Zoonoses and Public Health

WILEY

ORIGINAL ARTICLE OPEN ACCESS

Potential Zoonotic Infections Transmitted by Free-Ranging Macaques in Human–Monkey Conflict Areas in Thailand

Sarin Suwanpakdee[1,2] | Benjaporn Bhusri[2] | Aeknarin Saechin[2] | Chalisa Mongkolphan[2] | Siriporn Tangsudjai[2] | Parut Suksai[2] | Supakarn Kaewchot[3] | Rattana Sariwongchan[3] | Piya Sereerak[3] | Ladawan Sariya[2]

[1]Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand | [2]Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand | [3]Department of Nation Parks, Wildlife and Plant Conservation, Bangkok, Thailand

Correspondence: Ladawan Sariya (ladawan.sar@mahidol.edu)

Received: 20 August 2024 | **Revised:** 12 January 2025 | **Accepted:** 14 January 2025

Keywords: *Macaca* | macaque | nonhuman primate | zoonosis

ABSTRACT

Introduction: Nonhuman primates (NHPs) can transmit zoonotic diseases to humans because of their close genetic relationship, facilitating the cross-species transmission of certain pathogens. In Thailand, *Macaca* is the most common NHP genus and their inhabits area are in close proximity of human, particularly in urban and suburban areas, where frequent interactions with humans increase the risk of pathogen transmission. The risk is influenced by factors such as the type of pathogen, the mode of transmission (e.g. direct contact or vector-borne), and the density of human and macaque populations in the regions. This study aims to investigate potential zoonotic infections in free-ranging macaques residing in human–monkey conflict areas.

Methods: From 2014 to 2023, 2703 macaques across 29 provinces in Thailand were tested for 18 pathogens using PCR, RT-PCR, or real-time PCR. The associations between disease occurrence, demographic variables, and sample types of macaques were analysed using univariable and multivariable regression.

Results: The overall pathogen infection percentage was 35.7% (965/2703). Simian foamy virus (SFV) had the highest infection percentage at 52.5% (759/1446), followed by Herpesviridae at 41.4% (353/852), *Plasmodium* spp. at 1.8% (14/758), and hepatitis B virus at 0.1% (2/1403). Significant differences were observed among different sampling sites, macaque age groups, and species in infection proportion of SFV, and Herpesviridae.

Conclusions: Identifying the pathogens carried by macaques is crucial for preparing for potential disease epidemics and outbreaks.

1 | Introduction

Approximately 72% of emerging infectious disease events are caused by zoonotic diseases, the majority of which are transmitted by wildlife. The most common pathogens are bacteria or rickettsia (54.3%), followed by viruses or prions (25.4%), protozoa (10.7%), fungi (6.3%) and helminths (3.3%) (Jones et al. 2008). Over 20% (77/365 species) of nonhuman primate (NHP) species are zoonotic hosts (Han, Kramer, and Drake 2016). Zoonotic diseases can be prevalent in NHPs given their close genetic relationship with humans (genetic homology of 75.0%–98.5%) (Jiang et al. 2023). This close genetic similarity increases the risk of pathogen spillover between NHPs and humans, facilitating the transmission of infectious diseases across species boundaries. In Thailand, the most common NHP genus is *Macaca*, with six species endemic in

Abbreviations: HBV, hepatitis B virus; McHV-4, macacine herpesvirus 4; NHP, nonhuman primate; PCR, polymerase chain reaction; RFHV, retroperitoneal fibromatosis-associated herpesvirus; RT-PCR, reverse transcription PCR; SFV, simian foamy virus.

Summary

- With 72% of emerging infectious diseases attributed to wildlife-transmitted pathogens, zoonotic transmission between humans and NHPs is predominant because of their genetic similarity to humans.
- The close proximity between macaque habitats and human settlements has heightened the risk of zoonotic disease transmissions. This study identified 18 pathogens in 2703 macaques across 29 provinces in Thailand to determine the infection rates and assess the association between macaque demographics and zoonotic risk.
- A total of 35.7% of free-ranging macaques harbour potential zoonotic pathogens posing substantial health risks to humans were identified. Notable differences in Simian foamy virus and Herpesviridae detection proportions were observed among macaque demographics including age groups, species and sampling sites.

the country, namely, the crab-eating or long-tailed macaque (*Macaca fascicularis*), northern pig-tailed macaque (*M. leonine*), southern pig-tailed macaque (*M. nemestrina*), rhesus macaque (*M. mulatta*), assam macaque (*M. assamensis*), and stump-tailed macaque (*M. arctoides*) (Malaivijitnond 2005; Roos et al. 2014). A nationwide survey conducted in 2023 by the Wildlife Research Division, Department of National Parks, Wildlife and Plant Conservation, Thailand found that 64,055 monkeys resided in 306 human–monkey conflict zones outside conservation areas, including recreation parks, tourist sites, temples, and other areas near human communities. Specifically, the population consisted of 59,840 crab-eating macaques (93.42%), 1365 pig-tailed macaques (2.13%), 1310 rhesus macaques (2.04%), 1174 stump-tailed macaques (1.83%), 306 assam macaques (0.48%), and 60 unclassified macaques (0.09%).

Zoonotic diseases are a significant concern in macaques, with many infectious agents posing potential risks. These include viruses (such as herpes B virus, Ebola virus, hepatitis virus, lyssa virus, monkeypox virus, simian retroviruses, West Nile virus and Zika virus), bacteria (such as *Burkholderia*, *Campylobacter*, *Leptospira*, *Mycobacterium* and *Salmonella*), protozoa (*Hepatocystis* and *Plasmodium*) and gastrointestinal parasites (Vonfeld et al. 2022; Wachtman and Mansfield 2012; WOAH 2021). Pathogenic transmission can occur through direct contact (biting, scratching, or contact with faecal matter) or indirectly via vectors or intermediate hosts. In Asian countries, most studies on zoonotic pathogens in *Macaca* species have focused on vector-borne protozoa, especially *Hepatocystis* and *Plasmodium*, followed by gastrointestinal parasites, viruses, bacteria, and fungi. No studies have investigated various pathogens in the same study (Patouillat et al. 2024). Similarly, in Thailand, several reports have identified diseases in macaques. However, these investigations have only focused on a few specific diseases and were only conducted in areas where macaques are found (Balasubramaniam et al. 2021; Juttuporn, Hengpraprom, and Rattananupong 2023; Kaewchot et al. 2022; Kosoltanapiwat et al. 2022). Herein, we investigated 18 potential zoonotic infections in 2703 free-ranging macaques residing in 29 provinces across Thailand. Because macaque habitats in study areas are located close to human settlements, human–monkey encounters frequently occur, increasing the risk of potential zoonotic disease transmission. Therefore, identification of the pathogens carried by macaques is crucial to prevent possible epidemics and outbreaks.

2 | Materials and Methods

2.1 | Sampling Data

This study was conducted using data collected from the database of the Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals (MoZWE), Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand, from 2014 to 2023. In total, 2703 healthy macaques underwent basic physical (body temperature, body condition score, weight, and clinical signs) examination and specimen collection of oral and/or rectal swabs and/or EDTA blood. The study population included 2603 crab-eating macaques (*M. fascicularis*), 55 assam macaques (*M. assamensis*), 35 rhesus macaques (*M. mulatta*), and 10 southern pig-tailed macaques (*M. nemestrina*). These macaques were sampled from 87 human–monkey conflict areas, which included residential area, Buddhist temples, schools and markets, in 29 provinces in each region across Thailand (Figure 1). These areas are defined as human–monkey conflict zones because of their close proximity to human settlements, which encourages frequent and problematic interactions between monkeys and communities. This definition was established by the Wildlife Research Division, Department of National Parks, Wildlife and Plant Conservation, which collected the samples and submitted them to MoZWE. Pathologic data and other details on the specimens collected (sampling area, species, sex and age) were also retrieved from the MoZWE database without collecting any additional samples from animals.

2.2 | Disease Detection

The samples collected from macaques were analysed using polymerase chain reaction (PCR) or reverse transcription PCR (RT-PCR) or real time-PCR for 18 pathogens (16 viruses, 1 bacterium and 1 protozoan), with a total of 9765 tests conducted. Details of the tests and the number of tested samples in each pathogen are shown in Data S1 and Table 1. The amount of template DNA or RNA used during each conventional PCR or real-time PCR assay was 25–100 ng. A positive control was included in each PCR run to ensure the accuracy of the amplification process. Several measures were implemented to prevent contamination, including the use of filter tips and separate workstations for sample preparation, PCR setup and post-PCR analysis. Reagent blank control was also included in each PCR run to monitor for contamination. Furthermore, all surfaces and equipment were routinely decontaminated using RNase AWAY Surface Decontaminant (Thermo Fisher Scientific, MA, USA) to remove nuclease and nucleic acid residues and thus minimise the risk of crossover.

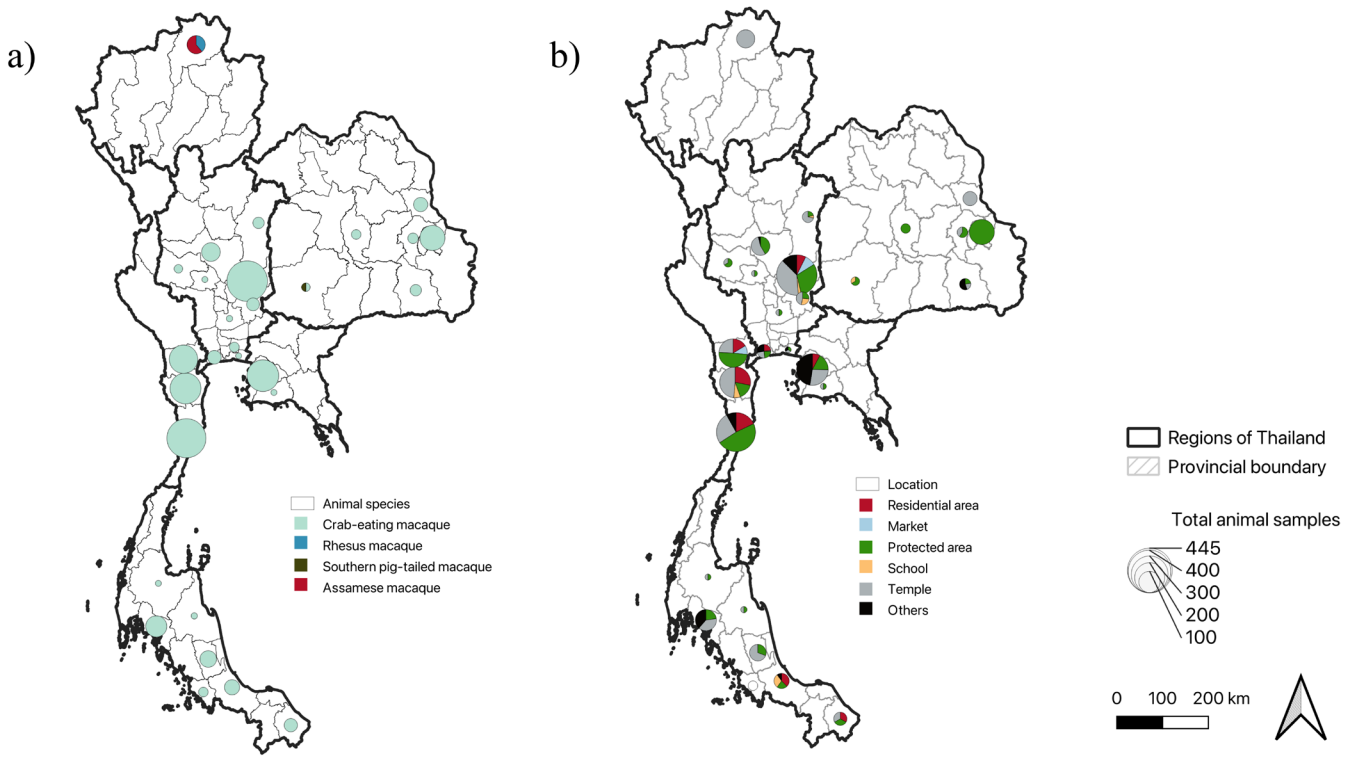

FIGURE 1 | Geographic distribution of the macaque habitat sampling sites. Each sampling site is categorised by macaque species (a) and location type (b). All sampling areas are in close proximity to human settlements.

2.3 | Statistical Analysis

Descriptive statistics were used to analyse the specimens collected from macaque populations. Binary logistic regression was used to assess the crude associations between disease prevalence and each independent variable, including demographic information of macaques (sampling region, age, sex, species) and sample type. Outcomes were expressed as odds ratios (OR) and 95% confidence intervals (CI). Variables with a p value < 0.05 from univariable analysis were included in the multivariable analysis. Multiple logistic regression was performed to identify independent predictors of outcomes. Model fit was evaluated using the Hosmer–Lemeshow test. Multicollinearity among predictors was assessed using variance inflation factors. Binary logistic regression analysis was conducted using SPSS software (version 29). Furthermore, the geographic distribution of the macaques' sample collection and the prevalence of positive pathogens detected in each sampling area categorised using equal intervals were visualised using QGIS v 3.8.3, a free and open-source geographic information system. A Sankey diagram was constructed using R program version 4.4.1. (R Foundation for Statistical Computing, Vienna, Austria) to illustrate the flow path of individual samples from macaques.

3 | Results

The overall proportion of pathogen infection was 35.7% (965/2703), with the highest infection proportion attributed to simian foamy virus (SFV) at 52.5% (759/1446), followed by Herpesviridae at 41.4% (353/852), *Plasmodium* spp. at 1.8% (14/758), and hepatitis B virus (HBV) at 0.1% (2/1403) (Table 1). Notably, of the 759 SFV-positive macaques, 131 (17.3%) were coinfected with other pathogens. Among the coinfected macaques, 120 were infected with Herpesviridae and SFV, 9 were infected with *Plasmodium* spp. and SFV, and 2 were infected with HBV and SFV. The samples flow path is shown in Figure 2.

SFV and Herpesviridae have spread across macaque populations throughout Thailand (Figure 3). In univariable analysis, SFV was 15.67 times more likely to be detected in NHP in the northern region compared with the central region (OR: 15.67; 95% CI: 3.72–66.01), representing the highest observed risk. In contrast, the eastern region was significantly 48% less likely to detect SFV compared to the central region (OR: 0.52; 95% CI: 0.35–0.78). Herpesviridae was significantly 2.91 times more likely to be detected in the southern region compared with the central region (OR: 2.91; 95% CI: 1.95–4.32) (Table 2). Adult macaques were significantly 1.3 times more likely to be infected with SFV (OR: 1.3; 95% CI: 1.02–1.65) compared with the unknown age group, whereas subadult macaques had a lower likelihood of infection (OR: 0.73; 95% CI: 0.55–0.98). The likelihood of infection with SFV (OR: 15.52; 95% CI: 3.71–64.91) and Herpesviridae (OR: 3.39; 95% CI: 1.60–7.19) was higher in *M. mulatta* (rhesus macaque) compared to *M. fascicularis* (crab-eating macaque). In total, 2703 macaques were assessed in this study, including 1472 males, 746 females and 485 macaques without sex information. The odds of SFV and Herpesviridae infection between male and female macaques did not differ significantly ($p > 0.05$) on univariable regression (Table 2).

Variables associated with SFV and Herpesviridae infections, identified through univariable regression, were further

TABLE 1 | Number of samples and pathogen detections by year.

Pathogen	Number of samples tested in each year[a]										
	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	Total
Dengue virus	—	—	51	54	—	—	—	—	—	—	105
Ebola virus and Marburg virus	49	51	81	—	—	—	—	—	—	—	181
Hantavirus	50	51	—	—	—	—	—	—	—	—	101
Hepatitis A virus	50	51	51	54	—	—	—	—	—	—	206
Hepatitis B virus	50	51	81	156	262	648 (2)	100	—	40	15	1403 (2)
Hepatitis C virus	50	51	51	—	—	—	—	—	—	—	152
Hepatitis E virus	50	51	51	—	—	—	—	—	—	—	152
Herpes B virus	50	51	114	198	—	649	122	—	20	15	1219
Herpes simplex virus	50	50	11	102	—	—	—	—	20	—	233
Herpesviridae	—	51 (44)[b]	81	185 (63)[b]	262 (68)[b]	—	100 (58)	173 (120)	—	—	852 (353)
Japanese encephalitis virus	—	—	64	54	—	—	—	—	—	—	118
Lyssavirus	50	51	53	54	262	150	221	70	125	—	1036
Monkeypox virus	—	—	—	—	—	—	—	—	—	15	15
Nipah virus	50	51	51	—	—	—	—	—	—	—	152
Simian foamy virus	—	—	—	—	262 (186)	649 (367)	342 (130)	173 (70)	20 (6)	—	1446 (759)
Zika virus	—	—	131	150	90	—	—	—	—	—	371
Mycobacterium spp.	—	—	—	—	262	649	342	—	12	—	1265
Plasmodium spp.	—	—	—	—	—	648 (14)[b]	—	—	110	—	758 (14)

[a]Numbers in parentheses indicate the number of positive samples.
[b]Positive samples were sequenced for species identification.

analysed using multiple logistic regression. Notably, after adjusting for all other variables, both SFV and Herpesviridae were significantly more likely to be detected in macaques from the northern region compared to the central region. SFV showed an adjusted odds ratio (aOR) of 25.32 (95% CI: 5.92–108.33), while Herpesviridae had an aOR of 4.22 (95% CI: 1.89–9.43). Additionally, Herpesviridae detection was more likely in the southern region than in the central region (aOR: 3.04; 95% CI: 1.02–4.59). SFV was more likely to be identified in pooled blood and swab samples than in individual swabs. Regarding age, adult macaques were significantly more likely to be infected with SFV (aOR: 1.88; 95% CI: 1.44–2.44) than those of unknown age. Conversely, Herpesviridae infections were less likely in adults compared with individuals of unknown age (aOR: 0.57; 95% CI: 0.40–0.82). The infections were also significantly less likely in *M. assamensis* (assam macaque) compared with *M. fascicularis* (aOR: 4.22; 95% CI: 1.89–9.43) (Table 3).

Univariable and multivariable analyses were omitted for *Plasmodium* spp. and HBV because of sample imbalance or disproportionate event distribution across the testing outcomes. *Plasmodium* spp. was restricted to the southern (Stun and Krabi Provinces, 6.78%, 8/118) and central (Bangkok and Saraburi Provinces, 2.32%, 6/259) regions of Thailand, with the significantly highest prevalence occurring in the southern provinces (Figure 3 and Data S2). HBV was identified in one sample from the central region (Bangkok Province, 0.26%, 1/379) and one from the northeastern region (Si Sa Ket Province, 0.52%, 1/193); however, the difference was not statistically significant (Figure 3 and Additional file 2).

The positive samples from *Plasmodium* spp. and Herpesviridae were sequenced for species identification by Sanger sequencing. Ten of fourteen *Plasmodium* spp. specimens were *P. inui*, while the remaining samples were *P. coatneyi*. For Herpesviridae, out of a total of 353 samples, 159 were identified as macacine herpesvirus 4 (McHV-4) or cercopithecine herpesvirus 15 in the genus *Lymphocryptovirus*, 13 as retroperitoneal fibromatosis-associated herpesvirus (RFHV) in the genus *Rhadinovirus*. However, 181 samples were not sequenced for species

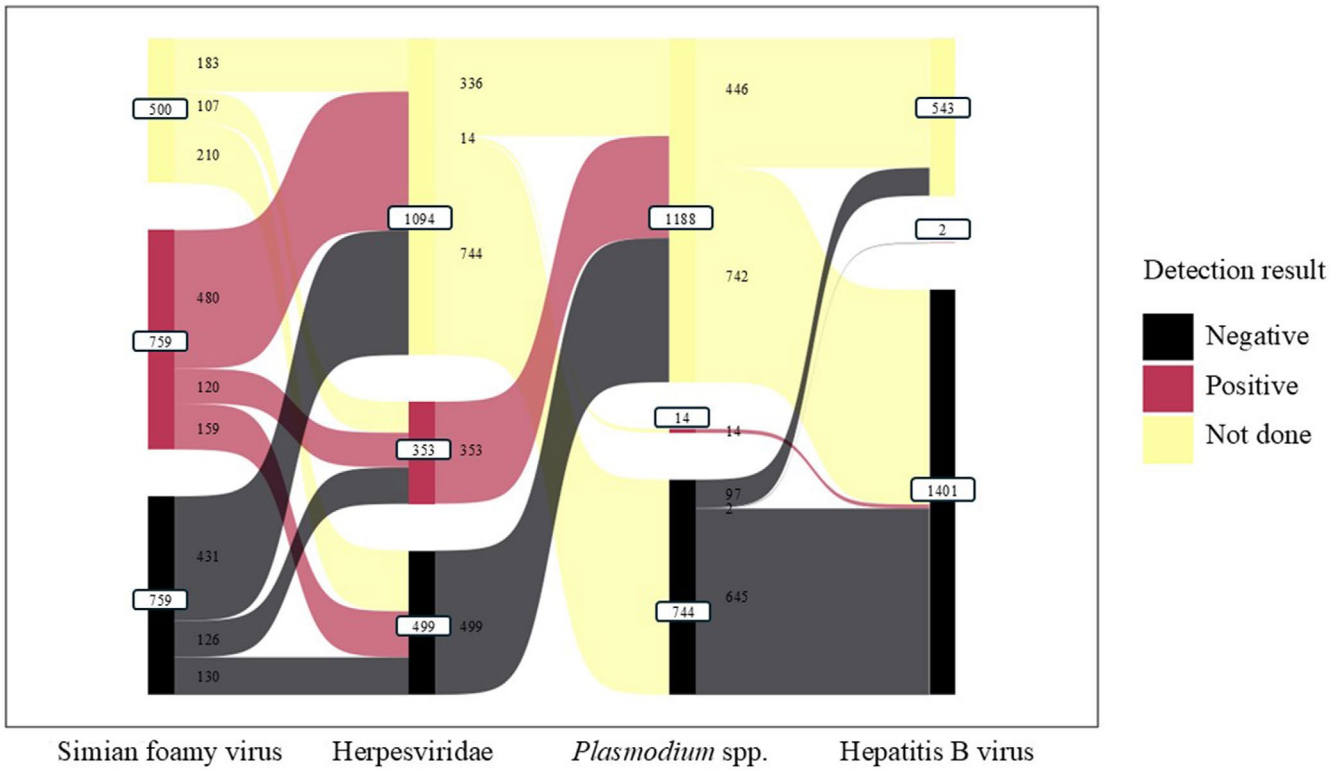

FIGURE 2 | Sankey diagram depicting the flow path of individual samples from macaques detected for simian foamy virus, Herpesviridae, *Plasmodium* spp. and hepatitis B virus.

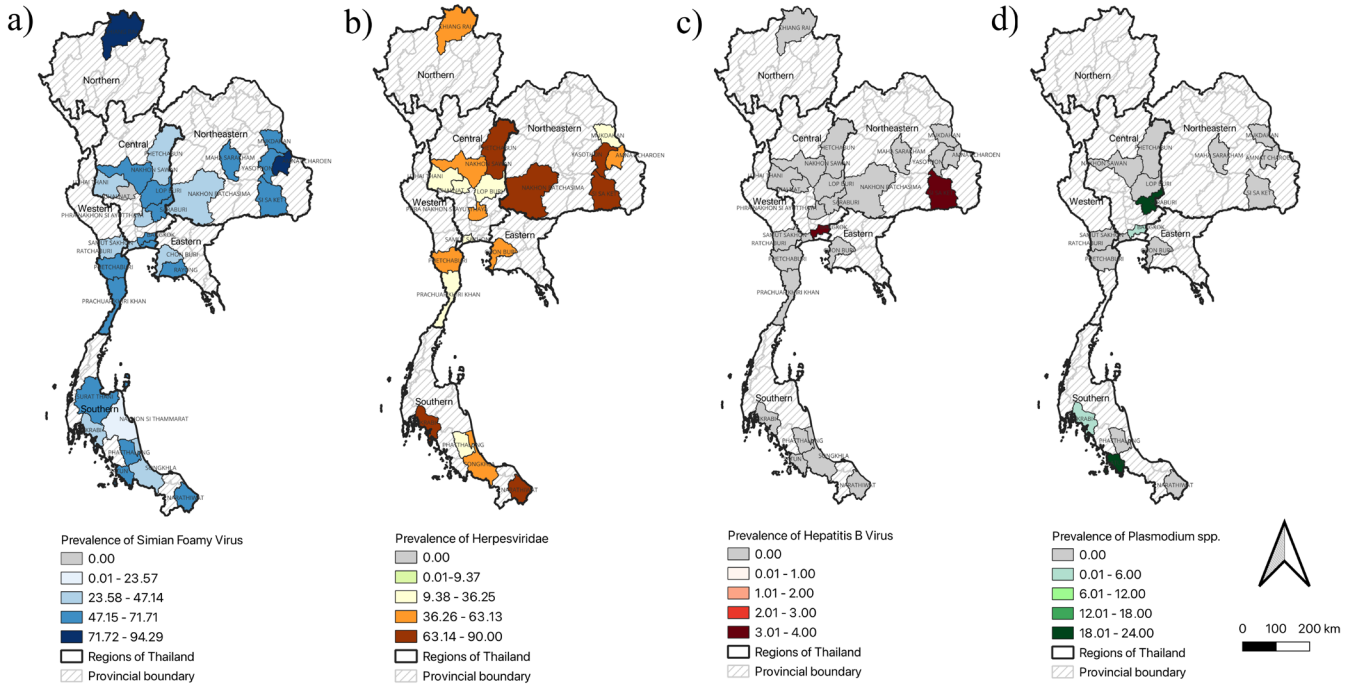

FIGURE 3 | Prevalence of positive pathogens detected in each sampling area. The prevalence of (a) simian foamy virus, (b) Herpesviridae, (c) hepatitis B virus and (d) *Plasmodium* spp.

identification (Table 4). Some sequences of *Plasmodium*- and Herpesviridae-positive samples were deposited in GenBank with the following accession numbers: PQ482493-PQ482495 for *Plasmodium* and MH938751–MH938806 for Herpesviridae.

4 | Discussion

In Thailand, most habitats of free-ranging macaques outside conservation areas are close to human communities, potentially

increasing the risk of transmission of infectious diseases between macaques and humans. In this study, the overall infection proportion of potential zoonotic diseases was 35.7%. Of this, SFV had the highest infection proportion (52%). Prevalence of SFV in *Macaca* can vary between 10% and 100% (Meiering and Linial 2001). In Cambodia, the prevalence of SFV was 48% as determined by serological surveillance and 17% based on PCR detection (Ayouba et al. 2013). By contrast, seroprevalence in Thailand and Singapore was 87% and 100%, respectively (Jones-Engel et al. 2007), whereas molecular detection by PCR was 57% and 90% in Thailand and Singapore, respectively (Jones-Engel et al. 2007; Kaewchot et al. 2022). The seroprevalence of SFV was higher than that detected by PCR because serological tests can indicate long-term exposure of the virus, whereas the PCR results may vary based on the timing of sample collection relative to viral shedding. Our study demonstrated that SFV was more likely to be detected in pooled oral and blood samples compared with individual swab samples, a pattern consistently supported by the odds ratios observed in both univariable and multivariable analyses. These results suggested that pooling samples likely increased the viral load, thereby enhancing the sensitivity for virus detection compared to individual samples. Previous studies have detected proviral DNA of SFV from multiple organs and blood of infected NHPs, indicating widespread latent infection. High levels of viral RNA have also been found in the oral mucosa, which is the main site of SFV replication. High viral shedding in saliva facilitates highly efficient transmission among NHP populations (Falcone et al. 1999; Murray et al. 2008). Moreover, SFV infection is predominant in adults, a development stage associated with increasing aggression (Al-Hakim et al. 2023), indicates that their behaviour poses a risk for exposure. The virus in the saliva can be passed on through

TABLE 2 | Univariable analysis of the associations between demographic variables, sample types and disease occurrence in macaques.

	SFV		Herpesviridae	
Variable	***p* value**	**OR (95% CI)**	***p* value**	**OR (95% CI)**
Region				
Northern	< 0.001*	15.672 (3.721–66.008)	0.067	1.606 (0.967–2.666)
Northeastern	0.006*	1.572 (1.139–2.169)	0.005*	1.970 (1.221–3.179)
Eastern	0.001*	0.520 (0.349–0.776)	0.010*	1.888 (1.166–3.058)
Western	0.580	1.088 (0.808–1.464)	0.937	0.981 (0.616–1.564)
Southern	0.498	0.905 (0.677–1.209)	< 0.001*	2.906 (1.954–4.322)
Central	—	Ref.	—	Ref.
Sample type				
Blood	0.999	0.000 (0.000–∞)	0.998	0.000 (0.000–∞)
Swab	< 0.001*	0.295 (0.192–0.453)	0.589	0.891 (0.586–1.354)
Pool	—	Ref.	—	Ref.
Age				
Juvenile	0.999	0.000 (0.000–∞)	0.999	1.87E + 09 (0.000–∞)
Subadult	0.038*	0.734 (0.548–0.984)	0.150	0.621 (0.324–1.189)
Adult	0.032*	1.297 (1.022–1.647)	< 0.001*	0.564 (0.418–0.762)
Unknown	—	Ref.	—	Ref.
Sex				
Male	0.113	1.542 (0.902–2.633)	0.150	0.749 (0.505–1.111)
Female	0.266	1.367 (0.788–2.372)	0.947	1.015 (0.651–1.583)
Unknown	—	Ref.	—	Ref.
Species				
M. assamensis	N/A	N/A	0.011*	0.438 (0.231–0.829)
M. mulatta	< 0.001*	15.517 (3.709–64.915)	0.001*	3.392 (1.600–7.195)
M. nemestrina	0.471	0.627 (0.176–2.231)	0.927	0.942 (0.264–3.367)
M. fascicularis	—	Ref.	—	Ref.

Abbreviations: N/A = data not available; Pool = pooled blood and swab samples; Ref. = reference; Swab = oral or nasal or rectal swab.
*Indicates statistical significance (p value < 0.05).

the wound caused when an infected macaque bites another. SFV should be considered as a significant zoonotic agent because SFV infection has been reported worldwide in 1%–3% of individuals who worked with NHPs in zoos, primate centers, laboratories and bushmeat hunters (Betsem et al. 2011; Heneine et al. 1998; Jones-Engel et al. 2008; Sandstrom et al. 2000). In South and Southeast Asian countries, the overall prevalence of SFV in humans living or working around nonhuman primates was 2.6% (8/305), with 3 confirmed SFV-positive cases found in Thailand (Jones-Engel et al. 2008). In detail, two SFV infection cases in Thailand involved individuals with a history of being bitten or scratched by macaques (*M. nemestrina* and *M. assamensis*). In one case, an infected patient reported that an *M. assamensis* individual would enter his home a few times a year because of his proximity to a Buddhist temple where free-ranging macaques lived. However, he had no history of bites or scratches by macaques (Jones-Engel et al. 2008); thus, the infection may have occurred via contact with contaminated materials.

The second most prevalent pathogenic agents identified in this study were species under Herpesviridae, specifically, McHV-4 and RFHV. McHV-4 is closely related to and shares similar genomic organisation and biological properties with Epstein–Barr virus (EBV) in humans. It causes naturally occurring infections in both Old and New World monkeys (Kaewchot et al. 2019; Kamperschroer, Tartaro, and Kumpf 2014; Ohta 2023). Similar to EBV infection in humans, McHV-4 infection in macaques is typically asymptomatic or causes only mild symptoms and often resolves on its own in individuals with normal immune systems (Ohta 2023). However, experimental infection with McHV-4 in immunocompromised rhesus macaques has been associated with the development of various clinical conditions similar to those in EBV infection in immunosuppressed humans (Carville and Mansfield 2008). Based on the data, McHV-4 tends to be more virulent in immunocompromised animals. In this study, we also detected RFHV in macaques. A previous report found that distinct RFHV variants occur in each macaque species (Kaewchot et al. 2019). Macaque RFHV is closely related to human herpesvirus-8, which may be a necessary cofactor for the development of Kaposi's sarcoma in humans (Rose et al. 1997). Even though McHV-4 and RFHV have equivalent human

TABLE 3 | Multivariable analysis of the associations between demographic variables, sample types, and disease occurrence in macaques.

	SFV		Herpesviridae	
Variable	***p* value**	**aOR (95% CI)**	***p* value**	**aOR (95% CI)**
Region				
Northern	< 0.001*	25.318 (5.917–108.329)	< 0.001*	4.218 (1.887–9.429)
Northeastern	0.021*	1.521 (1.066–2.172)	0.084	1.578 (0.940–2.650)
Eastern	0.009*	0.583 (0.388–0.874)	0.124	1.486 (0.897–2.460)
Western	0.080	1.315 (0.967–1.789)	0.855	0.957 (0.598–1.532)
Southern	0.765	0.955 (0.707–1.290)	< 0.001*	3.043 (2.017–4.592)
Central	—	Ref.	—	Ref.
Sample type				
Blood	0.999	0.000 (0.000–∞)	Excluded	Excluded
Swab	< 0.001*	0.295 (0.187–0.465)	Excluded	Excluded
Pool	—	Ref.	Excluded	Excluded
Age				
Juvenile	0.999	0.000 (0.000–∞)	0.999	9.33E + 08 (0.000–∞)
Subadult	0.391	1.149 (0.837–1.578)	0.195	0.606 (0.284–1.293)
Adult	< 0.001*	1.875 (1.437–2.445)	0.002*	0.571 (0.399–0.816)
Unknown	—	Ref.	—	Ref.
Species				
M. assamensis	N/A	N/A	0.002*	0.202 (0.072–0.565)
M. mulatta	N/A	N/A	N/A	N/A
M. nemestrina	0.552	0.672 (0.182–2.488)	0.660	0.742 (0.197–2.804)
M. fascicularis	—	Ref.	—	Ref.

Abbreviations: N/A = data not available; excluded = data not included in the multivariable analysis because univariable analysis did not show significant results; Pool = pooled blood and swab samples; Ref. = reference; Swab = oral or nasal or rectal swab.
*Indicates statistical significance (p value < 0.05).

TABLE 4 | Pathogens detected in this study.

Pathogen	Species	Number of sample (% positive)		
		Positive	Negative	Total
Hepatitis B virus		2 (0.1)	1401	1403 (0.1)
Simian foamy virus		759 (52.5)	687	1446 (52.5)
Herpesviridae	McHV-4	159 (18.7)	499	852 (41.4)
	RFHV	13 (1.5)		
	Not identified	181 (21.2)		
Plasmodium	*P. inui*	10 (1.3)	744	758 (1.8)
	P. coatneyi	4 (0.5)		

pathogens, no evidence of human McHV-4 and RFHV infections has been reported. Furthermore, our study found that the likelihood of Herpesviridae infection was lower in adult macaques compared to those of unknown age, while the odds of infection in juveniles were comparable to those in individuals of unknown age. A previous study suggested that juvenile macaques were more susceptible to RFHV than older individuals possibly because of their relatively immunocompromised state (Giddens et al. 1985; Lu et al. 2023). However, the limited data on the unknown age group in our study prevents a definitive conclusion.

In the present study, *P. inui* (1.3%) and *P. coatneyi* (0.5%) were found in *M. fascicularis* from the central and southern parts of Thailand, with a significantly higher prevalence in macaque samples from the southern region. These results were consistent with previous reports, which indicated that *Plasmodium* infections in macaques have been observed across Thailand, predominantly in the southern region (Fungfuang et al. 2020; Putaporntip et al. 2010; Seethamchai et al. 2008). Although the primers used in this study have been used to successfully detect the two most common zoonotic malaria species, *P. knowlesi* and *P. cynomolgi* (Braima et al. 2024), we did not find these *Plasmodium* species in our study. This could be attributed to the low prevalence of *Plasmodium* species in Thailand (Narapakdeesakul et al. 2023) and may be influenced by differences in the sensitivity of the tests and the primers used in each study. The overall prevalence of *Plasmodium* detected in this study was 1.8%, which is lower than that reported in previous studies. In Thailand, 6.9% (40/580) of *M. fascicularis* and *M. nemestrina* were infected with *Plasmodium*, as detected by nested PCR. Among these macaques, *P. inui* (5.5%) was the most prevalent species, followed by *P. cynomolgi* (1%) and *P. knowlesi* (0.3%) (Narapakdeesakul et al. 2023). By contrast, a study in Southeast Asia using nested PCR found a *Plasmodium* prevalence of 64.1% in *M. fascicularis*, with *P. cynomolgi* (53.3%) being the most dominant species, followed by *P. coatneyi* (20.4%), *P. inui* (12.3%), *P. fieldi* (3.4%) and *P. knowlesi* (0.4%) (Zhang et al. 2016). In Southeast Asia, simian malaria, *P. knowlesi*, *P. cynomolgi*, *P. coatneyi* and *P. inui* are potential agents of zoonotic malaria, which can be transmitted from macaques to humans through infected-mosquito (*Anopheles*) bites (Dian et al. 2022). Malaria infection in macaques presents a potential public health risk to the local population. Therefore, vector control should be incorporated into strategies for the prevention, reduction, and elimination of simian malaria transmission to humans.

HBV infection had the lowest pathogen prevalence (0.1%) in this study. The partial S gene of HBV-positive samples was sequenced by Kaewchot et al. (2022), who found that the positive samples showed 100% similarity to HBV sequences from Thai orangutans. Interspecies transmission between humans and apes (orangutans, gorillas, chimpanzees, and gibbons) is well documented (Grethe et al. 2000; Lanford et al. 2000). In contrast with apes, Old World monkeys, including baboons and macaques, were regarded as unsusceptible hosts for human HBV infection or vice versa (Müller et al. 2018). However, macaques infected with HBV have been reported in wild *M. fascicularis* living in Mauritius Island with 26% prevalence (Dupinay et al. 2013). The whole genome sequence of *M. fascicularis* HBV showed greater than 98% similarity to that of human HBV, indicating the simian variety might have been transmitted from humans (Dupinay et al. 2013).

One limitation of this study is that healthy macaques were tested, which may limit the understanding of the relationship between health status and disease prevalence. To strengthen the robustness of our findings, future investigations should analyse various health statuses, including symptomatic and asymptomatic individuals, thereby enabling a more nuanced analysis of disease prevalence and its epidemiological implications for macaque populations. Furthermore, although age significantly influenced the likelihood of SFV and Herpesviridae infection in macaques, approximately 49% of individuals lacked age data, limiting our ability to accurately assess age-related infection patterns within this population. Finally, although substantial associations were observed between a higher likelihood of SFV and Herpesviridae infection and *M. mulatta*, the majority of samples were collected from *M. fascicularis*, with limited samples from *M. leonina* and *M. arctoides*. A larger sample size across all macaque species found in Thailand would yield valuable information for identifying potential reservoir species.

In conclusion, our study found that 35.7% of free-ranging macaques harbour potential zoonotic pathogens that pose significant health risks to humans. This crucial finding underscores the need for control measures in areas where macaques and

human populations coexist. Recommended interventions include regular health checks and monitoring of macaque populations and humans (especially those who come into close contact with macaques), public education on the risks and preventive measures associated with close contact with macaques, and strict enforcement of hygiene and sanitation practices in areas of human–macaque interactions.

Author Contributions

Sarin Suwanpakdee: investigation, methodology, visualisation, data curation, writing – original draft. Benjaporn Bhusri: methodology, writing – original draft. Aeknarin Saechin: formal analysis. Chalisa Mongkolphan: methodology. Siriporn Tangsudjai: data curation. Parut Suksai: investigation. Supakarn Kaewchot: resources. Rattana Sariwongchan: resources. Piya Sereerak: resources. Ladawan Sariya: conceptualization, validation, visualisation, writing – review and editing. This manuscript has been reviewed by all authors.

Acknowledgements

We would like to thank the Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals, Faculty of Veterinary Science, Mahidol University for providing informative data in this study.

Ethics Statement

This study was performed after obtaining verbal informed consent from the sample owners and approved by the animal ethics committee of the Faculty of Veterinary Science, Mahidol University.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the Supporting Information of this article.

References

Al-Hakim, R. R., S. B. Hastuti, E. K. Nasution, I. G. A. A. R. Puspitasari, C. D. Imtiyaaz, and S. Rukayah. 2023. "Social Behaviour of Endangered Macaque Species at Kalisalak Forest, Central Java-Indonesia." *IOP Conference Series: Earth and Environmental Science* 1220, no. 1: 012021. https://doi.org/10.1088/1755-1315/1220/1/012021.

Ayouba, A., L. Duval, F. Liégeois, et al. 2013. "Nonhuman Primate Retroviruses From Cambodia: High Simian Foamy Virus Prevalence, Identification of Divergent STLV-1 Strains and no Evidence of SIV Infection." *Infection, Genetics and Evolution* 18: 325–334. https://doi.org/10.1016/j.meegid.2013.04.015.

Balasubramaniam, K. N., S. Malaivijitnond, T. Kemthong, et al. 2021. "Prevalence of Enterobacteriaceae in Wild Long-Tailed Macaques (*Macaca fascicularis*) in Thailand." *International Journal of Primatology* 42, no. 3: 337–341. https://doi.org/10.1007/s10764-021-00209-3.

Betsem, E., R. Rua, P. Tortevoye, A. Froment, and A. Gessain. 2011. "Frequent and Recent Human Acquisition of Simian Foamy Viruses Through apes' Bites in Central Africa." *PLoS Pathogens* 7, no. 10: e1002306. https://doi.org/10.1371/journal.ppat.1002306.

Braima, K. A., K. A. Piera, I. N. Lubis, et al. 2024. "Improved Limit of Detection for Zoonotic Plasmodium Knowlesi and P. Cynomolgi Surveillance Using Reverse Transcription for Total Nucleic Acid Preserved Samples or Dried Blood Spots." *medRxiv*. https://doi.org/10.1101/2024.04.04.24305339.

Carville, A., and K. G. Mansfield. 2008. "Comparative Pathobiology of Macaque Lymphocryptoviruses." *Comparative Medicine* 58, no. 1: 57–67.

Dian, N. D., M. Rahim, S. Chan, and Z. M. Idris. 2022. "Non-human Primate Malaria Infections: A Review on the Epidemiology in Malaysia." *International Journal of Environmental Research and Public Health* 19, no. 13: 7888. https://doi.org/10.3390/ijerph19137888.

Dupinay, T., T. Gheit, P. Roques, et al. 2013. "Discovery of Naturally Occurring Transmissible Chronic Hepatitis B Virus Infection Among Macaca Fascicularis From Mauritius Island." *Hepatology* 58, no. 5: 1610–1620. https://doi.org/10.1002/hep.26428.

Falcone, V., J. Leupold, J. Clotten, et al. 1999. "Sites of Simian Foamy Virus Persistence in Naturally Infected African Green Monkeys: Latent Provirus Is Ubiquitous, Whereas Viral Replication Is Restricted to the Oral Mucosa." *Virology* 257, no. 1: 7–14. https://doi.org/10.1006/viro.1999.9634.

Fungfuang, W., C. Udom, D. Tongthainan, K. A. Kadir, and B. Singh. 2020. "Malaria Parasites in Macaques in Thailand: Stump-Tailed Macaques (*Macaca arctoides*) are New Natural Hosts for Plasmodium Knowlesi, Plasmodium Inui, Plasmodium Coatneyi and Plasmodium Fieldi." *Malaria Journal* 19, no. 1: 350. https://doi.org/10.1186/s12936-020-03424-0.

Giddens, W. E., Jr., C. C. Tsai, W. R. Morton, H. D. Ochs, G. H. Knitter, and G. A. Blakley. 1985. "Retroperitoneal Fibromatosis and Acquired Immunodeficiency Syndrome in Macaques. Pathologic Observations and Transmission Studies." *American Journal of Pathology* 119, no. 2: 253–263.

Grethe, S., J.-O. Heckel, W. Rietschel, and T. Hufert Frank. 2000. "Molecular Epidemiology of Hepatitis B Virus Variants in Nonhuman Primates." *Journal of Virology* 74, no. 11: 5377–5381. https://doi.org/10.1128/jvi.74.11.5377-5381.2000.

Han, B. A., A. M. Kramer, and J. M. Drake. 2016. "Global Patterns of Zoonotic Disease in Mammals." *Trends in Parasitology* 32, no. 7: 565–577. https://doi.org/10.1016/j.pt.2016.04.007.

Heneine, W., W. M. Switzer, P. Sandstrom, et al. 1998. "Identification of a Human Population Infected With Simian Foamy Viruses." *Nature Medicine* 4, no. 4: 403–407. https://doi.org/10.1038/nm0498-403.

Jiang, X., Z. Fan, S. Li, and H. Yin. 2023. "A Review on Zoonotic Pathogens Associated With Non-human Primates: Understanding the Potential Threats to Humans." *Microorganisms* 11, no. 2: 246. https://doi.org/10.3390/microorganisms11020246.

Jones, K. E., N. G. Patel, M. A. Levy, et al. 2008. "Global Trends in Emerging Infectious Diseases." *Nature* 451, no. 7181: 990–993. https://doi.org/10.1038/nature06536.

Jones-Engel, L., C. C. May, G. A. Engel, et al. 2008. "Diverse Contexts of Zoonotic Transmission of Simian Foamy Viruses in Asia." *Emerging Infectious Diseases* 14, no. 8: 1200–1208. https://doi.org/10.3201/eid1408.071430.

Jones-Engel, L., K. A. Steinkraus, S. M. Murray, et al. 2007. "Sensitive Assays for Simian Foamy Viruses Reveal a High Prevalence of Infection in Commensal, Free-Ranging Asian Monkeys." *Journal of Virology* 81, no. 14: 7330–7337. https://doi.org/10.1128/jvi.00343-07.

Juttuporn, A., S. Hengpraprom, and T. Rattananupong. 2023. "Incidences and Characteristics of Monkey-Related Injuries Among Locals and Tourists in Mueng District, Lopburi Province, 2013–2017." *Chulalongkorn Medical Journal* 64, no. 1: 29–37.

Kaewchot, S., C. Mongkolphan, P. Suksai, et al. 2019. "Occurrence of Oral Shedding of Retroperitoneal Fibromatosisassociated Herpesvirus

and Lymphocryptovirus in Free-Ranging Macaques, Thailand." *Thai Journal of Veterinary Medicine* 49, no. 4: 369–375.

Kaewchot, S., S. Tangsudjai, L. Sariya, et al. 2022. "Zoonotic Pathogens Survey in Free-Living Long-Tailed Macaques in Thailand." *International Journal of Veterinary Science and Medicine* 10, no. 1: 11–18. https://doi.org/10.1080/23144599.2022.2040176.

Kamperschroer, C., K. Tartaro, and S. W. Kumpf. 2014. "Quantitative PCR Assays Reveal High Prevalence of Lymphocryptovirus as Well as Lytic Phase Gene Expression in Peripheral Blood Cells of Cynomolgus Macaques." *Journal of Virological Methods* 207: 220–225. https://doi.org/10.1016/j.jviromet.2014.07.015.

Kosoltanapiwat, N., J. Tongshoob, S. Ampawong, et al. 2022. "Simian Adenoviruses: Molecular and Serological Survey in Monkeys and Humans in Thailand." *One Health* 15, no. 100: 434. https://doi.org/10.1016/j.onehlt.2022.100434.

Lanford, R. E., D. Chavez, R. Rico-Hesse, and A. Mootnick. 2000. "Hepadnavirus Infection in Captive Gibbons." *Journal of Virology* 74, no. 6: 2955–2959. https://doi.org/10.1128/jvi.74.6.2955-2959.2000.

Lu, J., Y. Long, J. Sun, and L. Gong. 2023. "Towards a Comprehensive View of the Herpes B Virus [Review]." *Frontiers in Immunology* 14: 1281384. https://doi.org/10.3389/fimmu.2023.1281384.

Malaivijitnond, S. 2005. "The Current Distribution and Status of Macaques in Thailand." *Natural History Journal of Chulalongkorn University* 1: 35–45.

Meiering, C. D., and M. L. Linial. 2001. "Historical Perspective of Foamy Virus Epidemiology and Infection." *Clinical Microbiology Reviews* 14, no. 1: 165–176. https://doi.org/10.1128/cmr.14.1.165-176.2001.

Müller, S. F., A. König, B. Döring, D. Glebe, and J. Geyer. 2018. "Characterisation of the Hepatitis B Virus Cross-Species Transmission Pattern via na+/Taurocholate Co-Transporting Polypeptides From 11 New World and Old World Primate Species." *PLoS One* 13, no. 6: e0199200. https://doi.org/10.1371/journal.pone.0199200.

Murray, S. M., L. J. Picker, M. K. Axthelm, K. Hudkins, C. E. Alpers, and M. L. Linial. 2008. "Replication in a Superficial Epithelial Cell Niche Explains the Lack of Pathogenicity of Primate Foamy Virus Infections." *Journal of Virology* 82, no. 12: 5981–5985. https://doi.org/10.1128/jvi.00367-08.

Narapakdeesakul, D., T. Pengsakul, M. Kaewparuehaschai, et al. 2023. "Zoonotic Simian Malaria Parasites in Free-Ranging Macaca Fascicularis Macaques and Human Malaria Patients in Thailand, With a Note on Genetic Characterization of Recent Isolates." *Acta Tropica* 248, no. 107: 030. https://doi.org/10.1016/j.actatropica.2023.107030.

Ohta, E. 2023. "Pathologic Characteristics of Infectious Diseases in Macaque Monkeys Used in Biomedical and Toxicologic Studies." *Journal of Toxicologic Pathology* 36: 95–122. https://doi.org/10.1293/tox.2022-0089.

Patouillat, L., A. Hambuckers, S. Subrata, M. Garigliany, and F. Brotcorne. 2024. "Zoonotic Pathogens in Wild Asian Primates: A Systematic Review Highlighting Research Gaps." *Frontiers in Veterinary Science* 11: 1386180. https://doi.org/10.3389/fvets.2024.1386180.

Putaporntip, C., S. Jongwutiwes, S. Thongaree, S. Seethamchai, P. Grynberg, and A. L. Hughes. 2010. "Ecology of Malaria Parasites Infecting Southeast Asian Macaques: Evidence From Cytochrome b Sequences." *Molecular Ecology* 19, no. 16: 3466–3476. https://doi.org/10.1111/j.1365-294X.2010.04756.x.

Roos, C., R. Boonratana, J. Supriatna, et al. 2014. "An Updated Taxonomy and Conservation Status Review of Asian Primates." *Asian Primates Journal* 4: 2–38.

Rose, T. M., K. B. Strand, E. R. Schultz, et al. 1997. "Identification of Two Homologs of the Kaposi's Sarcoma-Associated Herpesvirus (Human Herpesvirus 8) in Retroperitoneal Fibromatosis of Different Macaque Species." *Journal of Virology* 71, no. 5: 4138–4144. https://doi.org/10.1128/jvi.71.5.4138-4144.1997.

Sandstrom, P. A., K. O. Phan, W. M. Switzer, et al. 2000. "Simian Foamy Virus Infection Among Zoo Keepers." *Lancet* 355, no. 9203: 551–552. https://doi.org/10.1016/s0140-6736(99)05292-7.

Seethamchai, S., C. Putaporntip, S. Malaivijitnond, L. Cui, and S. Jongwutiwes. 2008. "Malaria and Hepatocystis Species in Wild Macaques, Southern Thailand." *American Journal of Tropical Medicine and Hygiene* 78, no. 4: 646–653. https://doi.org/10.4269/ajtmh.2008.78.646.

Vonfeld, I., T. Prenant, B. Polack, J. Guillot, and B. Quintard. 2022. "Gastrointestinal Parasites in Non-human Primates in Zoological Institutions in France." *Parasite* 29: 43. https://doi.org/10.1051/parasite/2022040.

Wachtman, L., and K. Mansfield. 2012. "Viral Diseases of Nonhuman Primates." In: *Nonhuman Primates in Biomedical Research*, edited by C. R. Abee, K. Mansfield, S. Tardif, and T. Morris, 2nd ed., 1–104. Boston MA: Academic Press. https://doi.org/10.1016/B978-0-12-381366-4.00001-8.

WOAH. 2021. "Zoonoses Transmissible From Non-Human Primates." In *WOAH Terrestrial Manual 2021*, 1–5. Paris, France: World Organisation for Animal Health (WOAH). https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/.

Zhang, X., K. A. Kadir, L. F. Quintanilla-Zariñan, et al. 2016. "Distribution and Prevalence of Malaria Parasites Among Long-Tailed Macaques (*Macaca fascicularis*) in Regional Populations Across Southeast Asia." *Malaria Journal* 15, no. 1: 450. https://doi.org/10.1186/s12936-016-1494-0.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.