were aware of the option to vaccinate their horse(s) against WNV (85%). However, just over half (50.67%) of the horse owners would consider to vaccinate or were already vaccinating their horse(s) yearly. Yearly vaccination was described as a double vaccination in the first year, followed by a yearly single vaccination of approximately €55, excluding call-out fees. Respondents were then asked whether they applied preventive measures against insect bites for their horse (not specifically against mosquitoes). For this question, the most frequently chosen

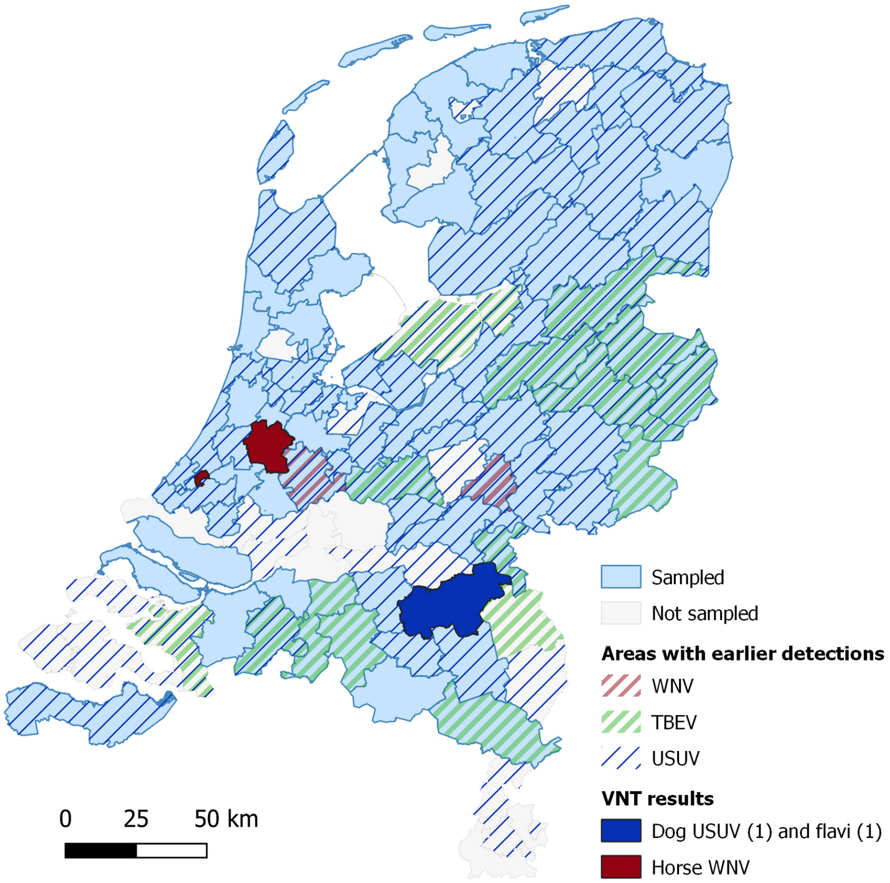

FIGURE 1 Serosurvey results shown in two-digit postal code areas. Earlier detections (colour shaded areas) published up to the end of the serosurvey in May 2022 are included (Esser et al., 2022; Oude Munnink et al., 2020; Sikkema et al., 2020; Vlaskamp et al., 2020). Map was created by using QGIS, version 3.22.5.

multiple-choice options 'use of a fly mask' (72.33%), 'daily use of insect repellent spray' (54%) and 'use of a fly banket' (57.33%). Additionally, 33 respondents used the 'other' textbox to describe their preventive measures. These responses included a wide range of answers, such as spraying insecticides in the stable, changing feed or supplements and encouraging natural enemies (e.g., *Hirundinidae* species). A few respondents gave answers unrelated to prevention of insect bites, such as vaccination and prednisone administration. Twenty-three horse owners (7.67%) did not take any preventive measures. Respondents were asked for which symptom(s) in their horse(s) they would contact their veterinarian. Answers to this question, shown in Figure 2, indicate that most horse owners would contact their veterinarian for symptoms such as fever and neurological symptoms. Only two respondents (0.7%) indicated they would not contact their veterinarian for any of the mentioned symptoms.

Social media (22.3%) and online equine fora (24.3%) (Bokt.nl in our case) were the most frequently mentioned sources of information for mosquitoes and MBVs. The (equine) veterinarian is another important information source (19.3%). Interestingly, 45.8% of horse owners did not recently read or receive any information about MBVs via any of the proposed information channels.

Figure 3 shows the 7-point Likert scale responses to the two remaining questions. A majority felt that their veterinarian was responsible for informing them about MBVs in horses. About half of the respondents removed breeding sites in the stabling area.

4 | DISCUSSION

In this study, we investigated the seroprevalence of WNV and USUV in domestic horses and dogs and studied the MBV knowledge and perceptions of horse owners in the Netherlands. We found a low observed seroprevalence for USUV and WNV between May 2021 and May 2022. The MosquitoWise survey revealed a high knowledge level for horse owners and high awareness of WNV vaccination but a lower intention to vaccinate.

4.1 | Serosurvey

The required sample size as calculated prior to the study was not met for both horses and dogs. Veterinarians received monthly reminders to submit samples, but many practices mentioned the effect of COVID-19 pandemic measures and personnel shortage on their ability to cooperate. Some equine veterinarians actively promoted WNV vaccination at the start of 2021, lowering the number of horses suitable for inclusion in the study.

This was the first study to investigate the seroprevalence of WNV and USUV in domestic animals in the Netherlands. In 2018, Zaaijer et al. (2019) performed an USUV screening study in Dutch blood donors and found that 0.45% of the donors showed USUV IgG responses in September and a similar result was found in Dutch bird ringers in 2021 (De Bellegarde De Saint Lary et al., 2023). Our findings are in

TABLE 2 Results of the serosurvey owner questionnaire, *n* (%) for horses and dogs separately.

	Species	
	Horse, *N* = 364	**Dog, *N* = 258**
Age	12 (6, 17)[a]	4 (1, 8)[a]
Sex		
Female	196 (54%)	139 (54%)
Male	168 (46%)	119 (46%)
Housing area		
Agricultural	273 (75%)	43 (17%)
Agricultural/Nature	8 (2.2%)	2 (0.8%)
Urban	51 (14%)	204 (79%)
Urban/Nature	3 (0.8%)	3 (1.2%)
Nature	28 (7.7%)	5 (1.9%)
Amount of time spent outside per day (per 24 h)		
0–6 h	92 (25%)	203 (79%)
6–12 h	139 (38%)	40 (16%)
12–18 h	30 (8.2%)	7 (2.7%)
>18 h	103 (28%)	6 (2.3%)
Use of repellent in vector season		
No	236 (65%)	88 (35%)
Yes	128 (35%)	166 (65%)
Tick(s) removed within 12 months prior to sampling		
No	328 (90%)	171 (67%)
Yes	35 (9.6%)	83 (33%)
Type of outdoor space		
Paddock	33 (9.1%)	–
Pasture	154 (42%)	–
Both	176 (48%)	–
Use of a blanket during vector season (April–November)		
No	224 (62%)	–
Yes	140 (38%)	–
Number of horses on premise		
1–2	51 (14%)	–
3–10	141 (39%)	–
10–20	32 (8.8%)	–
>20	140 (38%)	–

[a]Median (Inter Quartile Range).

line with both studies in humans. The low seroprevalence for USUV is interesting considering the circulation of the virus since 2016 and high impact on the bird community (Rijks et al., 2016). Possible explanations for this finding are that antibody titres of our study animals had already waned or that animals were never infected, potentially due to housing conditions or specific host-preferences of vectors (Clé et al., 2019).

Horses are extensively used for WNV seroprevalence studies throughout Europe (Metz et al., 2021). In eastern Germany, Ganzenberg et al. (2022) found a seroprevalence of 5.8% for WNV in horses in 2020. It is noteworthy that they also included animals with a foreign background. In eastern Austria, seroprevalence in horses was 15.5% for TBEV, 5.3% for WNV and 0% for USUV in 2017. Again, some horses had questionable countries of origin or travel history. Therefore, they also mention an 'autochthonous prevalence' of 1.2% for WNV (De Heus et al., 2021). We also noted the exclusion of six horses as owners initially did not declare the right country of origin or vaccination status. This highlights the fact that our data, and also that from other studies, should be interpreted with caution. Equine WNV seroprevalence in Spain is much higher, namely 19.7% in western Spain (in 2018–2019) and even 25.0% (in 2020) in feral horses in the south-west (Guerrero-Carvajal et al., 2021; Magallanes et al., 2023). Multiple countries have used dogs to study WNV, TBEV and (to a lesser extent) USUV seroprevalence (García-Bocanegra et al., 2018). In France, an USUV seroprevalence of 1.08% was found in dogs between 2016 and 2020. This seroprevalence is slightly higher than our findings. In the same study, a higher seroprevalence was found for both USUV (3.83%) and WNV (13.19%) in horses (Constant et al., 2022). Differences in seroprevalence between countries and animal species can be explained by many factors, such as endemicity of the virus, sampling strategy, demographics and housing conditions of the animals. Furthermore, there are notable differences in types of diagnostic methods used, which hampers comparability of seroprevalence estimates between countries (García-Bocanegra et al., 2018; Metz et al., 2021).

The majority of doubtful and positive ELISA sera turned out to be negative in all VNTs, while specificity is presumed to be high for horse sera (Beck et al., 2017). An explanation for this may be waned antibody titres below detection limits of the VNTs. Orthoflavivirus antibody persistence differs per virus and for each host species. Evidence suggests long-term persistence of antibodies for WNV and TBEV in multiple species, including horses (Klaus et al., 2014; Percivalle et al., 2020; Trachsel et al., 2021). No studies have been published on orthoflavivirus antibody waning in dogs. Furthermore, the higher starting dilution of TBEV compared to that of USUV and WNV might have resulted in false-negative outcomes for sera in the TBEV VNT. Another explanation may be that these ELISA positives could be due to circulation of other viruses not tested for neutralizing antibodies in this study, such as BAGV or LIV. This was also suggested by a recent study performed

TABLE 3 Health belief model construct and total scores for horse owners.

Construct	Mean [25%–75%] median score, *N* = 300
Perceived susceptibility	4.34 [3.75–5.00], 4.25
Perceived severity	5.48 [4.67–6.00], 5.67
Perceived benefits	4.81 [4.33–5.33], 5.00
Perceived barriers	4.85 [4.00–6.00], 5.00
Cues to action	5.28 [4.67–6.00], 5.33
Self-efficacy	4.65 [4.00–5.50], 4.75
HBM total score	29.41 [27.29–31.58], 29.46

Note: The construct score range is 1–7. The total HBM score range is 6–42.

Abbreviation: HBM, Health Belief Model.

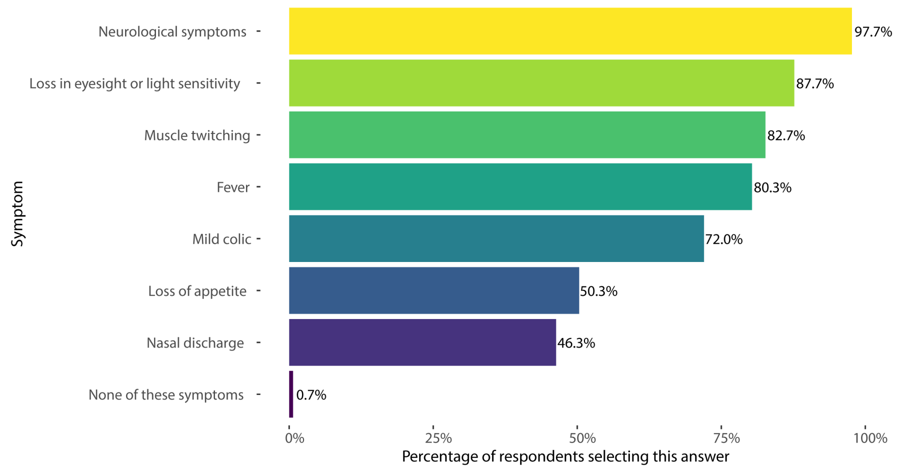

FIGURE 2 Bar plot of respondents' answers to the question "For which of these symptoms would you contact your veterinarian?" Neurological symptoms were described as weakness in hind limbs, paresis, paralysis and convulsions.

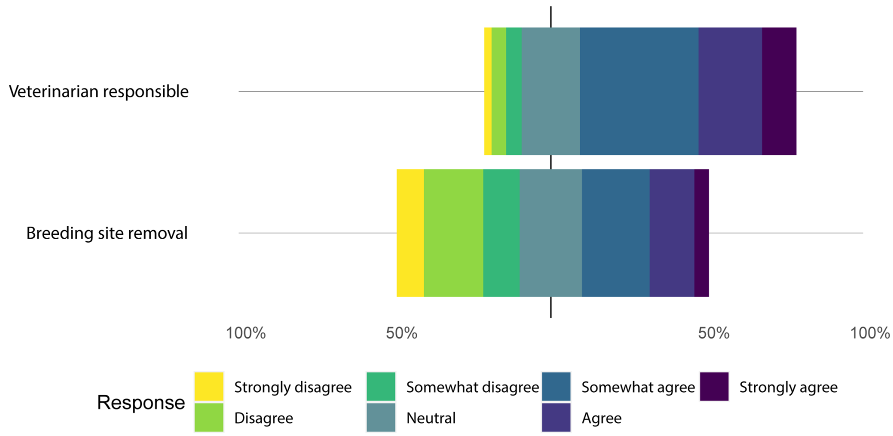

FIGURE 3 Likert scale results for horse owner questions: 'Veterinarian responsible' is short for "I think my veterinarian is responsible for informing me about mosquito-borne viruses in horses." And 'Breeding site removal' is short for "During mosquito season (March through September), I will ensure there are no mosquito breeding sites around my horse's stable."

by Laidoudi et al. (2023) in France. However, published BAGV detections in Europe are limited to Spain and Portugal (Benzarti et al., 2019). Furthermore, there are no publications of BAGV infections in dogs and horses. LIV infections in horses have been described, but cross-reactions with WNV or USUV have not been investigated nor reported (Hyde et al., 2007; Timoney et al., 1976).

The owner questionnaire revealed interesting results on possible risk factors, even though a formal risk factor analyses could not be performed. The combination of a relatively high percentage (65%) of repellent use in dogs, combined with the limited amount of time they spend outside, may be the cause of the low seroprevalence observed in this study. Interestingly, dog owners reported more frequent tick removals (33%) than horse owners (9.6%). This observation may be explained by the fact that dog owners actively search for ticks, and dogs may spend more time in tick-infested vegetation compared to horses. Moreover, a smaller percentage of horse owners (35%) indicated that they used insect repellents compared to dog owners (65%). However, it should be noted that we did not ask owners what type of repellents were used. Repellency and efficacy differ per repellent type and, thus, may influence the results (Pfister & Armstrong, 2016).

Other studies claimed the use of military or hunting animals as suitable species for arbovirus surveillance because of their frequent exposure to vectors (Durand et al., 2016; García-Bocanegra et al., 2018; Laidoudi et al., 2023; Montagnaro et al., 2019). A significant difference between seroprevalence in pet dogs and hunting dogs was found by García-Bocanegra et al. (2018) in Spain. Future studies to investigate the seroprevalence of orthoflaviviruses in the Netherlands could focus on animals that spend more time outdoors or are used for hunting or military work. Another option would be to investigate the potential of other animal species, such as (captive) birds, for example, chickens, goat, cattle or wildlife.

4.2 | MosquitoWise

The MosquitoWise survey in horse owners revealed a higher mean knowledge score compared to the Dutch general public. Abourashed et al. (2024) found a mean knowledge score of 4.34, while the horse owner mean score was 5.96 on a scale from 0 to 9. Additionally, all mean and median scores for separate constructs were higher, except for the perceived benefits of prevention measures. The higher perceived reversed barriers construct score implies horse owners experience lower barriers to use prevention measures, compared to the Dutch panel. The total mean HBM score in horse owners is 1.3 points higher than in the Dutch panel, which indicates that horse owners have more health belief and more intent to show preventive behaviour (Abourashed et al., 2024). Scores may be influenced by the fact that horse owners are a specific subpopulation of the Dutch general public. Of our respondents, 93% were female, and the median age of 41 was lower compared to the Dutch median of 49. The majority live in rural areas (68%) and a substantial proportion works in healthcare-related professions. The already mentioned bias in gender, age and profession is also found in other studies from the UK (Boden et al., 2013; Chapman et al., 2018).

There are few published studies on knowledge or attitudes towards MBVs in horse owners in Europe. Chapman et al. (2018) found that 65.4% of horse owners correctly identified mosquitoes from an image. The majority (83.1%) of their respondents were aware that WNV can affect horses, which is similar to the percentage of respondents in our study (85%) when asking about awareness of the possibility of WNV vaccination. Horse owner intent to vaccinate against WNV was much higher (80.1%) compared to our results (50.67%), but it should be noted that we did not ask specifically for vaccination intent 'in the case of a WNV outbreak event' in contrast to their study. Factors limiting willingness to vaccinate can be related to concerns about side effects and efficacy, lack of risk of disease and associated cost, among others (Chapman et al., 2018; Manyweathers et al., 2017).

Owners feel their veterinarian is responsible for providing them with information about MBVs, which also aligns with earlier research (Chapman et al., 2018). Most horse owners will contact their veterinarian if their horse shows symptoms associated with WNV infection, such as fever, neurological symptoms and muscle twitching. This contact between owner and veterinarian may aid in early detection of WNV cases. However, this also requires awareness of, and rapid notification of, suspected cases by veterinarians.

5 | CONCLUSIONS

The low seroprevalences of WNV and USUV indicate many dogs and horses remain susceptible, offering opportunities for trend analysis and surveillance. However, despite multiple recent detections of WNV, USUV and TBEV in humans, the role of dogs and horses in early detection of human cases is debatable. High awareness among horse owners and the absence of detected equine WNV cases highlight this uncertainty. Continued surveillance is crucial for detecting increased virus circulation and protecting both animal and human health. Additionally, veterinarians' awareness and intentions to notify suspected cases need further investigation.

ACKNOWLEDGEMENTS

The authors acknowledge Jenny Hesson, Åke Lundkvist and Reina Sikkema for critically reviewing the manuscript. Additionally, we thank Kees van Maanen (Royal GD) for providing us with WNV reference sera and the Virology department of Wageningen University & Research for the WNV strain used in the virus neutralization test. The authors thank all participating veterinarians and horse and dog owners for participating in this study.

FUNDING INFORMATION

This publication is part of the project 'Preparing for vector-borne virus outbreaks in a changing world: a One Health Approach' (NWA.1160.1S.210), which is (partly) financed by the Dutch Research Council (NWO). The research was partially funded by the European Union's Horizon 2020 research innovation programme (Grant no. 874735 (VEO)) and the Ministry of Agriculture, Nature and Food Quality (WOT-A-1600003002 (Preparing for Emerging Pathogens)).

CONFLICT OF INTEREST STATEMENT

The authors do not declare any conflicts of interest.

DATA AVAILABILITY STATEMENT

Data used in this manuscript are available in a pseudonymized format in Appendix S1.

ETHICS APPROVAL STATEMENT

Animals in this study were sampled under legislation (licence no. AVD40100202114384) of the Dutch Central Authority for Scientific procedures on Animals (CCD). The experimental plan was approved by the Animal Welfare Body of Wageningen University and Research before sampling started. For the MosquitoWise survey, the Erasmus University Medical Center Medical Ethics Committee determined that the methods were not subject to the Dutch Medical Research Involving Human Subject's Act and could therefore be carried out without further review (Ref. MEC-2021-0586 dated 21 September 2021).

PATIENT CONSENT STATEMENT

Animal owners signed a consent form before participation in the serosurvey. Horse owners filled out the MosquitoWise survey in LimeSurvey. In LimeSurvey, participants were informed about the study aim, their right to withdraw from the study and assured their data would be stored anonymously.

ORCID

Kiki Streng https://orcid.org/0000-0001-6153-1428

REFERENCES

Abourashed, A., De Best, P. A., Doornekamp, L., Sikkema, R. S., Van Gorp, E. C. M., Timen, A., Bartumeus, F., Palmer, J. R. B., & Koopmans, M. P. G. (2024). Development and validation of the MosquitoWise survey to assess perceptions towards mosquitoes and mosquito-borne viruses in Europe. *Scientific Reports*, *14*(1), 1777. https://doi.org/10.1038/s41598-024-52219-9

Adjadj, N. R., Vervaeke, M., Sohier, C., Cargnel, M., & De Regge, N. (2022). Tick-borne encephalitis virus prevalence in sheep, wild boar and ticks in Belgium. *Viruses*, *14*(11), 2362. https://doi.org/10.3390/v14112362

Beck, C., Jimenez-Clavero, M., Leblond, A., Durand, B., Nowotny, N., Leparc-Goffart, I., Zientara, S., Jourdain, E., & Lecollinet, S. (2013). Flaviviruses in Europe: Complex circulation patterns and their consequences for the diagnosis and control of West Nile disease. *International Journal of Environmental Research and 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

Benzarti, E., Linden, A., Desmecht, D., & Garigliany, M. (2019). Mosquito-borne epornitic flaviviruses: An update and review. *Journal of General Virology*, *100*(2), 119–132. https://doi.org/10.1099/jgv.0.001203

Boden, L. A., Parkin, T. D., Yates, J., Mellor, D., & Kao, R. R. (2013). An online survey of horse-owners in Great Britain. *BMC Veterinary Research*, *9*(1), 188. https://doi.org/10.1186/1746-6148-9-188

Braks, M., & van den Kerkhof, J. (2021). *Westnijlvirus in Nederland: Aanpak surveillance en respons 2021–2023*. Rijksinstituut voor Volksgezondheid en Milieu RIVM.

Calisher, C. H., Karabatsos, N., Dalrymple, J. M., Shope, R. E., Porterfield, J. S., Westaway, E. G., & Brandt, W. E. (1989). Antigenic relationships between flaviviruses as determined by cross-neutralization tests with polyclonal antisera. *Journal of General Virology*, *70*(1), 37–43. https://doi.org/10.1099/0022-1317-70-1-37

Cavalleri, J. V., Korbacska-Kutasi, O., Leblond, A., Paillot, R., Pusterla, N., Steinmann, E., & Tomlinson, J. (2022). European College of Equine Internal Medicine consensus statement on equine flaviviridae infections in Europe. *Journal of Veterinary Internal Medicine*, *36*(6), 1858–1871. https://doi.org/10.1111/jvim.16581

CBS. (2022a). *Dashboard Arbeidsmarkt Zorg en Welzijn (AZW)*. https://dashboards.cbs.nl/v4/AZWDashboard/

CBS. (2022b). *Population dashboard: Age distribution*. https://www.cbs.nl/en-gb/visualisations/dashboard-population/age/age-distribution

Chapman, G. E., Baylis, M., & Archer, D. C. (2018). Survey of UK horse owners' knowledge of equine arboviruses and disease vectors. *Veterinary Record*, *183*(5), 159. https://doi.org/10.1136/vr.104521

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. https://doi.org/10.1017/S0950268819001213

Constant, O., Gil, P., Barthelemy, J., Bolloré, K., Foulongne, V., Desmetz, C., Leblond, A., Desjardins, I., Pradier, S., Joulié, A., Sandoz, A., Amaral, R., Boisseau, M., Rakotoarivony, I., Baldet, T., Marie, A., Frances, B., Reboul Salze, F., Tinto, B., … Simonin, Y. (2022). One health surveillance of West Nile and Usutu viruses: A repeated cross-sectional study exploring seroprevalence and endemicity in southern France, 2016 to 2020. *Eurosurveillance*, *27*(25), 2200068. https://doi.org/10.2807/1560-7917.ES.2022.27.25.2200068

De Bellegarde De Saint Lary, C., Kasbergen, L. M. R., Bruijning-Verhagen, P. C. J. L., Van Der Jeugd, H., Chandler, F., Hogema, B. M., Zaaijer, H. L., Van Der Klis, F. R. M., Barzon, L., De Bruin, E., Ten Bosch, Q., Koopmans, M. P. G., Sikkema, R. S., & Visser, L. G. (2023). Assessing West Nile virus (WNV) and Usutu virus (USUV) exposure in bird ringers in The Netherlands: A high-risk group for WNV and USUV infection? *One Health*, *16*, 100533. https://doi.org/10.1016/j.onehlt.2023.100533

De Heus, P., Kolodziejek, J., Hubálek, Z., Dimmel, K., Racher, V., Nowotny, N., & Cavalleri, J.-M. V. (2021). West Nile virus and tick-borne encephalitis virus are endemic in equids in eastern Austria. *Viruses*, *13*(9), 1873. https://doi.org/10.3390/v13091873

Dekker, M., Laverman, G. D., de Vries, A., Reimerink, J., & Geeraedts, F. (2019). Emergence of tick-borne encephalitis (TBE) in The Netherlands. *Ticks and Tick-borne Diseases*, *10*(1), 176–179. https://doi.org/10.1016/j.ttbdis.2018.10.008

Dibevo. (2022). *Huisdieren in Nederland, 2021*. https://dibevo.nl/kenniscentrum/huisdieren-in-nederland

Durand, B., Haskouri, H., Lowenski, S., Vachiery, 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), 1857–1864. https://doi.org/10.1017/S095026881600011X

ECDC. (2023). Surveillance, prevention and control of West Nile virus and Usutu virus infections in the EU/EEA. *EFSA Supporting Publications*, *20*(9), 11–12. https://doi.org/10.2903/sp.efsa.2023.EN-8242

Escribano-Romero, E., Lupulović, D., Merino-Ramos, T., Blázquez, A.-B., Lazić, G., Lazić, S., Saiz, J.-C., & Petrović, T. (2015). West Nile virus serosurveillance in pigs, wild boars, and roe deer in Serbia. *Veterinary Microbiology*, *176*(3–4), 365–369. https://doi.org/10.1016/j.vetmic.2015.02.005

Esser, H. J., Lim, S. M., De Vries, A., Sprong, H., Dekker, D. J., Pascoe, E. L., Bakker, J. W., Suin, V., Franz, E., Martina, B. E. E., & Koenraadt, C. J. M. (2022). Continued circulation of tick-borne encephalitis virus variants and detection of novel transmission foci, the Netherlands. *Emerging Infectious Diseases*, *28*(12), 2416–2424. https://doi.org/10.3201/eid2812.220552

Falcão, M., Barros, M., Duarte, M. D., Santos, F. A. D., Fagulha, T., Henriques, M., Ramos, F., Duarte, A., Luís, T., Parreira, R., & Barros, S. C. (2023). Genome characterization and spaciotemporal dispersal analysis of Bagaza virus detected in Portugal, 2021. *Pathogens*, *12*(2), 150. https://doi.org/10.3390/pathogens12020150

Ganzenberg, S., Sieg, M., Ziegler, U., Pfeffer, M., Vahlenkamp, T. W., Hörügel, U., Groschup, M. H., & Lohmann, K. L. (2022). Seroprevalence and risk factors for equine West Nile virus infections in eastern Germany, 2020. *Viruses*, *14*(6), 1191. https://doi.org/10.3390/v14061191

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 and Emerging Diseases*, *65*(3), 765–772. https://doi.org/10.1111/tbed.12801

Garcia-Vozmediano, A., Bellato, A., Rossi, L., Hoogerwerf, M. N., Sprong, H., & Tomassone, L. (2022). Use of wild ungulates as sentinels of TBEV circulation in a Naïve area of the northwestern Alps, Italy. *Lifestyles*, *12*(11), 1888. https://doi.org/10.3390/life12111888

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 and Emerging Diseases*, *68*(3), 1432–1444. https://doi.org/10.1111/tbed.13810

Hubálek, Z., Rudolf, I., & Nowotny, N. (2014). Arboviruses pathogenic for domestic and wild animals. *Advances in Virus Research*, *89*, 201–275.

Hyde, J., Nettleton, P., Marriott, L., & Willoughby, K. (2007). Louping ill in horses. *Veterinary Record*, *160*(15), 532. https://doi.org/10.1136/vr.160.15.532

Jahfari, S., de Vries, A., Rijks, J. M., Van Gucht, S., Vennema, H., Sprong, H., & Rockx, B. (2017). Tick-borne encephalitis virus in ticks and roe deer, the Netherlands. *Emerging Infectious Diseases*, *23*(6), 1028–1030. https://doi.org/10.3201/eid2306.161247

Jeffries, C. L., Mansfield, K. L., Phipps, L. P., Wakeley, P. R., Mearns, R., Schock, A., Bell, S., Breed, A. C., Fooks, A. R., & Johnson, N. (2014). Louping ill virus: An endemic tick-borne disease of Great Britain. *Journal of General Virology*, *95*(5), 1005–1014. https://doi.org/10.1099/vir.0.062356-0

Joó, K., Bakonyi, T., Szenci, O., Sárdi, S., Ferenczi, E., Barna, M., Malik, P., Hubalek, Z., Fehér, O., & Kutasi, O. (2017). Comparison of assays for the detection of West Nile virus antibodies in equine serum after natural infection or vaccination. *Veterinary Immunology and Immunopathology*, *183*, 1–6. https://doi.org/10.1016/j.vetimm.2016.10.015

Klaus, C., Ziegler, U., Kalthoff, D., Hoffmann, B., & Beer, M. (2014). Tick-borne encephalitis virus (TBEV) – Findings on cross reactivity and longevity of TBEV antibodies in animal sera. *BMC Veterinary Research*, *10*(1), 78. https://doi.org/10.1186/1746-6148-10-78

KNHS. (2016). *Netherlands, land of horses*. KNHS. https://www.knhs.nl/media/11820/netherlands-land-of-horses-v2.pdf

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 and Emerging Diseases*, *2023*, 1–8. https://doi.org/10.1155/2023/8779723

Llorente, F., García-Irazábal, A., Pérez-Ramírez, E., Cano-Gómez, C., Sarasa, M., Vázquez, A., & Jiménez-Clavero, M. Á. (2019). Influence of flavivirus co-circulation in serological diagnostics and surveillance: A model of study using West Nile, Usutu and Bagaza viruses.

Transboundary and Emerging Diseases, *66*(5), 2100–2106. https://doi.org/10.1111/tbed.13262

Magallanes, S., Llorente, F., Ruiz-López, M. J., Martínez-de La Puente, J., Soriguer, R., Calderon, J., Jímenez-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. https://doi.org/10.1016/j.onehlt.2023.100578

Manyweathers, J., Field, H., Longnecker, N., Agho, K., Smith, C., & Taylor, M. (2017). "Why won't they just vaccinate?" horse owner risk perception and uptake of the Hendra virus vaccine. *BMC Veterinary Research*, *13*(1), 103. https://doi.org/10.1186/s12917-017-1006-7

Metz, M. B. C., Olufemi, O. T., Daly, J. M., & Barba, M. (2021). Systematic review and meta-analysis of seroprevalence studies of West Nile virus in equids in Europe between 2001 and 2018. *Transboundary and Emerging Diseases*, *68*(4), 1814–1823. https://doi.org/10.1111/tbed.13866

Monaco, F., Purpari, G., Di Gennaro, A., Mira, F., Di Marco, P., Guercio, A., & Savini, G. (2019). Immunological response in horses following West Nile virus vaccination with inactivated or recombinant vaccine. *Veterinaria Italiana*, *55*(1), 73–79. https://doi.org/10.12834/VetIt.1820.9611.1

Montagnaro, S., Piantedosi, D., Ciarcia, R., Loponte, R., Veneziano, V., Fusco, G., Amoroso, M. G., Ferrara, G., Damiano, S., Iovane, G., & Pagnini, U. (2019). Serological evidence of mosquito-borne flaviviruses circulation in hunting dogs in Campania region, Italy. *Vector-Borne and Zoonotic Diseases*, *19*(2), 142–147. https://doi.org/10.1089/vbz.2018.2337

Oude Munnink, B. B., Münger, E., Nieuwenhuijse, D. F., Kohl, R., van der Linden, A., Schapendonk, C. M. E., van der Jeugd, H., Kik, M., Rijks, J. M., Reusken, C. B. E. M., & Koopmans, M. (2020). Genomic monitoring to understand the emergence and spread of Usutu virus in The Netherlands, 2016–2018. *Scientific Reports*, *10*(1), 2798. https://doi.org/10.1038/s41598-020-59692-y

Percivalle, E., Cassaniti, I., Sarasini, A., Rovida, F., Adzasehoun, K. M. G., Colombini, I., Isernia, P., Cuppari, I., & Baldanti, F. (2020). West Nile or Usutu virus? A three-year follow-up of humoral and cellular response in a Group of Asymptomatic Blood Donors. *Viruses*, *12*(2), 157. https://doi.org/10.3390/v12020157

Pfister, K., & Armstrong, R. (2016). Systemically and cutaneously distributed ectoparasiticides: A review of the efficacy against ticks and fleas on dogs. *Parasites & Vectors*, *9*(1), 436. https://doi.org/10.1186/s13071-016-1719-7

Rathore, A. P. S., & St. John, A. L. (2020). Cross-reactive immunity among flaviviruses. *Frontiers in Immunology*, *11*, 334. https://doi.org/10.3389/fimmu.2020.00334

Rijks, J., Kik, M., Slaterus, R., Foppen, R., Stroo, A., IJzer, J., Stahl, J., Gröne, A., Koopmans, M., van der Jeugd, H., & Reusken, C. (2016). Widespread Usutu virus outbreak in birds in the Netherlands, 2016. *Eurosurveillance*, *21*(45), 1–6. https://doi.org/10.2807/1560-7917.ES.2016.21.45.30391

RIVM. (2020). *Patient infected with West Nile virus in the Arnhem region*. https://www.rivm.nl/en/news/patient-infected-with-west-nile-virus-in-arnhem-region

RIVM. (2022). *Grey heron infected with West Nile virus*. National Institute for Public Health and the Environment. https://www.rivm.nl/node/210071

RIVM. (2023). *Tekenencefalitis (TBE)*. National Institute for Public Health and the Environment. https://www.rivm.nl/tekenencefalitis

Sergeant. (2018). *Epitools Epidemiological Calculators* [Computer software]. http://epitools.ausvet.com.au

Sikkema, R. S., Schrama, M., van den Berg, T., Morren, J., Munger, E., Krol, L., van der Beek, J. G., Blom, R., Chestakova, I., van der Linden, A., Boter, M., van Mastrigt, T., Molenkamp, R., Koenraadt, C. J., van den Brand, J. M., Oude Munnink, B. B., Koopmans, M. P., & van der Jeugd, H. (2020). Detection of West Nile virus in a common whitethroat (*Curruca communis*) and culex mosquitoes in The Netherlands, 2020. *Eurosurveillance*, *25*(40), 2001704. https://doi.org/10.2807/1560-7917.ES.2020.25.40.2001704

Simonin, Y. (2024). Circulation of West Nile virus and Usutu virus in Europe: Overview and challenges. *Viruses*, *16*(4), 599. https://doi.org/10.3390/v16040599

Steenbekkers, A., Vermeij, L., & van Houwelingen, P. (2017). *Dorpsleven tussen stad en land: Slotpublicatie Sociale Staat van het Platteland*. Sociaal en Cultureel Planbureau.

Timoney, P. J., Donnelly, W. J. C., Clements, L. O., & Fenlon, M. (1976). Encephalitis caused by Louping ill virus in a Group of Horses in Ireland. *Equine Veterinary Journal*, *8*(3), 113–117. https://doi.org/10.1111/j.2042-3306.1976.tb03311.x

Trachsel, D. S., Drozdzewska, K., Bergmann, F., Ziegler, U., & Gehlen, H. (2021). Bestätigte West-Nil-Virus-Infektion bei einem Pferd mit minimalen neurologischen Symptomen und günstigem klinischem Verlauf. *Tierärztliche Praxis. Ausgabe G, Grosstiere/Nutztiere*, *49*(4), 281–286. https://doi.org/10.1055/a-1519-4547

Van Heuverswyn, J., Hallmaier-Wacker, L. K., Beauté, J., Gomes Dias, J., Haussig, J. M., Busch, K., Kerlik, J., Markowicz, M., Mäkelä, H., Nygren, T. M., Orlíková, H., Socan, M., Zbrzeźniak, J., Žygutiene, M., & Gossner, C. M. (2023). Spatiotemporal spread of tick-borne encephalitis in the EU/EEA, 2012 to 2020. *Eurosurveillance*, *28*(11), 2200543. https://doi.org/10.2807/1560-7917.ES.2023.28.11.2200543

Vlaskamp, D. R., Thijsen, S. F., Reimerink, J., Hilkens, P., Bouvy, W. H., Bantjes, S. E., Vlaminckx, B. J., Zaaijer, H., van den Kerkhof, H. H., Raven, S. F., & Reusken, C. B. (2020). First autochthonous human West Nile virus infections in The Netherlands, July to august 2020. *Eurosurveillance*, *25*(46), 2001904. https://doi.org/10.2807/1560-7917.ES.2020.25.46.2001904

Weissenböck, H., Bakonyi, T., Rossi, G., Mani, P., & Nowotny, N. (2013). Usutu virus, Italy, 1996. *Emerging Infectious Diseases*, *19*(2), 274–277. https://doi.org/10.3201/eid1902.121191

Wijburg, S. R., Fonville, M., de Bruin, A., van Rijn, P. A., Montizaan, M. G. E., van den Broek, J., Sprong, H., & Rijks, J. M. (2022). Prevalence and predictors of vector-borne pathogens in Dutch roe deer. *Parasites & Vectors*, *15*(1), 76. https://doi.org/10.1186/s13071-022-05195-w

WOAH. (2018). West Nile fever. In *OIE terrestrial manual* (8th ed., ch. 3.1.24, pp. 697–710). Office International des Epizooties. ISBN: 978-92-95108-18-9

Zaaijer, H. L., Slot, E., Molier, M., Reusken, C. B. E. M., & Koppelman, M. H. G. M. (2019). Usutu virus infection in Dutch blood donors. *Transfusion*, *59*(9), 2931–2937. https://doi.org/10.1111/trf.15444

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Streng, K., Hakze-van der Honing, R. W., Graham, H., van Oort, S., de Best, P. A., Abourashed, A., & van der Poel, W. H. M. (2024). Orthoflavivirus surveillance in the Netherlands: Insights from a serosurvey in horses & dogs and a questionnaire among horse owners. *Zoonoses and Public Health*, *00*, 1–11. https://doi.org/10.1111/zph.13171