

## Serological detection of Nipah virus in domestic animals in Malaysia between 2012 and 2023

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### ABSTRACT

Malaysia experienced the Nipah virus outbreak in Negeri Sembilan, Perak, and Selangor from September 1998 to May 1999 and was granted Nipah-free status by World Organisation for Animal Health (WOAH) in 2001. In response to the severity of Nipah virus infections in both pigs and humans, the Department of Veterinary Services (DVS) Malaysia launched a Nationwide Surveillance Program designed to monitor pigs and domestic animals for the presence of the virus. Between 2012 and 2023, a total of 44,755 serum samples were received from cats (3,359), dogs (3,713), pigs (26,507), and horses (11,176) and tested using an in-house Indirect ELISA developed by the Veterinary Research Institute (VRI) of DVS Malaysia. No seropositive cases of Nipah virus IgG antibodies were detected in any of the tested samples. While this indicates that Malaysia has maintained its Nipah-free status, the absence of detectable antibodies does not eliminate the possibility of sporadic infections or the presence of the virus in other wildlife or animal reservoirs. This highlights the importance of ongoing surveillance efforts and emphasizes the need to understand zoonotic risks for both veterinary and public health in Malaysia.

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## 1. INTRODUCTION

Nipah virus (NiV) is a highly pathogenic paramyxovirus first recognized during a devastating outbreak of viral encephalitis among pig farmers and abattoir workers in Malaysia in 1998–1999 (Chua et al., 2000). Belonging to the Henipavirus genus within the *Paramyxoviridae* family, NiV is closely related to Hendra virus and is capable of causing severe respiratory and neurological disease in humans and animals (DeBuysscher et al., 2013). The Malaysian outbreak was ultimately traced to the spillover of NiV from fruit bats (*Pteropus* spp.) to pigs, which subsequently acted as amplifying hosts (Tan & Chua, 2008), with risk factors for human infection identified through a case-control study conducted during the outbreak (Parashar et al., 2000). Retrospective analyses suggested that the introduction of NiV into pig populations was likely facilitated by the consumption of fruit contaminated by bat secretions (Chowdhury et al., 2023).

Clinically, NiV presents with species-specific manifestations. In pigs, infection is characterised by high fever, barking cough, and severe respiratory distress, with young piglets often experiencing nervous signs such as trembling and gait abnormalities (Khan et al., 2024). In humans, the clinical spectrum ranges from asymptomatic or mild influenza-like illness to acute and often fatal encephalitis.

During the Malaysian outbreak, most human patients presented with encephalitis and relatively few displayed respiratory signs (Goh et al., 2000). Key clinical descriptions are available (Ang et al., 2018; CDC, 2024). By contrast, subsequent outbreaks in Bangladesh and India have more frequently involved respiratory symptoms, including cough and acute respiratory distress syndrome, often alongside neurological involvement (Chimire et al., 2022). These differences have been attributed to genetic and phenotypic variation between NiV strains—NiV-Malaysia (NiV-M) and NiV-Bangladesh (NiV-B)—and to distinct transmission dynamics. While the Malaysian outbreak was largely pig-to-human, outbreaks in Bangladesh and India demonstrated direct human-to-human transmission, particularly in households and healthcare settings (Khan et al., 2024; Sakharkar, 2021).

Globally, NiV has caused at least 754 confirmed cases and 435 deaths across Bangladesh, India, Malaysia, Singapore, and the Philippines, yielding an average case fatality rate (CFR) of 58% (Khan et al., 2024). In Malaysia and Singapore, the 1998–1999 outbreak resulted in 294 cases and 110 deaths (39% CFR), with most cases occurring in Malaysia due to pig exposure. Since then, NiV has not re-emerged in either Malaysia or Singapore, but recurrent outbreaks in Bangladesh and India have kept the pathogen in global focus.

It has also been detected at the human–flying-fox interface in Cambodia (Duong et al., 2020). In 2014, a horse-associated outbreak in the Philippines caused 17 confirmed cases and 9 deaths (53% CFR), demonstrating that equines can also act as spillover hosts (Khan et al., 2024). These repeated outbreaks highlight NiV's adaptability and its capacity to exploit multiple transmission pathways.

The post-Malaysian outbreaks have revealed critical differences in transmission ecology. In Bangladesh, direct contamination of raw date palm sap by bats has been identified as a major transmission route (Chimire et al., 2022; Deka & Morshed, 2018). India has seen outbreaks characterized by nosocomial spread, highlighting the potential for prolonged chains of human-to-human transmission (Arunkumar et al., 2019). NiV is therefore recognized as a pathogen with epidemic and pandemic potential. Modelling studies and reviews indicate that deforestation, agricultural intensification, and wildlife trade further increase the probability of future spillovers (Clayton et al., 2013; Hilderink & Winter, 2021; Plowright et al., 2015). Regional modelling has also highlighted the potential risk of Nipah virus emergence and spread in East Asia, particularly China (Yu et al., 2018).

Recent analyses have quantified the scale of the threat. The World Bank (2023) projected that a single large outbreak could cost the global economy up to USD 2 billion, factoring in healthcare costs, productivity losses, trade restrictions, and containment measures. The World Health Organisation (WHO, 2019; WHO, 2024) has included NiV in its R&D Blueprint of priority pathogens requiring urgent countermeasure development, while Moore et al. (2024) emphasised NiV in setting research priorities for the coming decade. The Coalition for Epidemic Preparedness Innovations (CEPI, 2024) has initiated vaccine development programs, although no licensed vaccines or therapeutics are currently available. Recent reviews reaffirm NiV as one of the most serious re-emerging zoonoses, combining high mortality, a broad host range, and the potential for international spread (Branda et al., 2025; Faus-Cotino et al., 2024).

Fruit bats of the genus *Pteropus* are the principal natural reservoirs of NiV. Viral RNA and antibodies have been detected in *P. medius* and *P. lylei* across South Asia (McKee et al., 2022; Wacharapluesadee et al., 2010, 2021), and in *P. vampyrus* and *P. hypomelanus* in Malaysia (Rahman et al., 2013). The ecology of NiV within bat populations is influenced by seasonality, with shedding peaks associated with mating and birthing periods (Wacharapluesadee et al., 2010). Serological surveys have reported prevalences ranging from 12% to 54%, underscoring the virus's entrenched presence in chiropteran hosts (Hauser et al., 2021).

Transmission to humans occurs through various spillover pathways. In Malaysia, infection was mediated by pigs exposed to contaminated fruit, whereas in Bangladesh, it has occurred via contaminated food sources (Tan & Chua, 2008; Khan et al., 2024). Horses have also served as intermediate hosts in the Philippines (Khan et al., 2024). Importantly, peridomestic animals such as dogs and cats were found to be seropositive during the Malaysian outbreak, highlighting their potential role as incidental hosts and sentinels (Chua et al., 2000). Recent studies recommend including these species in surveillance programs because of their proximity to humans and livestock (McKee et al., 2022).

The emergence of novel henipa-like viruses in shrews (Caruso & Edwards, 2023; Zhang et al., 2022), bats from Madagascar (Madera et al., 2022), and fruit bats in Cameroon (Mbu'u et al., 2025) expands the known diversity of this viral group. These discoveries suggest that the true range of henipaviruses is underestimated and that surveillance must extend beyond *Pteropus* bats. This broader ecological context emphasizes the need for a One Health approach, integrating veterinary, human, and environmental health in surveillance strategies (Hilderink & Winter, 2021; Naguib et al., 2021).

The Malaysian outbreak prompted unprecedented control measures, including the culling of over one million pigs, closure of nearly 1,000 farms, strict animal movement restrictions, and large-scale compensation schemes (Chua, 2010; Chowdhury et al., 2021; EMJ, 2024). These interventions successfully halted transmission, but at a massive economic and social cost. More recently, the 2023 Kerala outbreak in India led to school closures, travel restrictions, and containment zones despite involving only six confirmed cases (Frontiers in Public Health, 2024), illustrating the disruptive socio-economic impact of even small outbreaks.

In Malaysia, the DVS, through the VRI, continues to coordinate nationwide surveillance. These efforts are legally mandated under the Animals Act 1953 and serve as the basis for the country's WOAH-certified Nipah-free status (WOAH, 2022). Surveillance includes routine monitoring of pigs, regulatory testing of horses, and submissions from cats and dogs. Wild boar surveillance has also been incorporated in recent years, reflecting recognition of their role as potential spillover hosts.

Internationally, NiV preparedness has gained traction within the WHO and CEPI frameworks. The WHO's Blueprint identifies NiV as a priority for accelerated research (WHO, 2019), while CEPI has invested in candidate vaccines that have entered preclinical and early clinical evaluation (CEPI, 2024). Moore et al. (2024) stressed the need for enhanced field diagnostics, vaccine stockpiles, and capacity-building in high-risk regions. Spatial modelling has also proven

useful for identifying high-risk zones and optimizing resource allocation (Deka & Morshed, 2018).

Despite the extensive global focus on NiV, there remains a paucity of long-term, multi-species surveillance data in Malaysia. Most published studies address outbreak investigations or ecological surveys in bats rather than systematic monitoring of domestic animals (Sendow et al., 2010; McKee et al., 2022). This knowledge gap is critical because domestic animals can act as both amplifying hosts and sentinels at the human–wildlife interface (Hauser et al., 2021).

The present study addresses this gap by retrospectively analysing 44,755 serum samples collected between 2012 and 2023 from pigs, horses, dogs, cats, and wild boar submitted to VRI. It uses an in-house indirect ELISA developed and standardized at VRI, in combination with the Serum Neutralization Test (SNT), to assess the serological status of multiple domestic species over a 12-year period.

## 2. MATERIALS AND METHODS

### 2.1. Sample sources

A total of 44,755 serum samples were submitted to the Veterinary Research Institute (VRI) between 2012 and 2023, with corresponding test records archived in the Laboratory Information Management System (LIMS) and retrieved for retrospective analysis. The samples represented domestic and peri-domestic species, including pigs (both commercial and wild boar), dogs, cats, and horses, and originated from various states across Malaysia as part of routine monitoring, active surveillance, diagnostic investigations, animal-movement verification, research projects, and import–export certification programs. The antigen used in the assay was prepared under biosafety conditions in a biosafety-cabinet (BSC) Level III laboratory at the Zoonotic Section, VRI, following established standard operating procedures (Zoonotic Section, 2011). The positive control was rabbit-derived hyperimmune serum produced in-house at VRI.

### 2.2. Sample testing

At the time of submission, all samples were screened for IgG antibodies against Nipah virus (NiV) using an in-house indirect enzyme-linked immunosorbent assay (ELISA) developed and validated by the Veterinary Research Institute (VRI). The assay was adapted from previously published henipavirus detection protocols (Chua et al., 2000; Daniels, Ksiazek, & Eaton, 2001; Yu et al., 2006) and optimized for Malaysian conditions. In brief, 96-well microplates were coated with inactivated NiV antigen, and diluted sera were added. Bound antibodies were detected using species-specific conjugates and chromogenic substrate reactions. Optical density (OD) values were measured

spectrophotometrically and converted into ELISA units (EU). A diagnostic cut-off of  $\geq 8\%$  EU was applied to identify potential reactors. This threshold was established through validation studies at VRI to minimize both false positives and false negatives and was intentionally conservative, reflecting the need to avoid spurious positives in a country certified as Nipah-free by WOA (WOA, 2022). As this study involved retrospective data retrieved from the laboratory information management system (LIMS), only the final interpreted ELISA results (positive or negative) were recorded. Samples that initially exceeded the 8% EU threshold were routinely re-evaluated using both infected and uninfected Vero cell lysates and confirmed by serum neutralization test (SNT). However, the exact number of borderline reactors was not individually traceable in the archived dataset.

### 2.3. Confirmatory testing by Serum Neutralisation Test (SNT)

To address potential false positives, ELISA-reactive samples were further assessed by SNT using an in-house protocol developed and validated at VRI under biosafety-level III conditions. The procedure followed VRI's internal quality-assurance and biosafety framework, consistent with international guidance in the WOA Terrestrial Manual (2022). This confirmatory step ensured diagnostic accuracy and reliability. The Australian Animal Health Laboratory (AAHL) serves as the reference laboratory receiving Nipah-virus ELISA reactors for additional confirmatory testing.

### 2.4. Data management and analysis

This study was based entirely on retrospective analysis of archived LIMS records from VRI. No new or prospective sampling was undertaken. The dataset was cleaned to remove incomplete or duplicate entries, and descriptive statistics were performed using Microsoft Excel (version 2021). Analyses included sample distributions by species, state, and year, as well as the proportion of submissions flagged as ELISA reactors and their outcomes following SNT. Temporal trends were examined to identify periods of increased surveillance activity, and submission volumes by state were compared with livestock-density data and ecological-risk patterns.

## 3. RESULT AND DISCUSSION

Between January 2012 and December 2023, a total of 44,755 serum submissions were analysed at the VRI. The samples represented multiple species: pigs ( $n = 25,976$ ; 58%), horses ( $n = 11,176$ ; 25%), dogs ( $n = 3,713$ ; 8%), cats ( $n = 3,359$ ; 8%), and wild boar ( $n = 531$ ; 1%). This diverse representation reflects both livestock-associated surveillance and peri-domestic monitoring, consistent with Malaysia's multi-species approach to NiV surveillance.

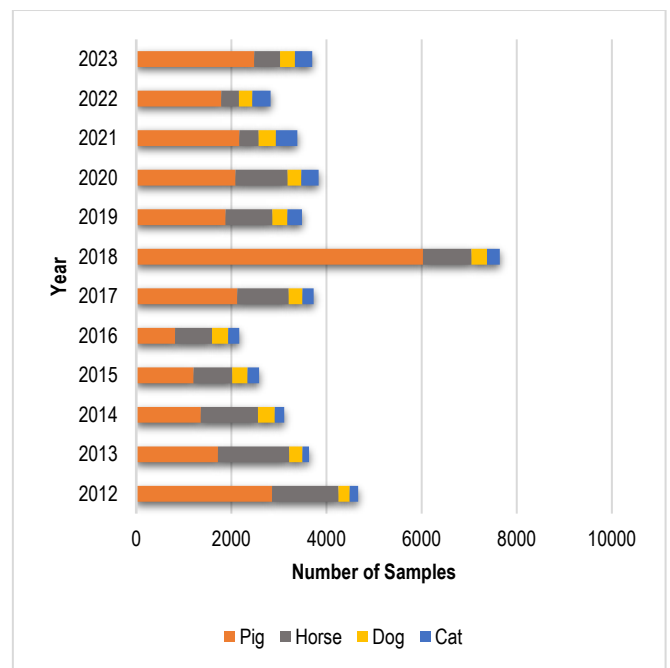
Geographically, the majority of submissions originated from states with intensive livestock production—Selangor, Perak, Sarawak, and Pulau Pinang—which together accounted for more than two-thirds of all samples. Smaller numbers were received from Sabah, Melaka, Johor, and Kuala Lumpur, while Putrajaya and Labuan contributed fewer than 0.1 % of samples. Table 1 summarizes the detailed state- and species-level distribution of submissions, highlighting the concentration of surveillance in high-risk zones where livestock density and human–animal contact is greatest.

**Table 1:** Distribution of serum samples submitted to the VRI for Nipah virus ELISA testing by state and animal species in Malaysia, 2012–2023.

State/ Federal Territory	Commercial pig (n)	Wild Boar (n)	Horse (n)	Dog (n)	Cat (n)	Total (n) (% Of Total)	ELISA/ SNT
Selangor	3,213	18	6,752	1,887	1,619	13,489 (30.1%)	Not detected
Perak	4,278	26	1,583	104	52	6,043 (13.5%)	Not detected
Sarawak	5,782	–	10	12	2	5,806 (13.0%)	Not detected
Pulau Pinang	3,968	107	72	348	223	4,718 (10.5%)	Not detected
Sabah	3,179	5	69	16	14	3,283 (7.3%)	Not detected
Melaka	2,857	61	41	10	11	2,980 (6.7%)	Not detected
Johor	1,842	31	741	117	96	2,827 (6.3%)	Not detected
W.P. K.Lumpur	9	24	614	795	1,026	2,468 (5.5%)	Not detected
Pahang	525	45	778	7	8	1,363 (3.0%)	Not detected
Negeri Sembilan	150	48	84	354	242	878 (2.0%)	Not detected
Terengganu	5	48	282	2	3	340 (0.8%)	Not detected
Kedah	167	42	23	53	44	329 (0.7%)	Not detected
Kelantan	1	55	106	–	–	162 (0.4%)	Not detected
Perlis	–	21	19	7	3	50 (0.1%)	Not detected
W.P. Putrajaya	–	–	–	–	16	16 (<0.1%)	Not detected
W.P. Labuan	–	–	2	1	–	3 (<0.1%)	Not detected
Grand Total	25,976	531	11,176	3,713	3,359	44,755 (100%)	Not detected

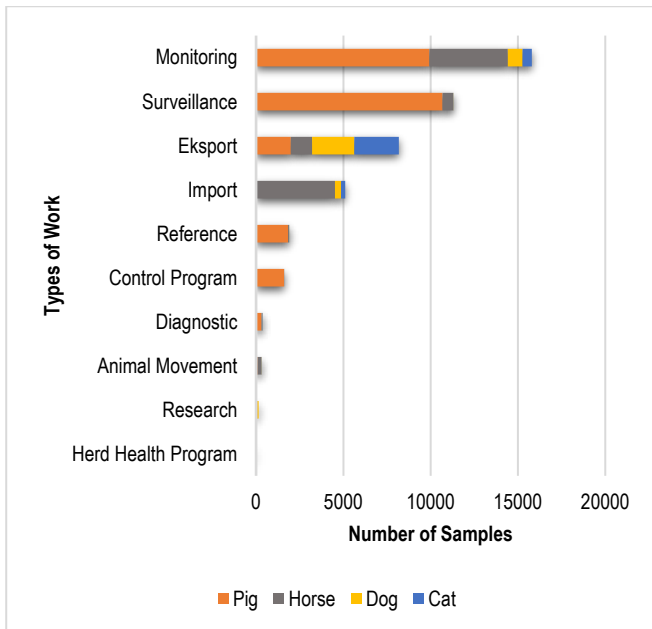
Despite extensive geographic and species coverage, no confirmed NiV-positive samples were identified by ELISA or SNT. A small number of borderline ELISA reactors with values near the 8 % cut-off were initially detected but subsequently confirmed negative after repeat testing with both infected and uninfected Vero-cell lysates and SNT. This multi-tier diagnostic approach minimized false positives and ensured that the observed absence of positives reflected true surveillance outcomes rather than technical artefacts, consistent with practices in Indonesia (Sendow et al., 2010) and Bangladesh (McKee et al., 2022).

Submission volumes varied across the study period. A marked increase in 2018 coincided with heightened awareness following the Kerala outbreak in India (23 cases, 21 deaths) (Arunkumar et al., 2019). Figure 1 shows annual sample distributions by species, demonstrating the program’s ability to respond quickly to regional events. Most submissions came from pig-farming states with greater human–animal interfaces—Selangor, Perak, Pulau Pinang, and Sarawak—which also correspond to ecological-risk zones associated with Pteropus bat populations. Sampling intensity in each state was proportionate to the relative population size of the target animal species, ensuring that higher-density livestock areas contributed more samples to the surveillance dataset. A spatial risk map was not generated, but these data indicate strong sampling coverage in known high-risk regions.



**Figure 1:** Yearly distribution of serum samples submitted to VRI for Nipah virus ELISA testing in Malaysia (2012 to 2023).

The dataset also captured submission purpose categories, including monitoring, active surveillance, diagnostic investigations, animal-movement verification, and permit-related testing. Monitoring and surveillance accounted for the majority of submissions, reflecting Malaysia’s emphasis on preventive action rather than outbreak response (Figure 2).



**Figure 2:** Distribution of serum sample submissions for Nipah virus ELISA testing by types of work in Malaysia, 2012–2023.

The absence of confirmed NiV positives in 44,755 samples over a 12-year period strongly supports Malaysia's continued Nipah-free status since its WOAHA certification in 2001 (WOAHA, 2022). This finding is consistent with national reports and reaffirms the effectiveness of ongoing surveillance systems coordinated by the DVS. The absence of serological evidence in multiple domestic species, including pigs, horses, cats, dogs, and wild boar, strengthens confidence that NiV circulation has not re-emerged in domestic animal populations in Malaysia.

The Malaysian data contrast with reports from Bangladesh and India, where repeated spillovers and occasional human-to-human transmission maintain NiV as a persistent public-health threat (Chimire et al., 2022; Nikolay et al., 2019). In the Philippines, a horse-associated outbreak in 2014 demonstrated that species beyond pigs can mediate human infection (Khan et al., 2024). Malaysia's ongoing absence of cases underscores the success of strict biosecurity, movement control, and long-term monitoring implemented since 1999 (Chua, 2010; Chowdhury et al., 2021).

The inclusion of dogs and cats represents an innovative extension of NiV surveillance. Although not primary hosts, their close contact with humans and livestock makes them valuable peri-domestic sentinels (McKee et al., 2022; Hauser et al., 2021). During the 1998–1999 outbreak, both species exhibited NiV antibodies—likely spillover from infected pigs (Chua et al., 2000). Their continued inclusion demonstrates Malaysia's adoption of a One Health perspective, ensuring that secondary transmission pathways are not overlooked.

Wild-boar surveillance ( $n = 531$ ), though smaller in scale, adds important ecological coverage because wild boar shares habitats with *Pteropus* bats and may serve as spillover hosts. Integrating this wildlife component strengthens risk assessment at the livestock–wildlife interface (Rahman et al., 2013).

Malaysia's two-decade Nipah-free record illustrates that strong veterinary oversight, multi-species surveillance, and One Health collaboration can sustain disease freedom even in high-risk ecosystems. This model offers valuable guidance for neighbouring countries confronting henipavirus threats.

At the global level, novel henipa-like viruses continue to emerge—from shrews (Caruso & Edwards, 2023), fruit bats in Madagascar (Madera et al., 2022), and synanthropic bats and rodents in Cameroon (Mbu'u et al., 2025)—showing that henipavirus diversity extends well beyond current surveillance frameworks. These discoveries underscore the importance of maintaining vigilance and expanding surveillance to unexpected reservoirs.

Preparedness is not only a scientific issue but also an economic and political imperative. The World Bank (2023) estimated that a single NiV outbreak could result in multi-billion-dollar losses. Moore et al., (2024) and Branda et al., (2025) identified NiV as a priority pathogen for the coming decade, requiring expanded vaccine pipelines, rapid field diagnostics, and strengthened international collaboration. Faus-Cotino et al., (2024) further described NiV as a multidimensional threat to health, trade, and food security.

This study was retrospective and relied on archived diagnostic records, introducing potential sampling bias due to uneven submissions among states and species. Although the in-house ELISA was supported by internal controls and confirmed by SNT, quantitative estimates of diagnostic sensitivity and specificity were unavailable. Formal determination of diagnostic sensitivity and specificity is ongoing, as confirmed positive field sera are limited. Nonetheless, the in-house ELISA has been thoroughly optimized using negative sera and internal positive controls, showing consistent and reproducible results under VRI's quality-assurance framework. This process follows WOAHA-recommended validation principles and ensures the assay's reliability for routine surveillance use. The absence of spatial risk assessment also limited the detection of very low-level or localized exposures. Nonetheless, the large dataset and long surveillance period provide robust evidence supporting the reliability of the negative findings.

## 4. CONCLUSION

This study reviewed 44,755 serum samples from pigs, horses, dogs, cats, and wild boar collected between 2012 and 2023. The results showed no evidence of Nipah-virus antibodies in any tested animals, confirming that Malaysia has remained Nipah-free since 1999. These findings reaffirm the strength and continuity of Malaysia's national surveillance system, which integrates multi-species monitoring and applies a One Health framework to detect potential spillovers early at the human–animal–environment interface. While Malaysia remains free from the Nipah virus, outbreaks in Bangladesh, India, and the Philippines demonstrate that the risk of re-emergence persists regionally. The discovery of related henipa-like viruses in bats, rodents, and shrews worldwide further underscores the need for continued vigilance and adaptive surveillance. To ensure continued protection, Malaysia should sustain risk-based surveillance, strengthen monitoring in high-density livestock and wildlife-overlap areas, and maintain close collaboration with international partners. These combined efforts will safeguard animal and human health and contribute to global preparedness against the Nipah virus and other emerging zoonoses.

## DECLARATION

Ethics approval: This study involved the analysis of existing laboratory data and did not require the collection of new animal samples or procedures. The Veterinary Research Institute Ethics Committee has confirmed that no ethical approval is required.

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