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REVIEW ARTICLE

Prevalence of West Nile Virus in Zoonotic Animal Species in Asia (2000–2024): A Systematic Review and Meta-Analysis

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ABSTRACT

West Nile virus (WNV), a mosquito-borne pathogen causing encephalomyelitis in animals, is an emerging health concern in Asia. Despite its public and veterinary health significance, data on WNV prevalence remain scarce in countries such as Cambodia, Kazakhstan, Pakistan, and Singapore, with only isolated or zero-prevalence reports. This systematic review and meta-analysis aimed to assess the prevalence of WNV in various animal species across Asia from 2000 to 2024. A comprehensive search of PubMed (n = 636), Web of Science (n = 364), and Google Scholar (n = 542) was conducted using predefined keywords and strict inclusion criteria. Out of 1,542 records screened, 38 studies met the criteria for final analysis, encompassing 93,268 animals, including equines, bovines, small ruminants, swine, poultry, felines, canines, and wild birds. The overall estimated prevalence of WNV in animals was 7.52%. Prevalence varied across studies, influenced by differences in sampling strategies, diagnostic methods, and host species. Temporal analysis revealed fluctuating prevalence trends over the two decades, some aligning with documented WNV outbreaks. Study heterogeneity was associated with variability in geographical coverage, study designs, and ecological factors. Climate change, vector abundance, and migratory bird routes were identified as potential factors contributing to the spread of WNV. Risk factors included (i) ecological intensity (wetlands, irrigated lands), (ii) climatic factors (temperature, rainfall), (iii) vector abundance (Culex spp. density), and (iv) migratory bird flyways. These findings highlight the need for standardised surveillance systems and coordinated monitoring across the region. Enhanced vector control, systematic reporting, and increased research efforts are vital for mitigating WNV risks. Strengthening intersectoral collaboration between veterinary and public health services is essential for effective disease control and prevention. This study provides a foundation for improved understanding of WNV transmission dynamics and supports evidence-based strategies for managing this growing zoonotic threat in Asia.

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1. INTRODUCTION

West Nile virus (WNV) is a mosquito-borne zoonotic virus, first identified in the Flavivirus genus of the Flaviviridae family (Zhang et al., 2021). WNV was originally isolated from a human in the West Nile region of Uganda in 1937 (Pattan et al., 2009). The virus circulates primarily in an enzootic cycle between birds and mosquitoes, with birds serving as the main reservoir hosts and mosquitoes, predominantly from the Culex spp., alongside Anopheles spp. and Aedes spp. acting as vectors (Habarugira et al., 2020). The ecology of this virus is complex, with different mosquito species playing varying roles in its transmission across geographic regions (Rochlin et al., 2019). Birds act as amplifying hosts, where the virus replicates to high levels, allowing mosquitoes to become infected when they feed on viraemic birds. Infected mosquitoes can then transmit the virus to other animals, including humans and horses, which are considered dead-end hosts due to insufficient viraemia for further transmission (Petersen & Marfin, 2021). The wide host range of WNV, along with its ability to survive in diverse ecosystems, makes it a significant emerging infectious disease globally (Gossner et al., 2022). The horse industry plays a pivotal role in the national economies of several Asian countries, particularly through exports to the Middle East and Europe. Countries like Japan, South Korea, and China are major players, with thousands of horses exported annually (WOAH, 2023). The epidemiology of WNV in Asia is influenced by factors such as suitable vectors, migratory bird patterns, and ecological conditions that favour transmission. Susceptibility in livestock varies by species. Horses are the most commonly affected domestic animals, often exhibiting severe neurological symptoms (Bertram et al., 2020). In contrast, other animals, such as cattle, goats, and sheep, show exposure to the disease but typically experience milder or asymptomatic infections

(Carpenter et al., 2023; Selim et al., 2022). Transmission involves complex interactions between birds, mosquitoes, and mammals. Primary vectors are Culex spp., which thrive in both rural and urban environments (Rochlin et al., 2019). Migratory birds play a key role in cross-border spread (Malik et al., 2021). Risk is heightened where birds/mosquitoes coexist with livestock (Solgi et al., 2020). The economic impact is severe in rural areas reliant on horses for agriculture, transportation, and tourism (Pepperell et al., 2023). Outbreaks cause significant losses; for example, the 2002 US outbreak resulted in ~15,000 horse deaths, reduced tourism in Israel, and millions of dollars in veterinary costs (Yeh et al., 2011a). Social disruption affects breeders, veterinarians, and related industries (Davis et al., 2015). While primarily mosquito-borne, potential human infection routes include handling infected animal fluids or meat, though risks are mitigated by meat maturation processes (Bunning et al., 2002). Detection in milk is minimal, with no major transmission concerns. No evidence supports transmission via faeces, urine, nasal, or lachrymal secretions (Petersen & Marfin, 2021). In horses, WNV causes severe neurological disease (ataxia, tremors) with up to 30% fatality (Bertram et al., 2020). Survivors may have long-term deficits (Aleman et al., 2021). Cattle/pigs are typically asymptomatic (Carpenter et al., 2023). Companion animals, such as cats and dogs, exhibit rare clinical cases, with cats potentially experiencing more severe conditions (Nguyen et al., 2022). Birds exhibit neurological symptoms and high mortality (Gamino & Höfle, 2013). Humans experience symptoms from mild fever to neuroinvasive disease (Petersen & Marfin, 2021). Surveillance combines serological and molecular techniques. The World Organisation for Animal Health (WOAH) Terrestrial Manual (2023) recommends the ELISA, Virus Neutralisation Test (VNT), and Plaque Reduction Neutralisation Test (PRNT) for seroprevalence studies. ELISA is scalable but lacks specificity in regions with co-circulating flaviviruses (e.g., USUV, TBEV), necessitating confirmation via PRNT/VNT (Luhken et al., 2022). Molecular methods, such as RT-PCR, are critical for the detection of acute phases and outbreak management (WOAH, 2023; Zhang et al., 2021).

The primary aim of this study is to conduct a systematic review and meta-analysis to estimate the overall prevalence of WNV in animals across Asia from 2000 to 2024. By comparing the prevalence in different countries and evaluating changes over two distinct time periods — 2000 to 2010 and 2011 to 2024 — this study aims to provide a comprehensive understanding of WNV dynamics in the region. This information will be crucial to alarm policy makers in the aspect of disease management strategies, particularly as the risk of WNV spreading to new areas continues to rise.

2. MATERIALS AND METHODS

2.1. Search strategy

The research consisted of a systematic literature review following the precepts of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) methodology (Moher et al., 2011). A systematic search strategy was conducted in the databases PubMed and Scopus to identify all published studies reporting the prevalence of WNV in animals in Asia from April 1, 2024, to May 30, 2024 (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 characterisation OR investigation) AND (WNV OR West Nile virus OR Flavivirus) AND (animals OR poultry OR cattle OR feline OR equine OR porcine). In Scopus, the search terms were applied to the title, abstract and keywords. In PubMed, the search terms were applied in all fields. No language restrictions were applied.

2.2. Selection criteria

A comprehensive literature search identified 1,542 potentially relevant articles published in English, including peer-reviewed journal articles and theses. These articles were screened for relevance based on predetermined inclusion and exclusion criteria. Studies were included if they reported data on the positivity of West Nile Virus (WNV) in various hosts within the Asian continent. Conversely, studies were excluded if they lacked WNV prevalence data or were conducted outside the specified geographical scope. After applying these criteria, the remaining articles were evaluated for their relevance to the study.

2.3 Quality of Study and Bias Assessment

Inter-rater agreement is a critical factor in ensuring the reliability of quality assessments across research constructs. Aiken's V was employed to summarise agreement ratings from a panel of expert judges, following established guidelines. To assess bias, two independent evaluators used a 6-item structured questionnaire, applying a modified risk of bias tool (Higgins et al., 2003; Schwarzer et al., 2015). The inter-rater agreement between these evaluators was measured using Aiken's V index, calculated as follows:

$$V = S/([n*(c-1)])$$

Where V is the item validity index, s is the scores assigned by each rater minus the lowest score in the used category, n is the number of raters, and c is the maximum score in the grading scale. The Aiken V index ranges from 0

to 1.0, with a score of 1.0 indicating perfect agreement among raters who assigned the highest possible score to an item. The questionnaires used a 5-point Likert scale, where one represented "very unlikely" and five represented "very likely". Additionally, Kappa statistics were employed to measure the inter-rater reliability between the two evaluators. The independent ratings of both evaluators were averaged to determine the final score for each study. Studies were considered of acceptable quality if their Aiken V Index exceeded 0.7. The review process was conducted with the rates being blinded to the study authors, institutions, and journals to reduce potential bias. Aiken's V Index was used to calculate the degree of agreement between the rating scores obtained by the two independent evaluators. If the Aiken V Index is≥ 0.7, the study quality is considered confirmatory and acceptable (Suresh et al., 2022). Ultimately, 40 studies were selected for the meta-analysis, as outlined in Table 1.

Table 1: Risk of bias tool used for inter-rater agreement checking between two raters.

No.	Domain	Evaluator 1 (Mean)	Evaluator 2 (Mean)	Kappa Value
External validation				
1	Did the study provide an accurate description of the type of validity tested?	4.46	4.46	0.60
2	Were the samples in the study representative of the sample population from which they were undertaken?	4.52	4.54	0.82
3	Was the probability of bias minimal?	4.55	4.48	0.70
Internal validation				
4	Was the study method used to measure the parameter valid and reliable?	4.47	4.43	0.78
5	Was the same mode of data collection used?	4.52	4.58	0.84
6	Summary of the overall risk of study bias	4.41	4.54	0.76

2.4 Statistical analysis method

Statistical analyses were performed using descriptive statistics to provide a simple summary of the reports and their apparent prevalence. A meta-analysis of prevalence data was conducted, pooling the estimates along with 95% confidence intervals (CIs). Given the anticipated substantial heterogeneity, a random-effects meta-analysis was applied, with heterogeneity estimates derived from the inverse-variance model (DerSimonian & Laird, 1986). To explore the heterogeneity further, subgroup analysis was performed by stratifying studies based on their geographic location, categorised as West Asia, Middle East Asia, Southern Asia, and Western Asia, as well as by affected hosts (either humans or animals). To assess publication bias, the Begg and Egger tests were employed, supplemented by visual inspection of the funnel plot. Meta-regression was used to explore factors that may contribute to heterogeneity between studies. In the univariable analysis, factors such as sample size (as both a continuous and categorical variable), study location, reported WNV prevalence type, study year, affected host, and diagnostic methods were examined. In the multivariable meta-regression, variables with a p-value < 0.05 in the univariable analysis were included, and only those significant at $p \leq 0.05$ were retained in the final model.

3. RESULT AND DISCUSSION

3.1 Article finding and screening

A total of 1542 articles were retrieved from PubMed (n =636), Web of Science (n =364) and Google Scholar (n = 542). In the first screening, 497 duplicates were identified and removed, and publications were excluded due to not being conducted in Asia (n = 318). Also eliminated were book chapters (n = 4) and articles unrelated to the targeted diseases (n = 288). Thus, a total of 435 articles were included in the full-text assessment (Figure 1). The list of articles (including titles, authors, abstracts and years of publication) is attached in the Annexe (Excel) file.

Subsequently, a total of 435 full-text articles were assessed in the second screening, where 364 articles were excluded because of the full text being unavailable (n = 27), the animal selection procedure being unclear (n = 228), or the results not being presented clearly (n = 109). Thus, 38 publications were included in the final qualitative synthesis.

3.2 Descriptions of articles

Most of the papers found were on studies conducted in Turkey (9/38), Israel (4/38), followed by China (2/38), India (1/38), Russia part of Siberia and Volgograd (2/38), Singapore (1/38), Indonesia (1/38), Iran (4/38), Japan (2/38), Malaysia (3/38), Pakistan (2/38), Qatar (1/38), South Korea (4/38), Cambodia (1/38) Kazakhstan (1/38), Vietnam (1/38) as depicted in Figure 2.

The systematic review encompassed a comprehensive analysis of studies investigating the prevalence of West Nile Virus (WNV) across various countries in Asia and surrounding regions. A total of 38 studies, spanning different time periods from 2000 to 2024, were evaluated. These studies encompassed diverse countries, including Israel, Turkey, Japan, South Korea, China, Pakistan, India, Iran, Malaysia, Singapore, Qatar, Kazakhstan, Cambodia, and Russia. The prevalence of WNV varied widely

across these studies, ranging from 0% in studies conducted by Yeh et al. (2011b) in South Korea to 0% in studies conducted by Zohaib et al.

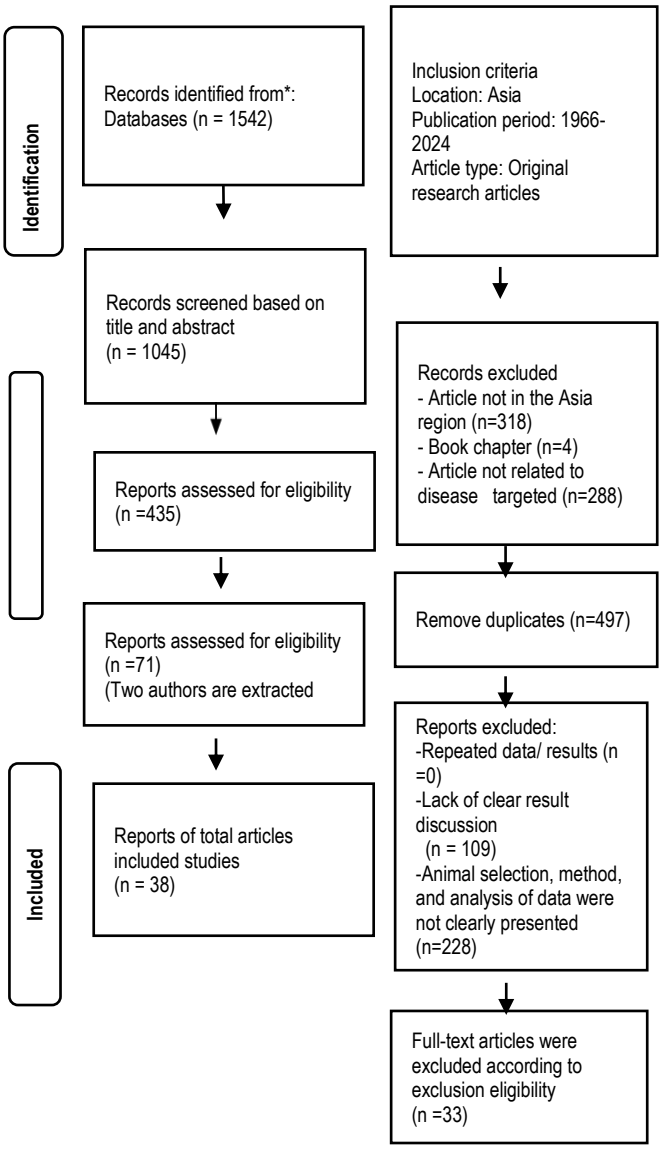

Figure 1: Schematic flow chart of the literature selection for the review on West Nile diseases in Asia.

(2019) in Pakistan, and Soh, S., and Aik, J (2021) in Singapore, to as high as 69.13% reported by Pham-Thanh et al. (2021) in Vietnam. Its disparities likely stem from differences in diagnostic methods (PCR vs. ELISA), species sampled (wild birds vs. domestic dogs), sample sizes, and ecological suitability (Vietnam's wetland–mosquito abundance vs. urban Singapore). Smaller sample sizes and reliance on single methods (e.g., ELISA) often yielded higher prevalence rates, whereas large-scale molecular testing (e.g., Singapore, with over 41,000 samples by RT-PCR) returned near-zero prevalence rates, underscoring the methodological influence. The diagnostic methodologies employed in these studies included enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), reverse transcription-PCR (RT-PCR), plaque reduction neutralisation test (PRNT), virus neutralisation test (VNT), haemagglutination inhibition (HI), and the focus reduction neutralisation test (FRNT). ELISA was the most frequently used screening tool, either alone or in combination with confirmatory methods such as PRNT or PCR. For instance, Murata et al. (2011) employed FRNT to evaluate WNV prevalence in Japan, while Kuwahara et al. (2012) combined ELISA and PRNT in the same country. The reliability of these diagnostic tests varied depending on the co-circulation of other flaviviruses in the regions under study, as cross-reactivity among flaviviruses is a well-known issue in serological diagnostics. To mitigate this, studies often employed multiple tests, including confirmatory assays like PRNT and VNT, to ensure diagnostic accuracy.

In many instances, the number of positive samples was relatively small compared to the total number tested, reflecting the sporadic nature of WNV outbreaks in certain regions. Notably, Zohaib et al. (2015) in Pakistan and Pham-Thanh et al. (2021) in Vietnam reported strikingly high prevalence rates of 65.03% and 69.13%, respectively, suggesting potential WNV hotspots or the occurrence of localised outbreaks.

The variability in WNV prevalence observed across studies may be attributed to several factors, including differences in the year of sample collection, geographic distribution, sample size, species under investigation, and the diagnostic methods employed (Table 2). Additionally, the environmental and climatic conditions in different regions could influence vector abundance and viral transmission dynamics, potentially contributing to the observed heterogeneity in prevalence rates. Some studies, such as Soh, S., and Aik, J (2021), incorporated climatic factors into their analysis, reflecting the growing recognition of the role that climate plays in shaping the epidemiology of vector-borne diseases like WNV.

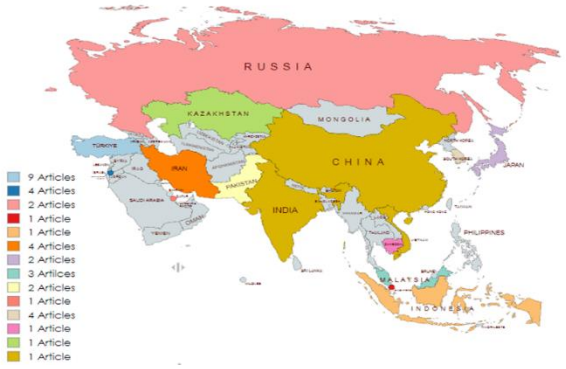

Figure 2: Geographical distribution of studies on zoonotic pathogens in Asia. Map showing Asia countries for which data were included in the systematic review and created using https://mapchart.net/europe.html with different colour shading indicating the number of studies performed in each country.

Table 2: Characteristics of studies included in the systematic review.

No	Publication	Country	No. tested	Prevalence (%)	Screening test
1	Ozkul et al., 2006	Turkey	764	13.35	PRNT
2	Kalaycioglu et al., 2012	Turkey	47	25.52	ELISA, PRNT
3	Murata et al.,2011	Japan	145	14.48	FRNT
4	Yeh et al., 2011a	South Korea	2275	0	RT-PCR
5	Lan et al., 2013	China	436	1.14	ELISA, PRNT
6	Kuwahara et al., 2012	Japan	4000	1.2	ELISA, PRNT
7	Ozkul et al., 2013	Turkey	180	31.67	ELISA, PCR, PRNT
8	Ergunay et al., 2014	Turkey	1702	9.11	PRNT
9	Myint et al., 2014	Indonesia	406	0.99	ELISA, RT-PCR
10	Marlina et al.,2014	Malaysia	742	1.21	ELISA
11	Zohaib et al., 2015	Pakistan	449	65.03	ELISA
12	Kim et al.,2016	South Korea	75	4	ELISA
13	Monaco et al.,2016	Turkey	27	55.56	ELISA
14	Basal et al., 2017	Israel	3,145	11.13	ELISA
15	Ergünay et al., 2017	Turkey	12,711	4.32	PCR
16	Lustig et al., 2017a	Israel	1318	18.28	ELISA
17	Lustig et al., 2017b	Israel	149	14.81	ELISA, RT-PCR
18	Khan et al.,2017	India	1154	32.32	RT-PCR
19	Shahhosseini et al., 2017	Iran	32 317	0.33	RT-PCR
20	Ziyaeyan et al.,2018	Iran	494	20.65	ELISA
21	Akıner et al., 2019	Turkey	75	13.33	PCR
22	Kim et al.,2017	South Korea	75	4	ELISA
23	Zohaib et al., 2019	Pakistan	4150	0	RT-PCR
24	Auerswald et al., 2020	Cambodia	620	29.2	HI, FRNT
25	Dargham et al., 2021	Qatar	1,948	10.42	ELISA
26	Nurmakhanov et al., 2021	Kazakhstan	454	4.63	ELISA
27	Schvartz et al., 2020	Israel	37	29.73	RT-qPCR
28	Shahhosseini et al., 2020	Iran	5028	57.3	RT-PCR
29	Stacy Soh and Joel Aik, 2021	Singapore	41,170	0	CLIMATIC FACTORS
30	Bakhshi et al., 2021	Iran	220	14.55	ELISA
31	Chatterjee et al.,2021	Korea	1877	1.12	RT-nPCR
	Korobitsyn et al., 2021	Russia	412	26.94	PCR
32	Mohammed et al., 2021	Malaysia	80	62.5	ELISA, RT-PCR
33	Pham-Thanh et al., 2021	Vietnam	486	69.13	ELISA
34	Zhang et al., 2021	China	2876	1.91	ELISA & PRNT
35	Natasha et al., 2022	Malaysia	285	12.3	RT-nPCR
36	Shartova et al., 2022	Russia	1283	0.32	RT-PCR
37	Taskin et al., 2023	Turkey	416	0.72	ELISA
38	Bektore et al., 2024	Turkey	442	2.26	ELISA & VNT

N.S., not specified

ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; RT-PCR, reverse transcription-PCR; RT-qPCR, Quantitative reverse transcription polymerase chain reaction; PRNT, plaque reduction test; VNT, virus neutralisation test; FRNT 80, 80% focus reduction neutralisation test; HI, Haemagglutination Inhibition; RT-nPCR, reverse transcription-based nested PCR.

3.3 Sub-group and sensitivity analysis

To perform subgroup analysis, the countries were sub-grouped into six regions including East Asia (China, Japan and Korea), South Asia (India and Pakistan) Southeast Asia (Malaysia, Cambodia, Indonesia, Singapore and Vietnam), West Asia (Qatar, Israel, Iran and Turkey), Central Asia (Kazakhstan), and in North Asia (Russia part of Siberia and Volgograd) region, tests are subjected to subgroup analysis with a level of sample size under sub-grouped into period 2000 to 2010 and 2011 to 2024 (Table 3). Sensitivity analysis was used in this study to identify the influential study by eliminating one study at a time. The prevalence of West Nile Virus (WNV), as determined by different diagnostic tests across various geographical regions and time periods, highlights significant spatiotemporal variations. Linked to intensified surveillance post-2010, improved diagnostics, increased vector densities due to irrigation expansion, and more migratory bird stopovers. The data spans two decades (2000-2010 and 2010-2024), and the diagnostic tests considered include ELISA, VNT, and PCR.

Table 3: Prevalence of West Nile Virus according to diagnostic tests with samples for subgroup analysis.

Particulars	2000-2010			
	Prevalence proportion (95% CI)	I^2 (%)	τ^2	P-Value
East Asia region				
ELISA	0	0	0	0
Neutralisation test	0.24[0.12;0.39]	95	0.0253	0.046
PCR	0.15[0.12;0.18]	77	0.0029	0.051*
Overall Prevalence	0.15[0.11; 0.19]	98	0.0477	0.036
South East Asia				
ELISA	0.29[0.10;0.52]	93	0.0767	0.008
Neutralisation test	0	0	0	0
PCR	0	0	0	0
Overall Prevalence	0.16[0.04;0.37]	97	0.0482	0.023
South Asia				
ELISA	0	0	0	0
Neutralisation test	0	0	0	0
PCR	0	0	0	0
Overall Prevalence	0	0	0	0
West Asia				
ELISA	0.08[0.04;0.11]	87	0.0107	0.021
Neutralisation test	0.33[0.15;0.54]	93	0.0663	0.052*
PCR	0	0	0	0
Overall Prevalence	0.20[0.14;0.27]	99	0.0062	0.009
Middle East Asia				
ELISA	0	0	0	0
Neutralisation test	0	0	0	0
PCR	0.02[0.01;0.04]	94	0.0058	0.045
Overall Prevalence	0.24 [0.16;0.32]	93	0.0477	0.018
Central Asia				
ELISA	0	0	0	0
Neutralisation test	0	0	0	0
PCR	0.39[0.27;0.52]	97	0.1198	0.083*
Overall Prevalence	0.20[0.14;0.27]	88	0.0062	0.028
All regions Overall Prevalence	0.075 (0.071,0.079)	99	0.0868	0.041

Particulars	2010-2024			
	Prevalence proportion (95% CI)	I^2 (%)	τ^2	P-Value
East Asia region				
ELISA	0.75[0.60;0.88]	96	0.0538	0.024
Neutralisation test	0.33[0.15;0.54]	93	0.0663	0.015
PCR	0.30[0.11;0.53]	93	0.0767	0.045
Overall Prevalence	0.32[0.11;0.56]	95	0.0324	
South East Asia				
ELISA	0.14[0.10;0.39]	96	0.0153	0.023
Neutralisation test	0.18[0.12;0.18]	87	0.0039	0.021
PCR	0.12[0.11; 0.19]	97	0.0487	0.024
Overall Prevalence	0.39[0.27;0.52]	99	0.1198	0.030
South Asia				
ELISA	0.42[0.21;0.66]	98	0.0424	0.014
Neutralisation test	0.02[0.01;0.04]	94	0.0058	0.048
PCR	0.24 [0.16; 0.32]	93	0.0477	0.145*
Overall Prevalence	0.16[0.04;0.35]	97	0.0482	0.052
West Asia				
ELISA	0.42[0.21;0.66]	98	0.0424	0.014
Neutralisation test	0.65[0.50;0.78]	96	0.0638	0.024
PCR	0.32[0.13;0.55]	92	0.0867	0.205*
Overall Prevalence	0.24 [0.16; 0.32]	93	0.0058	0.032
Middle East Asia				
ELISA	0.19[0.02;0.42	93	0.0867	0.306*
Neutralisation test	0.08[0.04;0.11]	87	0.0107	0.008
PCR	0.43[0.25;0.64]	94	0.0673	0.025
Overall Prevalence	0.35[0.22;0.548	97	0.0298	0.023
Central Asia				
ELISA	0.16[0.04;0.35]	92	0.0482	0.036
Neutralisation test	0	0	0	0
PCR	0.24[0.18;0.29]	92	0.0061	0.508*
Overall Prevalence	0.39[0.27;0.52]	99	0.1198	0.036
All regions Overall Prevalence	0.26[0.21;0.31]	95	0.0768	0.018

Note: Species includes bovines, porcines, felines, canines, birds, non-ruminants, small ruminants and pets

* $p > 0.05$

In the East Asia region, the prevalence of WNV detected by ELISA remained at 0% from 2000 to 2010, but significantly increased to 75% (P = 0.024) in the subsequent decade (2010-2020). This change is consistent with the rising prevalence rates detected by the VNT and PCR, which also increased from 24% (P=0.046) to 33% (P=0.015) and from 15% (P=0.051) to 30% (P=0.045), respectively. The heterogeneity between studies, as indicated by the I^2 statistic, was substantial, with values exceeding 90% for all tests, underscoring significant variability between studies. In the Southeast Asia region, a similar trend was observed, with ELISA showing a decrease in prevalence from 29% (P = 0.008) in 2000-2010 to 14% (P = 0.023) in 2010-2020.

However, the VNT and PCR showed modest prevalence during the second decade, with prevalence proportions of 18% (P = 0.021) and 12% (P = 0.024), respectively. South Asia exhibited an even more dramatic change, where WNV prevalence remained undetectable across all tests from 2000 to 2010 but increased sharply from 2010 to 2020, particularly for ELISA (42%, P = 0.014) and PCR (24%, P = 0.145). West Asia, which includes countries such as Turkey, exhibited a notable increase in the overall prevalence of West Nile Virus (WNV) over the two decades. ELISA-based detection rose significantly from 8% (P = 0.021) to 42% (P = 0.014), while virus neutralisation tests (VNT) showed an increase from 33% (P = 0.052) to 65% (P = 0.024). Molecular detection methods also reflected a rise, with prevalence increasing from 0% in the early 2000s to 32% during 2010–2020 (P = 0.205). In the Middle East, where WNV prevalence was low or undetectable between 2000 and 2010, the subsequent decade showed substantial increases, particularly in PCR-based detection, which rose to 43% (P = 0.025). In Central Asia (Kazakhstan), PCR prevalence declined from 39% (P = 0.083) to 24% (P = 0.508), despite an overall increase in WNV prevalence from 20% (P = 0.028) to 39% (P = 0.036) between the two decades. Central Asia, although PCR-based detection of WNV decreased from 39% to 24% between the two decades, this change was not statistically significant (P = 0.083 and P = 0.508, respectively). However, the overall WNV prevalence in the region increased significantly from 20% (P = 0.028) to 39% (P = 0.036), suggesting a broader trend of increasing WNV presence not fully captured by PCR alone.

North Asia has seen a noticeable rise in the detection of West Nile Virus (WNV). PCR-based prevalence rose sharply from 2% (P = 0.045) to 43% (P = 0.025), and the overall WNV prevalence increased from 24% (P = 0.018) to 35% (P = 0.023), suggesting a significant emergence or improved detection capacity in the region during the second decade. ELISA tests remained negative throughout, while VNT detection appeared only in the later decade (P = 0.008), further highlighting the need for increased surveillance.

The overall prevalence across all regions increased sharply from 7.5% (P = 0.036) in 2000-2010 to 26% (P = 0.018) in 2010-2020. This substantial increase is indicative of heightened WNV activity in the latter decade, with rising detection rates across regions, particularly in East Asia, West Asia, and South Asia. The high heterogeneity (I^2 > 90%) and between-study variance (τ^2) indicate the presence of substantial variability in WNV prevalence across different diagnostic methods, regions, and study designs. The inclusion of diverse species such as bovines and small ruminant populations further highlights the widespread nature of WNV and the importance of continuous surveillance across different geographic and biological contexts.

3.4 Publication bias

Publication bias is a significant concern in systematic reviews and meta-analyses, as it can undermine the validity and generalisability of conclusions. This study assessed the presence and impact of publication bias in a meta-analysis by focusing on the inclusion of studies with a high likelihood of publication due to positive results or the use of new confirmatory tests. The extent of publication bias was evaluated using a funnel plot, where the Y-axis represented each study's standard error and the X-axis indicated the country of the study. A well-distributed funnel would suggest no bias, with high-accuracy studies following the regular line and low-accuracy studies distributed symmetrically around it. However, the results showed a clear dispersion of studies, with only a few falling into the funnel, indicating the presence of publication bias. Egger's test was conducted to further assess the level of bias. To address this bias, we employed meta-regression, using sample size as a bias component, which confirmed the presence of publication bias but established its significance as (p = 0.01), suggesting a limited influence on the overall findings.

The forest plot depicted in the image presents a meta-analysis summarising the effect sizes of studies related to a particular intervention or exposure across multiple regions, with significant heterogeneity among the findings (Figure 3). Each blue square in the plot represents the estimated effect size for individual studies, where the size of the square is proportional to the weight of the study in the overall analysis. The horizontal lines extending from these squares indicate the 95% confidence intervals of each study's effect size. The dashed red line marks the estimated overall effect size, while the diamond at the bottom reflects the cumulative effect size, with its width representing the confidence interval.

A striking feature of this analysis is the degree of heterogeneity, as reflected by key statistical parameters. The tau-squared value (τ^2) of 221098.20 indicates substantial variance between the effect sizes, not merely attributable to chance. Similarly, the I^2 statistic of 1.00 (100%) demonstrates that almost all of the variation between study results is due to heterogeneity rather than random error, highlighting the complexity of synthesising these studies. This is further corroborated by the Q-value for homogeneity (Q = 775864.97, p = 0.00), which rejects the null hypothesis of homogeneity, confirming that the studies are significant in their reported outcomes. The diamond at the bottom of the plot represents the overall effect size estimate from the random-effects model, showing that the aggregated result remains statistically significant (z = 2.43, p = 0.01). This model appropriately accounts for between-study variance, giving more conservative yet robust overall estimates. The confidence interval surrounding this overall estimate suggests a reliable summary effect across diverse populations and methodologies.

Overall, the forest plot underscores a high level of variability across the studies included in the analysis. Despite this heterogeneity, the meta-analysis reveals a significant overall effect, suggesting that the intervention or exposure has a meaningful impact. This result emphasises the importance of accounting for both study-specific differences and methodological variances in the context of meta-analyses, particularly in diverse and complex datasets.

The funnel plot presented illustrates the potential for publication bias in the meta-analysis of the included studies (Figure 4). In this plot, the vertical axis represents the standard error of the effect size estimate, which is inversely related to study size. In contrast, the horizontal axis indicates the positive effect size for each study. Each blue circle symbolises an individual study, with smaller studies, reflected by larger standard errors, appearing towards the bottom of the plot, while larger studies are concentrated towards the top.

Ideally, in the absence of publication bias, studies would be symmetrically distributed around the vertical line representing the overall effect size, as larger studies tend to cluster near the centre while smaller studies should scatter more widely due to greater variability. However, the funnel plot here exhibits some degree of asymmetry, particularly with more studies appearing on one side of the overall effect size line. This asymmetry suggests that there may be publication bias, where smaller studies with non-significant or negative findings might be underreported or excluded from the analysis, skewing the results towards a more positive outcome.

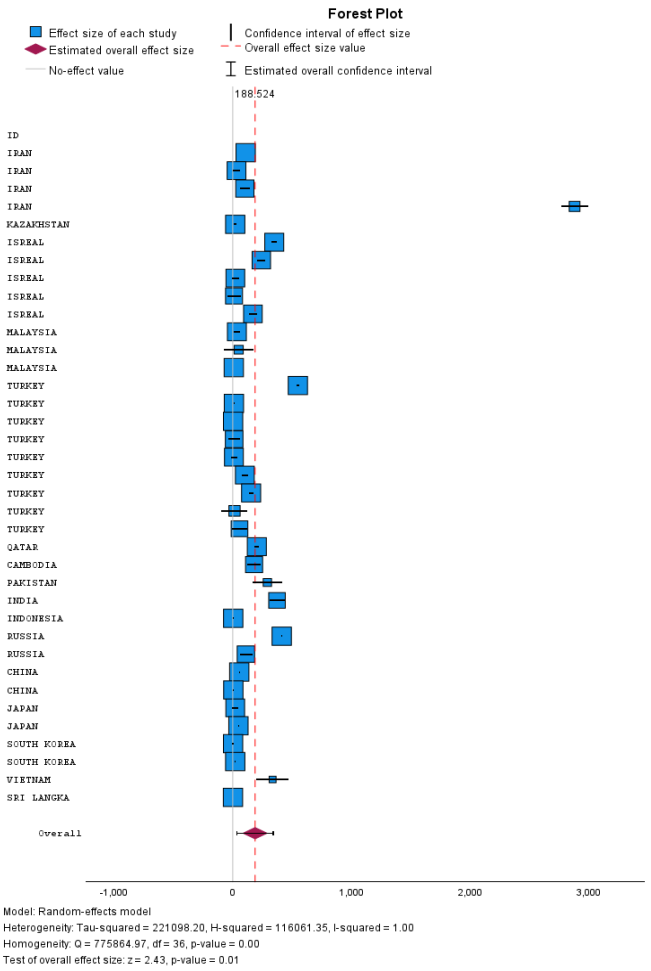

Figure 3: Forest plot for the prevalence of West Nile Virus (WNV) in the Asian continent based on a test with a sample size.

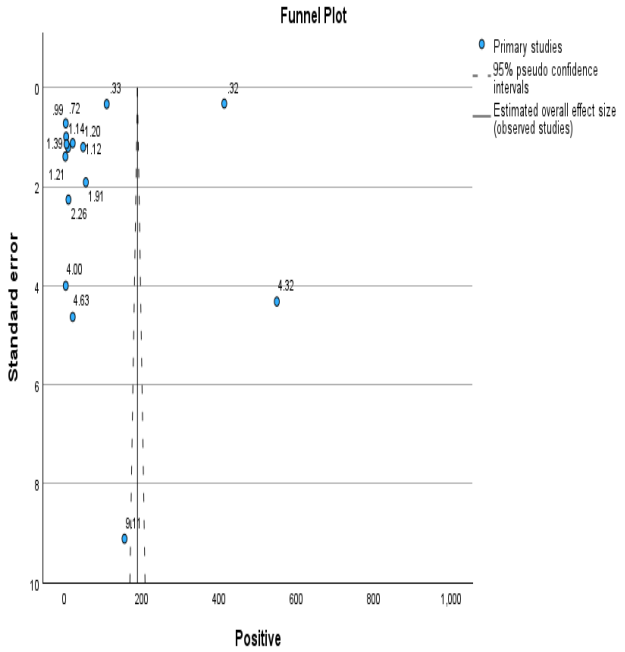

Figure 4: A funnel plot did not identify significant publication bias.

Moreover, the dashed lines indicate the 95% pseudo-confidence intervals, which provide a reference range for the expected distribution of studies. Several studies fall outside these lines, particularly towards the bottom-right quadrant, suggesting that these may be outliers or studies with larger effect sizes than expected based on their sample sizes. The presence of potential publication bias is critical to acknowledge, as it may artificially inflate the overall effect size, leading to overly optimistic conclusions. To address this, further sensitivity analyses, such as Egger's regression or the trim-and-fill method, could be employed to quantify and correct for the bias, thereby providing a more accurate estimate of the actual effect. Despite these concerns, the plot also highlights the robustness of the larger studies, which cluster towards the centre, suggesting that the overall effect estimate may still hold validity despite potential bias in smaller studies.

This systematic review aimed to highlight key trends in the prevalence of WNV in animals across Asia. Data from 380studies, spanning the years 2000 to 2020, were analysed. The pooled prevalence of WNV for the period from 2000 to 2010 was 7.5% (P = 0.041), while from 2011 to 2024, it increased to 26% (P = 0.018). However, only a few studies reported using random sampling methods, necessitating caution when generalising these prevalence estimates to the broader target population. Significant heterogeneity (I^2 = 99.3%) limited the ability to draw meaningful conclusions about differences in prevalence across six geographic regions: East Asia, South Asia, Southeast Asia, West Asia, Central Asia, and North Asia.

West Nile virus (WNV) presents significant health risks to various animal species, including chickens, goats, and horses, particularly in tropical regions where mosquitoes breed abundantly in waterlogged areas. In these environments, the high prevalence of Culex mosquitoes, which are primary vectors for WNV, facilitates rapid virus transmission, leading to increased infection rates in livestock and potentially severe neurological outcomes in equines (Habarugira et al., 2020). The movement of infected animals and mosquitoes across regions, often exacerbated by human transportation networks, heightens the risk of disease spread, enabling WNV to emerge in new locations, including previously unaffected areas (Malik et al., 2021). This geographical expansion can severely impact eco-tourism; potential visitors may avoid regions experiencing WNV outbreaks, leading to substantial economic losses for communities reliant on tourism revenue. Furthermore, the economic implications extend to increased costs associated with veterinary care, surveillance, and disease management (Pealer et al., 2003). The dynamic nature of WNV underscores the need for robust early detection systems and immediate response strategies. Implementing surveillance programs that utilise serological and molecular methods can facilitate the timely identification of WNV in both animal populations and mosquito vectors, enabling swift interventions to mitigate outbreaks in animal health (Ducrocq et al., 2022).

4. CONCLUSION

In conclusion, West Nile virus (WNV) remains a significant public health challenge across Asia, with substantial implications for both animal and human populations. The findings of this systematic review underscore the urgent need for enhanced surveillance systems, particularly in regions where data is sparse or unreliable. Continued efforts to improve the collection of epidemiological data are essential for accurately assessing the prevalence and spread of WNV. Moreover, the implementation of effective prevention and control strategies is crucial to mitigate the impact of future outbreaks. Strengthening public health infrastructure and fostering regional collaboration will be key to addressing the ongoing threat of WNV and safeguarding animal health in Asia. Ultimately, addressing the complexities of WNV requires an integrated approach that combines veterinary public Health, environmental management, and economic considerations to minimise the virus's impact on ecosystems and local economies.

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